26.001 ACRIDINE ORANGE AGGREGATION IN HOMOGENOUS AND NONHOMOGENOUS AQUEOUS SOLUTIONS

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Introducao:

The Acridine Orange (AO) interactions with micelles is of a great importance for many industrial processes and in medicine as well. Due to its photophysical properties, mainly photoactivity, high quantum yield and lifetime of their triplet state, the AO has been intensively studied as a potential sensitizing agent inducing photochemical reactions in biological environment. An important feature of this cyanine is its tendency to aggregate already in homogeneous aqueous solution. The aggregation reduces the AO efficiency as a photosensitizer or fluorescent probe due to the reduction of the lifetimes and quantum yields of its electronic excited states. The interaction with nanoorganized systems such as biological membrane, polymers, micelles etc., can stimulate or reduce the AO aggregation.

Objetivos:

In this work we studied the mechanism and dynamics of AO aggregation in their interactions with Sodium Dodecyl Sulfate (SDS) micelles as a function of the dye and SDS concentrations, pH and ionic strength.

Metodos:

Analyses were performed using optical absorption and fluorescence spectroscopies in their steady-state and time-resolved form, resonance (RLS) and dynamic (DLS) light scattering techniques. The AO concentration were controlled spectrophotometrically using ε490nm = 3.9x104 M-1cm-1. The solutions were prepared in phosphate buffer with Milli-Q quality water, ionic strength of 7.5 mM and pH 6.8. Absorption spectra of the AO were monitored with Beckman Coulter DU 640. The fluorescence and RLS were realized with a Hitachi F-7000 spectrofluorimeter. The DLS was measured with Zetasizer 3000HSA. Each value was the result of five independent measurements.

Resultados:

In aqueous solution in the presence of SDS we observed the AO aggregation. The aggregation

changes the AO absorption spectrum, reduces its fluorescence intensity and increases the resonance light scattering intensity. Depending of the ratio [AO/SDS] SDS can stimulate the formation of mixed nAO-mSDS aggregates or their disaggregation. We found a dynamics of the nAO-mSDS aggregate formation, with the successive formation of 4 different types of aggregates continued from seconds to hours, depending on AO and SDS concentrations and the environment composition. Characteristics time of the three kinetic components of the [AO] (14-74 uM) in the presence of [SDS] (0,1-1 mM) vary: t1 from 1.3 to 3.0 minutes, t2 from 20 to 60 minutes, t3 from 200 to 560 minutes with experimental deviation ≈ 10%.

Conclusão:

The aggregation modifies the photophysical characteristics of the AO monomer. The aggregation quenches the AO fluorescence due to the increase of the probability of non-radiative energy dissipation and the resonant light scattering intensity as a result of the increase of the size of scattering particles. The AO monomers bound with SDS pre-micelles and/or micelles possess higher fluorescence intensity as compared with that in homogeneous aqueous solutions. The aggregation is a complex process with duration from seconds to hours. Therefore it should be taken into account at the AO practical applications.

Keywords: Acridine orange, Sodium Dodecyl Sulfate, aggregation kinetics, effects on spectral characteristics.

Apoio Financeiro:

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Keywords: Acridine orange, Sodium Dodecyl Sulfate, aggregation kinetics, effects on spectral characteristics.

Comitê de Ética:

Não se aplica.

26.002 AMPHIPHILIC ANTIMONY COMPLEX FOR ORAL AND TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS: INFLUENCE OF SUPRAMOLECULAR AGGREGATION STATE

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Introducao:

Pentavalent antimonial (SbV) drugs have been used to date in the treatment of all forms of leishmaniasis, besides the need for parenteral administration for long periods and severe side effects. An amphiphilic SbV complex was recently synthesized from octanoyl-N-methylglucamide (Sb-L8) with the purpose of enhancing the permeation of SbV across biological barriers and thereby achieving topically- and orally-active antimonial drugs.

Objetivos:

In the present work, two different formulations of Sb-L8, containing or not the low-polarity propylene glycol (PG) solvent, were compared for their supramolecular aggregation state, their percutaneous absorption, their pharmacokinetics after oral administration and their effectiveness in a murine model of cutaneous leishmaniasis (CL).

Metodos:

The size and supramolecular organization of Sb-L8 nanoassemblies were investigated by fluorescence probing of hydrophobic environment, Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) on hydrophilic and hydrophobic substrates. Percutaneous absorption of Sb (formulations at 1M Sb in Natrosol gel) was evaluated using Franz diffusion cells with skin of hairless HRS/J mice with or without stratum corneum (SC). The serum pharmacokinetics of Sb was evaluated in Swiss mice after oral administration at 200mgSb/Kg. Sb was determined by graphite furnace atomic absorption. In efficacy assays, the formulations were applied topically (12mg/application/day) or orally (200mg/Kg/day) for 30 days in Balb/c mice infected with Leishmania amazonensis (MHOM/BR/89/BA199) at the base of the tail and parasite burden was determined in the lesion by limiting dilution. Statistical analyses were performed using Mann-Whitney.

Resultados:

Upon addition of PG to Sb-L8 aqueous dispersion (1:1 water:PG, v/v), Sb-L8 nanoassemblies lose their hydrophobic nucleus and become more flexible, as evidenced by TEM, fluorimetry, and AFM. The percentage of Sb permeated across hairless skin after 8h from applied Sb-L8 gels was significantly greater in the presence than absence of PG, when using the skin without SC (median % [25-75% percentile]: 0.57 [0.48-0.98] vs. 0.16 [0.060-0.21], $p<0.05$, n = 5). The formulation of Sb-L8 in water:PG given orally to mice promoted higher and more sustained levels of Sb in the serum when compared to that in water (median serum Sb concentrations at 0.5 h: 4.8 mg/L [2.7-6.5] vs. 1.5 mg/L [1.3-2.5], $p<0.05$; at 24 h: 4.3 mg/L [1.1-6.2] vs.1.0 mg/L [0.7-1.2] $p<0.05$; n =7/time). Finally, the formulation containing PG was as effective as that prepared in water only, when applied topically, but was more effective when given by oral route (median parasite burden: 4.86 [4.86-4.92] vs. 2.24 [1.96-2.30], $p<0.05$, n = 3-4).

Conclusão:

This study establishes for the first time the efficacy of an amphiphilic Sb complex in a murine model of CL, as an optimized oral formulation containing PG and a topical formulation.

Apoio Financeiro:

This study establishes for the first time the efficacy of an amphiphilic Sb complex in a murine model of CL, as an optimized oral formulation containing PG and a topical formulation.

Comitê de Ética:

CETEA215/2009;318/2013;74/2014

26.003 PROTEIN ALBUMIN NANOPARTICLE: characterization and biotoxicity

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Departamento de Ciencia y Tecnología, - LBM
MPBio (LBM, LaMaBio), - LBM, UNQ, IMBICE ? CONICET,
2Laboratory of Protein Folding (LEPP) - LEPP, UNQ, IMBICE ? CONICET,

Introducao:

Protein nanoparticles are used to carry and deliver drugs to disease tissues. They constitute an alternative to classical protein–ligand complexes and Emodin is a novel drug that displays interesting anticancer activities. A BSA nanoparticle was synthesized by gamma irradiation and characterized biophysical and biochemically. This new nanoparticle is of 50-70 nm size, which is important when studying the affinity for Emodin and the Emodin- nanoparticle–BSA interactions as compared with the protein bioconjugate Emodin–BSA interactions.

Objetivos:

The aim of this work was to characterize structure-function of this new drug carrier system and establish its biotoxicity where the unit (BSA molecule) is known for the specific and non specific binding of hydrophobic drugs, in particular with Emodin but becomes more excruciating when there are many sites available, which is the case of the nanoparticle.

Metodos:

Different assays such as T.E.M, UV-visible, light scattering, FTIR, fluorescence, drug affinity for the carrier were carried out, as well as biotoxicity tests in a Zebrafish model.

Resultados:

Results showed by FTIR that the nanoparticle has a more compact structure if comparing data of main peaks that shifted to the blue region. No loss of functionality of the protein nanoparticle was observed. Parallel studies at different pH´s with Emodin confirmed the structure adopted by Emodin on binding to the nanoparticle in sites I and II. When the interaction Emodin– BSA nanoparticle was studied by UV-Vis, the value of the binding constant by static fluorescence is indicative of a high affinity (70%) and binding constant in the order of c.a. 104. Regarding biotoxicity assays, mobility, malformation, and heartbeat rhythm were studied. Collected data showed that when alone, both carriers (BSA nanoparticles vs molecular BSA protein) do not present significative differences. Whereas, bound to Emodin, the BSAn-Emodin biconjugate did not show serious secondary effects, and the drug toxicity effect is reduced. This was not the same for the BSA-Emodin biconjugate which was lethal in cardiotoxicity for the animal model studied. Biotoxicity is discussed in depth analyzing actual controversies on nanoparticles toxicity under present international regulatory dispositions.

Conclusão:

The results presented here will help to further understand the nanoparticles–protein–drug interactions and the role that Emodin-BSA nanoparticles may play in biomedical and pharmacological applications

Apoio Financeiro:

The results presented here will help to further understand the nanoparticles–protein–drug interactions and the role that Emodin-BSA nanoparticles may play in biomedical and pharmacological applications

Comitê de Ética:

26.004 ANÁLISE DO POTENCIAL ANTITUMORAL DE NOVOS CARDIOTÔNICOS ESTEROIDAIS SINTÉTICOS E SEUS EFEITOS NA NA,K-ATPASE

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Laboratório de Farmacologia Bioquímica e Molecular - UFRJ

Introducao:

O efeito antitumoral de cardiotônicos esteroidais (CTS) tem sido observado em diversas linhagens celulares. CTS, como a digoxina e digitoxina, em doses terapêuticas possuem um efeito inibitório da Na,K-ATPase. A caracterização biológica na modulação da atividade da Na,K-ATPase, e a avaliação do efeito antiproliferativo em células tumorais devem ser avaliados.

Objetivos:

Verificar o efeito citotóxico de novos compostos CTS sintéticos em células de carcinoma de colo uterino (HeLa) e cólon intestinal retal (RKO) e correlacionar o efeito antitumoral com a atividade da Na,K-ATPase.

Metodos:

As células HeLa e RKO foram cultivadas e o MTT foi realizado. As células HeLa foram tratadas com 6 novos CTS sintéticos nas concentrações de 150 nM e 10 μM por 24h. Como controle positivo de inibição da Na,K-ATPase, foi utilizado a digoxina (150 nM). Após o tratamento, foi realizada preparação de membrana plasmática, as células foram maceradas, sonicadas e centrifugadas a 4°C. Os compostos também foram incubados com preparação de membrana de hemisfério cerebral de rato e a atividade da Na,K-ATPase foi analisada a partir da dosagem espectrofotométrica do fosfato inorgânico de acordo com método de Fiske e Subbarow. A análise estatística foi realizada pela análise de variância seguida de teste de Dunnet utilizando o programa GraphPad Prism 5, e os valores foram expressos em média ± desvio padrão e valores significativos com valor de $p<0,05$.

Resultados:

Os novos CTS sintéticos DGB2, DGB3, DGB4, DGB5, DB6 e DGB7 apresentaram efeitos citotóxicos em células HeLa e RKO. Todos os compostos apresentaram IC50 na faixa de 50 µM, sendo o DGB5 o único que apresentou IC50 de 0,26 ± 0,06 μM. O tratamento de 24h com 150 nM digoxina provocou 70% de inibição da Na,K-ATPase nas células HeLa. Nenhum outro composto foi capaz de inibir a Na,K-ATPase após o tratamento por 24h. As preparações de membrana de hemisfério cerebral de rato demonstraram um IC50 da inibição da Na,K-ATPase para a digoxina foi

de 0,22 ± 0,04 µM. DGB2 e DGB4 apresentaram IC50 de 0.46 ± 0.10 e 0.66 ± 0.20 µM enquanto que o DGB3 e DGB5 apresentaram 18 ± 10 e 20 ± 7 µM, sendo que o composto DGB7 não apresentou efeito inibitório na enzima. Análise de correlação de Pearson foi realizada para os valores de IC50 da viabilidade celular e da inibição da atividade da Na,K-ATPase não apresentou significância estatística.

Conclusão:

Os efeitos citotóxicos foram observados para todos os glicosídeos cardíacos testados, sendo que o DGB5 apresentou o melhor IC50 para viabilidade celular. Entretanto, o efeito citotóxico não pode ser relacionado ao efeito de inibição da Na,K-ATPase. Isso abre possibilidades para que o efeito citotóxico encontrado não seja mediado por modulação iônica e sim através de vias de sinalização celular.

Apoio Financeiro:

Os efeitos citotóxicos foram observados para todos os glicosídeos cardíacos testados, sendo que o DGB5 apresentou o melhor IC50 para viabilidade celular. Entretanto, o efeito citotóxico não pode ser relacionado ao efeito de inibição da Na,K-ATPase. Isso abre possibilidades para que o efeito citotóxico encontrado não seja mediado por modulação iônica e sim através de vias de sinalização celular.

Comitê de Ética:

26.005 Toll-like receptor 4 activation promotes cardiac arrhythmias by decreasing the transient outward potassium current (Ito) through an IRF3-dependent and MyD88-independent pathway.

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Introducao:

Cardiac arrhythmias are one of the main causes of death worldwide. Several studies have shown that inflammation plays a key role in different cardiac diseases and Toll like receptors (TLRs) seem to be involved in cardiac complications.

Objetivos:

In the present study, we investigated whether the activation of TLR4 induces cardiac electrical remodeling and arrhythmias, and the signaling pathway involved in these effects.

Metodos:

Left ventricle from male Wistar rats were used. Ca2+ transients, as well as the L-type Ca2+ current (ICaL) and the transient outward K+ current (Ito) were recorded in isolated myocytes after 24h exposure to the TLR4 agonist ultrapure lipopolysaccharide (LPS, 1µg/ml).For action potential records, intracellular microelectrode were used; the ionic currents were studied using Patch-clamp experiments performed in the whole-cell configuration;Cell shortening was measured with a video-edge detector; The levels of mRNA expression of KChIP2, Kv1.4, Kv4.2 and Kv4.3 genes in left ventricular cardiac strips incubated or not during 24 h with LPS were measured by quantitative real-time RT-PCR (qRT-PCR).

Resultados:

TLR4 stimulation in vitro promoted a cardiac electrical remodeling that lead to action potential prolongation (in mean±SEM) (action potential duration at 90% of repolarization: CTRL: 58.5±7.3; LPS: 86.5±8.8; $P<0.05$) associated with arrhythmic events, such as delayed after depolarization and triggered activity. After 24h LPS incubation, Ito amplitude (CTRL: 19.9±1.1; LPS: 9.9±1.9; $P<0.001$), as well as Kv4.3 and KChIP2 mRNA levels were reduced (Kv 4.3 CTRL: 1.3±0.4; LPS: 0.02±0.001; KChIP2 CTRL: 1.1±0.2; LPS: 0.3±0.05; $P<0.001$). The Ito decrease by LPS was prevented by inhibition of interferon regulatory factor 3 (IRF3) (LPS+IRF3inh: 17.2±1.2; $P<0.001$), but not by inhibition of interleukin-1 receptor-associated kinase 4 (IRAK4) (LPS+IRAK4inh: 10.4±1.8) or nuclear factor kappa B (NF-kB) (LPS+NF-kBinh: 11.8±1.7; $P<0.001$). Ca2+ transients and ICaL were not affected by LPS; however, extrasystolic activity was present in 25% of the cells.

Conclusão:

We conclude that TLR4 activation decreased Ito, which increased AP duration and producedarrhythmia. The mechanism involved is MyD88- independent and IRF3-dependent.1- Activation of TLR4 induces cardiac arrhythmias.2- TRL4 activation-induces Ito current, as well as Kv4.3 and KChIP2 mRNA levels reduction. 3- The IRF-3 signaling pathway is involved in the TLR4-induced Ito current decrease. 4- Ca2+ transients and ICaL were not affected by LPS. 5- Extra-systolic activity was present in 25% of the LPS-treated cells.

Apoio Financeiro:

We conclude that TLR4 activation decreased Ito, which increased AP duration and producedarrhythmia. The mechanism involved is MyD88- independent and IRF3-dependent.1- Activation of TLR4 induces cardiac arrhythmias.2- TRL4 activation-induces Ito current, as well as Kv4.3 and KChIP2 mRNA levels reduction. 3- The IRF-3 signaling pathway is involved in the TLR4-induced Ito current decrease. 4- Ca2+ transients and ICaL were not affected by LPS. 5- Extra-systolic activity was present in 25% of the LPS-treated cells.

Comitê de Ética:

26.006 LIPID CHANGES IN THE RAT HIPPOCAMPUS AFTER TREATMENT WITH LIPOPOLYSACCHARIDE (LPS) AND OUABAIN

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Department of Pharmacology - USP-SP

Introducao:

Neuroinflammation is characterized by the response of microglia in the CNS on shares that have similar character of inflammation (J.Neurol. 51:1304,2004). A model of this process is to use induction treatment with LPS, which activates microglia to release neurotoxins directly on inducing the inflammatory process (Trends Pharmacol Sci, 24:395,2003). The use of ouabain causes a down regulation of glutamate receptors, thereby indicating a possible neuroprotection mediated by this cardiotonic steroid (Life Sci.,78:245.2005).

Objetivos:

The aim of this study is to evaluate the changes in lipid content of rat hippocampus membrane after treatment with ouabain and LPS.

Metodos:

Adult male Wistar rats were separated into 4 groups that received the following administration: 1. ouabain (1.8 mg/kg)(n=6), 2. saline (SAL)(n=6), 3. LPS alone (200 mg/kg, ip)(n=6) and 4. ouabain (1.8 mg/kg) and 20 minutes after LPS (200 mg/kg)(n=6). The animals were sacrificed after 2 hours and hippocampus samples were collected for analysis: protein concentration by the Hartree method (Analyt.Biochem. 48:422,1972); extraction and quantification of phospholipids by the Folch (J.Biol.Chem.,226:497,1957) and Chen (Anal. Chem.28:1756,1956) methods; total content of cholesterol was determined by the Higgins method (Oxford.1:104,1987); and total gangliosides was determined by the method of Svennerholm (Biochim.Biophys.Acta.24:604,1957). The statistical analysis was performed using GhaphPad Prism 5 program, expressing the values as mean ± standard deviations and subjected to analysis of variance (ANOVA), followed by post-test Turkey.

Resultados:

In all treatments no significant changes were observed in the content of total cholesterol. There was a reduction in the content of total phospholipids present in the membrane fraction of the hippocampus in the group treated with LPS alone (47.21 ± 2.77 nmol/µL*100) when compared to the control group (58.52 ± 6.74 nmol/µL*100). In addition, a significant increase in the phospholipids content in the group treated with ouabain alone (81.93 ± 7.76 nmol/µL*100) was observed when compared with the control group. Ouabain appear to inhibit the LPS effect, one time that the

ouabain and LPS treatment did not presented significant difference (63.79 ± 3.38 nmol/µL*100) compared to control. Gangliosides levels of the treated group treated with LPS alone (1.018 ± 0.11 nmol/µL*10) showed a significant increase of total gangliosides when compared to the control group (0.755 ± 0.028 mol/µL*10), and a significant increased was also observed in ouabain treated group(1.065 ± 0.008 nmol/ µL*10) by about. In the group treated with ouabain and LPS (0.746 ± 0.006 nmol/µL*10) no significant difference was observed compared to the control group.

Conclusão:

These results demonstrated that ouabain change the lipid constitution of rat hippocampal membrane prevented the effect caused by LPS. This data raise the possibility of ouabain playing a neuroprotective effect on hippocampus.

Apoio Financeiro:

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Comitê de Ética:

No 77 on pages. 72 book 02 app

26.007 DEVELOPMENT OF POLYMERIC NANOPARTICLES FOR THE TREATMENT OF BONE METASTASIS

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Laboratório de Nanorradiofármaco - UEZO

Introducao:

Cancer is a problem of worldwide concern and future perspectives are worrisome. After non-melanoma skin cancer, the two types of highest incidence are breast and prostate cancer. These ones leads with high frequency to bone metastasis. The treatment with radiopharmaceutical stands out as a palliative treatment which improves the quality of life and increases survival time. The 153Sm-EDTMP (ethylenediamine tetra(methylene phosphonic acid) treatment provides clear improvement in 70-80% of patients. The major disadvantage of this radiopharmaceutical is the requirement of multiple doses, that can lead to mielotoxicity. The side effects could be attenuated through drug encapsulation in nanoparticles, promising properties such as controlled, prolonged and sustained release of the active ingredient, reduction of the required dose for therapeutic effect and the toxic effects. Also, it is well known that in affected cancer tissue, the endotelium possess around 250 nm gaps between cells. That said, the size of the nanoparticles is an aspect of paramount importance, during its production.

Objetivos:

The aim of this study is to produce polymeric nanoparticles of EDTMP, evaluating the parameters associated with the size of the nanoparticles.

Metodos:

Nanoparticles: They were produced by the method of double emulsion using poly-lactic acid (PLA) and poly-vinyl alcohol (PVA) as polymers and EDTMP as the drug. During this process, the output power of sonication and time of sonication were analysed. The first ranged between 55W to 150W. The time of sonication to produce the first emulsion ranged between 1min to 4min. Characterization of the nanoparticles: The morphology and dimension of the nanoparticles were obtained by atomic force microscopy (AFM) using PeakForce Tapping Mode (PFQNM)®.

Resultados:

The nanoparticles exhibited spherical shapes, when analysed through AFM topography images. In the adhesion and elasticity images it was possible to determine a heterogeneous surface of the nanoparticles, probably because of the use of two polymers, PLA and PVA. The average size of the nanoparticles did not changed significantly with increase of the output power of sonication. The sizes were around 230±118nm. However, the size dispersion were really different between samples. The best output power was 60W with a mean size of 150±57nm with the lower size of 23nm and the higher size of 470nm. The size of the nanoparticles get smaller with increase of time. However, the topography image in the AFM showed that the higher the period of time of sonication, more deformed stays the nanoestructure. So, the best time to do the first emulsion is 1min.

Conclusão:

The nanoparticles were successfully manufactured by the double emulsion methodology and this new mode of analysis by AFM allows the evaluation of the nanoparticles physical properties. Also, it was possible to determine optimals output power sonication and time of sonication to do nanoparticles of PLA/PVA/EDTMP, which can be used in further studies including the radiopharmaceutical.

Apoio Financeiro:

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nanoparticles of PLA/PVA/EDTMP, which can be used in further studies including the radiopharmaceutical.

Comitê de Ética:

Não foram realizados nenhuma experimentação animal e humanos.

26.008 Effect of 21-benzylidene digoxin in tumor cells: a new synthetic cardiotonic steroid

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Laboratório de Bioquímica Celular - UFSJ
Laboratório de Síntese Orgânica - UFSJ

Introducao:

Recently, cardiotonic steroids (CTS), used in heart failure treatment, have shown interesting antitumor effects acting on different signaling pathways triggered by Na, K -ATPase.

Objetivos:

Assess the cytotoxic and antiproliferative effect of a new synthetic CTS (21-benzylidene digoxin - 21-BD).

Metodos:

HeLa cell (cervical carcinoma) proliferation assay was conducted from exclusion trypan blue and clonogenic assay. The MTT assay was evaluated in MDA-MD-231(breast cancer) and MDCK (dog kidney cell) cells. The apoptotic potential of 21-BD was analyzed by TUNEL assay on HeLa cells. RT-qPCR and western blotting analysis were performed to verify the mRNA and protein levels of α1 and β1 subunits of Na,K-ATPase. Finally, specific inhibitors were used for the EGFR signaling pathway (25nM Thyrphostin AG1478), MAPK (10µM PD98059), PI3K (LY290042 5μM) and Src (5μM PP2) and subsequent antiproliferative potential analysis of 21-BD was performed in order to assess a possible molecular mechanism of action.

Resultados:

From clonogenic assay, can be observed that the 21-BD has an antiproliferative effect, reducing about 20-30% the cell number compared to control at 10 and 20nM concentrations (100% of control). In 24 hours, 21-BD decreased the cell number in 30%, at 150nM and 5 , 25 and 50 μM, but in 48 hours of treatment, the antiproliferative effect was just found at concentrations above 25μM (about 50%). During 24 hours of treatment, the concentrations of 150nM and 5μM of 21- BD was able to induce antiproliferative effect. The cytotoxic effect of 21-BD was observed just in tumor cells (MDA -MD- 231) at a concentration of 50μM and not in normal kidney cells (MDCK), while digoxin (a template cardiotonic steroid) demonstrated to be a cytotoxic agent in both cell lines from 1.36μM. In MDCK cells was found an increase in the MTT conversion at concentrations of 5 to 100μM, which may be associated with increased mitochondrial metabolism. The TUNEL assay showed that as the cells treated with digoxin (0,2 and 2,2μM) as 21-BD (5,6 and 56μM) presented similar etoposide apoptotic effect (0,22μM). RT-qPCR assay indicated an increase in mRNA expression of α1 and β1 subunits of Na, K -ATPase. However,

western blotting assays showed no significant increases in protein expression of the α1 subunit of Na, K - ATPase. Only EGFR pathway showed some involvement in the antiproliferative effect of 21-BD, demonstrated by partial prevention effect due to EGFR inhibitor (Tyrphostin AG 1478).

Conclusão:

These results demonstrate that the new cardiotonic steroid has an antiproliferative effect on tumor cells and also a selective cytotoxic effect on tumor cells. The 21-BD showed an apoptotic potential and a possible molecular mechanism of action through EGFR pathway. The increase in mRNA expression of the subunits of Na,K-ATPase was not followed by an increase in protein expression, demonstrating the existence of a possible post-transcriptional control point to the process. These data demonstrate an interesting effect to this new CTS, enabling its use as a prototype in the rational development of anti-tumor drugs.

Apoio Financeiro:

These results demonstrate that the new cardiotonic steroid has an antiproliferative effect on tumor cells and also a selective cytotoxic effect on tumor cells. The 21-BD showed an apoptotic potential and a possible molecular mechanism of action through EGFR pathway. The increase in mRNA expression of the subunits of Na,K-ATPase was not followed by an increase in protein expression, demonstrating the existence of a possible post-transcriptional control point to the process. These data demonstrate an interesting effect to this new CTS, enabling its use as a prototype in the rational development of anti-tumor drugs.

Comitê de Ética:

Culture HeLa cells.

26.009 ANÁLISE ESTRUTURAL DA PROTEÍNA DO CAPSÍDEO DO VÍRUS DENGUE E SUA INTERAÇÃO COM MEMBRANAS LIPÍDICAS

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Centro de Ciências da Saúde - UFRJ

Introducao:

O vírus dengue (DENV) causa a principal arbovirose humana, afetando inúmeras pessoas anualmente. Até o momento, ainda não estão disponíveis vacina e medicamentos específicos para o tratamento desta infecção. Estudos prévios revelaram que a interação entre a proteína do capsídeo (C) do DENV e corpúsculos lipídicos (LDs), organelas derivadas do retículo endoplasmático, é essencial para a replicação viral. Além disso, experimentos de ressonância magnética nuclear (RMN) demonstraram que, não somente a região hidrofóbica da proteína C, mas também a região N-terminal desordenada apresentam importância fundamental para a interação desta proteína com LDs.

Objetivos:

Os objetivos do trabalho são: avaliar a estabilidade estrutural da proteína C; estimar a provável posição da região N-terminal na estrutura da proteína C; e analisar os parâmetros intrínsecos da ligação da proteína C a vesículas unilamelares grandes (LUVs) e LDs.

Metodos:

Para a avaliação da estabilidade da proteína C, o perfil de desnaturação foi observado na presença de um agente desnaturante (guanidina) através de espectroscopia de fluorescência. Para a determinação do envelope molecular da proteína C foi usado a técnica de espalhamento de raios-X a baixo ângulo (SAXS). Para a análise dos parâmetros da interação entre proteína C e LUVs foi utilizado calorimetria por titulação isotérmica (ITC).

Resultados:

A estabilidade da proteína C foi avaliada pela análise do perfil de desnaturação em diferentes pHs (3,0; 4,5; 6,0; 6,8 e 7,5). Os resultados sugeriram que diferentes pHs não alteram o perfil de desnaturação da proteína C. A partir destes resultados, também foi observado que a variação de energia livre (DG0) do estado enovelado (na ausência do agente desnaturante) é maior em pH 3,0 (-2,0 Kcal/mol) do que em pH 7,5 (-4,4 Kcal/mol), sugerindo que a proteína C apresenta uma conformação menos estável em pH 3,0. Além disso, foi determinado também o valor de m (proporcional à área de superfície de uma proteína exposta ao solvente após desnaturação) em diferentes pHs. O valor de m em pH 7,5 foi 3,1 Kcal/mol.M. Este valor é considerado relativamente menor do que o teoricamente determinado para uma proteína dimérica com 200 aminoácidos (m =

4,1 Kcal/mol.M), sugerindo que parte da proteína, provavelmente a região N-terminal desordenada, pode já se encontrar relativamente exposta em pH fisiológico, antes mesmo da exposição ao agente desnaturante. Experimentos de SAXS mostraram que o raio de giro da proteína C obtido experimentalmente, ou seja, incluindo o N-terminal desordenado, (Rg=22,52 A º) é maior do que o do envelope molecular teórico determinado por RMN, não incluindo o N-terminal desordenado, conforme o PDB 1R6R (Rg = 17,56 Aº). A estrutura da proteína C do DENV, que foi previamente determinada por RMN, e o envelope molecular da proteína C do DENV, obtido por SAXS, foram sobrepostos manualmente para avaliar as prováveis posições da região N-terminal desordenada.
Experimentos prévios de ITC demonstraram uma interação entre a proteína C e LUVs contendo 1-palmitoil-2-oleoil-fosfatidilserina (carga negativa), sugerindo que a interação proteína / vesícula seja mediada por interações eletroestáticas. Estes resultados juntos propõem que a análise por ITC seja uma metodologia adequada para analisar a interação da proteína C com membranas (inclusive LDs).

Conclusão:

Os resultados prévios proporcionaram novos conhecimentos sobre a estrutura tridimensional da proteína C, sugerindo a provável posição da região N-terminal desordenada, e sobre as propriedades biofísicas das interações entre proteína C e membranas lipídicas, o que irá contribuir para o estudo de novas estratégias para romper esta interação essencial para a replicação viral.

Apoio Financeiro:

Os resultados prévios proporcionaram novos conhecimentos sobre a estrutura tridimensional da proteína C, sugerindo a provável posição da região N-terminal desordenada, e sobre as propriedades biofísicas das interações entre proteína C e membranas lipídicas, o que irá contribuir para o estudo de novas estratégias para romper esta interação essencial para a replicação viral.

Comitê de Ética:

Para os experimentos foi utilizada proteína C recombinante.

26.010 Identification and modeling of GH3 and GH9 cellulases from Achatina fulica`s metagenome.

Werneck, K. A. A. , Gomes D. E. B. ,
DIMAV - INMETRO

Introducao:

Sugarcane processing generates large volumes of bagasse, a residue with impacts in profitability and environmental protection. A major obstacle for conversion of lignocellulosic biomass into biofuels is the efficient deconstruction of plant polysaccharides. The metagenome of the gastric juice of the giant African snail (Achatina fulica) has led to the identification of numerous sequences with probable glucosyl hydrolase function.

Objetivos:

The aim of this study is to probe the sequences from Achatina fulica`s gastric juice metagenome for identification of candidate cellulase enzymes and structural modeling using the technique of comparative modeling.

Metodos:

The sequences were identified through a database search with BLAST-NR, and annotated using records from UniProt, KEGG, MetaCyc, pSort, RPS-BLAST, PSIPRED and SignalP servers. The 3D molecular models were build using MODELLER after sequence alignment to similar sequences found in the Protein Databank. 1000 candidate models were build for each, refined and evaluated for validity though PROCHECK, DOPEscore and PyMOL

Resultados:

We identified the sequences as Glycoside Hydrolase from Families 3 (GH3) and 9 (GH9). GH3 was found identical to the beta-n-acetylhexosaminidase from Bacterioides sp. (WP_008641704.1), sharing 35% of its sequence with the crystal structure (PDB:2X42). GH9 was found identical to the endoglucanase from Phyllobacterium sp (WP_008122639.1), sharing 43% residues with the crystal structure (PDB:1UT9). The best 3D structural models (GH3#8, GH9#94) conjugated a high DOPEscore with excellent stereochemistry in Ramachandram plot, therefore allowing confident delimitation of the active site.

Conclusão:

We obtained a three-dimensional model of optimum quality. As a perspective, the research will involve docking of oligosaccharides to this binding site, followed by molecular dynamics simulations and estimation of the binding free energy.

Apoio Financeiro:

We obtained a three-dimensional model of optimum quality. As a perspective, the research will involve docking of oligosaccharides to this binding site, followed by molecular dynamics simulations and estimation of the binding free energy.

Comitê de Ética:

Animal subjects were not required for any of the experiments (Não há experimento com animais)

26.011 Study of interfacial properties of the antimicrobial and antitumor peptide by Langmuir-monolayer

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Department of Physics - IBILCE

Introducao:

The antimicrobial tetradecapeptide Polybia-MP1 (IDWKKLLDAAKQIL-NH2), extracted from a native wasp, exhibits antitumor activity on prostate and bladder cancer cell cultures and high specificity to leukemic T-lymphocyte. Its inhibitory effect on cancer cell proliferation is believed to be due to the presence of both PS and PE in the outer leaflet of these cells. The MP1 interaction with anionic vesicles suggested interfacial action inducing lipid segregation or domain formation.

Objetivos:

Here, this interfacial action was explored by investigating its effect on compression isotherms of PS, PE and mixed PE/PS monolayers monitored by fluorescent microscopy and by measuring the penetration of MP1 into these phospholipid monolayers.

Metodos:

Compression isotherms of lipid, peptide or premixed lipid-peptide at the air-water or air-buffer (HEPES 10mM, 150mM NaCl, pH. 7.4) interfaces were measured on a Langmuir film balance with a Wilhelmy-plate at 20oC. Penetration of the peptide into phospholipid monolayers was assessed monitoring the surface pressure changes when peptide was injected into the subphase of lipid monolayer at constant area.

Resultados:

The compression isotherms of monolayers of pure MP1 on air-water or air-buffer interfaces confirm that the peptide presents interfacial action. Compression isotherms of DMPE, DMPS or mixture DMPE:DMPS in water, showed a liquid-expanded to liquid-crystalline plateau. In saline buffer the plateau of the DMPS and DMPE:DMPS isotherms were shifted to higher surface pressures, suggesting screening of the electrostatic interactions of head groups. Peptide co-spread with lipids displaces the LE-LC coexistence toward LE. Fluorescence microscopy demonstrates that the peptide displaces the LE-LC equilibrium and imposes changes in the solid domains geometry. The higher penetration of MP1 into monolayer of DMPE and DMPS or mixtures DMPE:DMPS in buffer compared to water subphase reinforces the important role played by the electrostatic interactions in the peptide binding to the monolayers and on the structure of domains formation.

Conclusão:

MP1 partitions preferentially onto the fluid lipid phase, increasing the relative amount of this phase,

inducing increase of the line tension in DMPE and dipole repulsion in DMPS.

Apoio Financeiro:

MP1 partitions preferentially onto the fluid lipid phase, increasing the relative amount of this phase, inducing increase of the line tension in DMPE and dipole repulsion in DMPS.

Comitê de Ética:

In these experiments did not involve animals.

26.012 ENCAPSULATION OF MEGLUMINE AND ALOPURINOL IN LIPOSOMES AND ITS EFFECT ON Leishmania amazonensis PROMASTIGOTES.

Gaona, V. V. O. , Giorgio, S. , Silva, C. M. G. , de Paula, E. ,
Bioquimica e Biologia Tecidual - UNICAMP
Parasitologia - UNICAMP

Introducao:

Meglumine antimoniate (AME) is the drug of choice while allopurinol (ALP) is a coadjuvant medicine in the treatment of leishmaniasis.

Objetivos:

In order to improve the bioavilability of meglumine antimoniate and allopurinol, we have prepared liposomal formulations with passive encapsulation of the drugs, characterized them and tested their in vitro effect against Leishmania amazonensis promastigotes.

Metodos:

Large multilamellar liposomes (MLV) composed by egg-phosphatidylcholine,cholesterol and α-tocopherol, (4:3:0.07 mole%) were prepared in Hepes buffer 20mM, pH 7.4.

Resultados:

The optical properties of the drugs in the UV/VIS were determined and used to measure their partition coefficient (P) between liposomes/water at pH 7.4: 37 and 29 for AME and ALP, respectively. Such values guarantee an encapsulation efficiency of ca. 30%, for both drugs, in the assay condition. The morphology of the liposomes was checked through Transmission electron microscopy images. In vitro assays, with Leishmania amazonensis promastigotes in culture, revealed that encapsulation into the liposomes was more effective for AME than ALP, to reduce the parasite growth. Nevertheless, both liposomal formulations were successful: the effective drug concentration for 50% decrease in parasites number was three times lower than with each drug in solution.

Conclusão:

In conclusion, liposomes provided sustained release of meglumine antimoniate and allopurinol with potential application for the treatment of leishmaniosis.

Apoio Financeiro:

In conclusion, liposomes provided sustained release of meglumine antimoniate and allopurinol with potential application for the treatment of leishmaniosis.

Comitê de Ética:

26.013 Self-Assembling of Septin 2 Amyloid Fibrils

Sales, E. M. , Damalio, J. C. P. , Barbosa, L. R. S. , Spinozzi, F. , Mariani, P. , Araujo, A. P. U. , Itri, R. ,

Instituto de Física de São Carlos - IFSC

Instituto de Física da USP - IFUSP

Universita Politecnica delle Marche - UNIVPM

Introducao:

Septins are proteins from the GTP-binding family and participate in cell division cycle performing functions such as secretion and cytoskeletal division. They can also be found in neurodegenerative conditions as Alzheimer`s and Parkinson`s diseases and some kinds of cancer as leukemia, lymphoma and solid tumors.

Objetivos:

In this work, we investigated the influence of temperature and concentration on the septin2 GTPase domain(SEPT2G) aggregation using small angle x-ray scattering(SAXS).

Metodos:

SAXS data of the protein at 0.5 mg/mL and 1 mg/mL and temperatures between 4 and 45°C were analyzed

Resultados:

SAXS data of the protein at 0.5mg/mL showed that SEPT2G is a dimer in aqueous solution at 4°C and this condition is kept stable for approximately one hour of experimental observation. At 15°C,SAXS results revealed the coexistence of three populations in solution composed by 88% of dimers, 10% of cylinder-like smaller aggregates and 2% of aggregates bigger than the technique detection.At 25°C, the dimers percentage decreases to 70% with a contribution of circa 30% of bigger aggregates, even at the beginning of data acquisition. At temperatures of 37°C and 45°C, dimers and very large aggregates coexist in solution since the beginning of data acquisition, which equilibrium quickly shifts in such a way that after 20 minutes of observation the solution is mostly composed by very large aggregates, identified as amyloid structures by the thioflavine fluorescence technique, which intercalates in the cross-beta structures. At 1mg/mL and 4°C, the protein was stable over 1 hour of observation where an equilibrium of dimers (93%) and elongated structures in solution takes place. Increasing the temperature to 15°C, most of the protein remains dimeric. On the other hand, at 25°C the very large aggregates contribution is around 30% coexisting with dimers and oligomers. At 37°C and 45°C there is the formation of large aggregates, similar to what was observed at 0.5mg/mL.

Conclusão:

In conclusion, our SAXS results indicated that the aggregation process of SEPT2G in solution may follow different pathways depending on concentration and temperature.

Apoio Financeiro:

In conclusion, our SAXS results indicated that the aggregation process of SEPT2G in solution may follow different pathways depending on concentration and temperature.

Comitê de Ética:

No animal experimentation were performed.

26.014 DEVELOPING CRITERIA FOR DECONTAMINATION OF URBAN AREAS TO SUPPORT DECISION-MAKING PROCESSES AFTER NUCLEAR ACCIDENTS IN BRAZIL

Silva, D. N. G. , Rochedo, E. R. R. , Guimaraes, J. R. D. ,
Centro de Ciências da Saúde - UFRJ
Programa de Engenharia Nuclear - IME

Introducao:

After an accident involving releases of radioactive material, it is important that the decontamination procedures implemented are justified under the radiological protection point of view. During the Chernobyl accident, for example, it was verified that the procedures applied in the most contaminated areas were often inefficient and that decontamination in order to bring the population back to these areas would be extremely expensive and would lead to high exposure of large number of people.

Objetivos:

This work describes the main efforts to derive criteria for classifying technical aspects related to decontamination procedures to feed a multi-criteria tool to support decisions on remediation of urban areas after nuclear accidents.

Metodos:

A database on decontamination procedures was created, including the main characteristics to be used as part of the criteria for a decision. Then, the technical criteria were developed through simulations of accidents and the consequences of applying the procedures in the database. The scenarios for typical tropical residential urban environments were selected and main characteristics were estimated. Finally, the procedures were classified according to the different criteria to be included in the multi-criteria software.

Resultados:

The database includes 25 decontamination procedures that may be applied to urban environments, classified according to their main characteristics (cleaning, covering, removing procedures and uses restrictions). The description includes: general aspects; radiological protection aspects; infrastructure needed; wastes generated; and, the worldwide actual experience of the procedure. Five main scenarios were developed for different types of urban areas to raise data for public and occupational exposures.

Conclusão:

The development of numerical values to perform optimization shall be extended to rural areas and

surface waters, currently under development. It must be emphasized, however, that there are criteria that cannot be established in advance as they depend on the actual situation.

Apoio Financeiro:

The development of numerical values to perform optimization shall be extended to rural areas and surface waters, currently under development. It must be emphasized, however, that there are criteria that cannot be established in advance as they depend on the actual situation.

Comitê de Ética:

Trabalho não realizado com seres vivos.

26.015 BIOLOGICAL AND STRUCTURAL CHARACTERIZATION OF C-TERMINALS DOMAIN IN HUMAN SEPTINS: IMPLICATION IN THE FORMATION AND STABILITY OF THE FILAMENT.

Sala, F. A. , Garratt, R. C. ,
Química Orgânica e Biológica - IQSC
Cristalografia - IFSC

Introducao:

Septins form a conserved family of guanine-nucleotide binding proteins. Structurally they share common characteristics - a central GTP binding domain flanked by N- and C-terminal regions of variable length. Crystal structure of septin filament revealed the importance of GTP binding domains in filament formation, but no electron density was visible for the C-terminal domain and your structure and function are still poorly understood. Relevant function of this domain for C. elegans and S. Cerevisae motivated this study to comprehend C-terminal contribution to filament formation in human septins.

Objetivos:

Analysis of homo and heterointeraction in septins of groups II (SEPT6C and SEPT8C) and IV (SEPT7C) will be investigated.

Metodos:

In human septins the C-terminal domains from group II and IV were expressed in E. coli strain Rosetta and purified using affinity chromatography and SEC. The secondary structure was studied by circular dichroism spectroscopy (CD). Interactions were investigated by analytical ultracentrifugation (AUC) and thermal stability.

Resultados:

The CD spectroscopy at 4°C for all proteins were characterized by two minima at 208 and 222 nm what indicates the presence of an alfa-helical structure. Thermal unfolding/refolding transition was also studied by CD. The temperature was increased from 4°C to 80°C, the protein concentration used was 13µM. The melting temperature for homodimers were about 10°C fewer than that obtained for heterodimers indicating a considerable thermal stability in vitro. Sedimentation velocity experiments in AUC was performed for homo/heterotypical interactions for SEPT6C, SEPT7C and SEPT8C in the range of concentrations from 1,6µM-16,6µM, showed that the main component in solution were dimers with a standart sedimentation coefficient of 1,77(+/-0,1). Equilibrium sedimentation was carried out for homodimers in concentration of 15uM for SEPT6C and SEPT7C and provided a Kd of 9.05µM and 5,57µM, respectively.

Conclusão:

It has been suggested that specific coiled-coil interaction may be pertinent to guaranteeing correct filament assembly.

Apoio Financeiro:

It has been suggested that specific coiled-coil interaction may be pertinent to guaranteeing correct filament assembly.

Comitê de Ética:

Não são realizados experimentos em animais ou humanos.

26.016 A correlação estrutura/função – associando múltiplas faces à promiscuidade estrutural

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Departamento de Física - USP
Centre for Technological Development in Health (CDTS) - Fiocruz

Introducao:

A forma e a função do aparato de Golgi estão intimamente correlacionadas. As proteínas de organização e compactação do Golgi (do inglês, Golgi reassembly and stacking proteins - GRASP) foram identificadas através de ensaios in vitro como fatores necessários para o empilhamento das cisternas do aparato de Golgi e facilitador de loading das vesículas destinadas a se fundir com ele. Além disso, até o presente momento são associadas à família GRASP as funções: remodelamento do Golgi em células migratórias, progressão do ciclo celular (um checkpoint da fase G2/M?), apoptose e secreção não convencional de biomoléculas. Associada às múltiplas faces das GRASPs está contida a sua promíscua capacidade de interagir com uma gama de diferentes proteínas, interação que se torna essencial para a função a ser exercida. Embora muito se conheça sobre a importância das proteínas GRASPs em nível celular, pouco se conhece sobre sua estrutura e dinâmica em solução. Como uma proteína pode exercer múltiplas funções e interagir com múltiplos parceiros?

Objetivos:

O trabalho teve como objetivo geral produzir dados experimentais que possibilitem a correlação estrutura-função da proteína GRASP de C. neoformans (CnGRASP), dando um passo fundamental para que se possa compreender o papel de GRASP em nível celular.

Metodos:

Para a execução dos objetivos nos valemos de uma abordagem multidisciplinar e multitécnicas que envolveu desde métodos em biologia molecular e bioquímica, como a clonagem e expressão heteróloga da proteína, até técnicas em biofísica molecular, como fluorescência, dicroísmo circular, calorimetria diferencial de varredura e difração de raios-X.

Resultados:

O conjunto de resultados permitiram assinalar à proteína CnGRASP um conteúdo mensurável de estruturação secundária e um interior hidrofóbico bem definido, porém com alta acessibilidade ao solvente. Entretanto, CnGRASP possui múltiplos sítios de desordem estrutural e não possui compactação típica de proteínas globulares estruturadas. Além disso possui alta flexibilidade

estrutural e baixa cooperatividade na transição para o estado desenovelado.

Conclusão:

Baseado nos resultados obtidos, se observou que a proteína CnGRASP se comporta como uma proteína intrinsicamente desordenada (PID) do tipo molten globule na forma nativa em solução. A alta flexibilidade estrutural permite que PIDs possam atuar como hubs, bem como exercer múltiplas funções associadas a sua promiscuidade estrutural. As conclusões do trabalho podem ajudar a entender o porquê de a família GRASP possuir tantas funções distintas, em um momento de relevância por se descobrir estar associada a possíveis tratamentos de doenças, tais como a fibrose cística e a doença de Alzheimer.

Apoio Financeiro:

Baseado nos resultados obtidos, se observou que a proteína CnGRASP se comporta como uma proteína intrinsicamente desordenada (PID) do tipo molten globule na forma nativa em solução. A alta flexibilidade estrutural permite que PIDs possam atuar como hubs, bem como exercer múltiplas funções associadas a sua promiscuidade estrutural. As conclusões do trabalho podem ajudar a entender o porquê de a família GRASP possuir tantas funções distintas, em um momento de relevância por se descobrir estar associada a possíveis tratamentos de doenças, tais como a fibrose cística e a doença de Alzheimer.

Comitê de Ética:

Não foram utilizados modelos animais ou experimentação humana no presente trabalho

26.017 Extraction and characterization of sulfated polysaccharide derived from Ulva lactuca ulvan (Linnaeus).

Maceio, T. S. , Oliveira, V. P. , Silva, T. R. , Prast, A. E. ,
Departamento de Ecologia - UFRJ

Introducao:

Sulfated polysaccharides comprise a complex group of macromolecules with a wide range of biological properties. In this context, the polysaccharides of seaweeds stemmed stand out due to their structural diversity and is present in high concentrations in cell walls. Among the green seaweeds (Chlorophyta), is extracted ulvan sulfated polysaccharide, derived mainly from algae species Ulva lactuca (Linnaeus), in which huge range of biotechnological properties has been demonstrated. However, information about its extraction and characterization are scarce in the scientific literature.

Objetivos:

The present study aimed, perform the extraction of sulfated polysaccharide derived from Ulva Lactuca ulvan (Linnaeus), performing the calculation of extraction yield, and subsequently performing the characterization and the establishment of the organic chains of the same.

Metodos:

Collecting seaweed Ulva lactuca (Linnaeus) was held at Prainha in Arraial do Cabo / RJ; packed in a vacuum flask containing seawater for transport to the laboratory. Subsequently, a screening material cleaning was performed. Following the protocol PENGZHAN et al. (2003) - modified, held the extraction of sulfated polysaccharide ulvan. We also analyzed by the DNS method for the characterization of carbohydrates, and the Biuret and Lowry method, in order to characterize amino acids or conjugated proteins ulvan. To quantify the lipid ulvan was performed the method of Schmid-Bondzynski-Ratzlaff (1959).

Resultados:

The results indicate an extraction yield of 40.1% (± 0.3) of the dry weight. The process of characterization of the extract showed the absence of monosaccharides with carboxyl groups and / or free ketone, and proteoglycans, highlighting the full composition of the samples in hydrophilic polysaccharide ($r^2 = 0.99$). Thus, also found that ulvan extracted is presented as a free polysaccharide interactions with amino acids and proteins. And the process for quantifying the lipid was not detectable by the method used.

Conclusão:

With this study it can be concluded that marine macroalgae U. lactuca has a high potential to

produce ulvan. In addition, not associated with proteins and lipids possessing a high degree of purity, thus demonstrating the potential for producing products for biotechnological purposes.

Apoio Financeiro:

With this study it can be concluded that marine macroalgae U. lactuca has a high potential to produce ulvan. In addition, not associated with proteins and lipids possessing a high degree of purity, thus demonstrating the potential for producing products for biotechnological purposes.

Comitê de Ética:

Para o presente trabalho não foram utilizados animais.

26.018 Analogue to 3,4,5-trimethoxy-dihidrocinamic vehicled in different liposomes system: production, biophysical characterization and its application like a leishmanicide.

Cury, T. A. C. , Yoneda, J. S. , Calderon, L. A. , Stabeli, R. G. , Ciancaglini, P. ,
Chemistry Department - USP - RP
Fundação Oswaldo Cruz - Rondônia - Fiocruz Rondônia

Introducao:

Leishmaniasis is caused by the protozoan Leishmania parasites by the bite of infected sandflies and affects the poorest region on the planet. 1.3 million of new cases are estimated per year (WHO, 2014). The compound B2, a synthetic analogue of 3,4,5-trimethoxy-dihidrocinamic (TMPP) was isolated from Piper tuberculatum fruits and it has been tested as a potential leishmanicide. It is known that TMPP has this activity (Rev. Bras. Farmacogn. 20(6):1003-1006; 2010).

Objetivos:

Our objective was the preparation of liposomes systems containing B2 and analysis of the liposome-B2 interaction by Differential Scanning Calorimetry (DSC). To measure the stability and diameter of vesicles by Dynamic Light Scattering (DLS) and the citotoxicity in Leishmania amazonensis cultures (promastigote form).

Metodos:

The compound B2 was inserted into liposomes of Dipalmitoylphosphatidylcholine (DPPC), Dipalmitoylphosphatidylserine (DPPS) and Dioctadecyldimethylammonium bromide (DODAB), obtaining systems with neutral, negative and positive charges, respectively. Experiments of control were performed with liposome without B2. The DSC calorimeter used is from CSC. The DLS equipment is from Beckman Coulter. Cytotoxicity tests in promastigotes forms of L. amazonensis cultures were realized with 1mg of B2 in liposomes. The concentration of B2 utilized in culture was 0.2mg/mL. The tests and controls were made in triplicate with 5x105 parasites/mL during four days.

Resultados:

The diameters (around of 150nm) of liposomes were measured by DLS during four days and all samples were stable. The thermodynamic parameters of the phase transition: enthalpy variation (?H) and transition temperature (Tt) were not significantly altered, but the cooperativity (?T1/2) was changed in the presence of B2 (DPPC:DODAB-decrease of 2.2°C, DPPC:DPPS-increase of 2.0°C). This change indicates that there is an interaction between the liposomes and B2. In the citotoxicity tests, the best result (about 50% of growth reduction) was to DPPC:DPPS liposome. The best result to DPPC:DPPS can be explained by the presence of positive charge on promastigote forms in L. amazonensis, information obtained in the literature.

Conclusão:

Our first results showed that a liposome system has a leishmanicide response with a concentration 10 times less of B2 than when the pure compound is used and this can be a promising result.

Apoio Financeiro:

Our first results showed that a liposome system has a leishmanicide response with a concentration 10 times less of B2 than when the pure compound is used and this can be a promising result.

Comitê de Ética:

26.019 Biostimulation of the membrane protein Na, K-ATPase by low intensity laser: activity, structural and conformational properties.

Campos, G. S. M. , Ciancaglini, P. , Itri, R. ,
Physics - IF - USP
Chemistry - FFCLRP - USP

Introducao:

This project is focused on the structure determination by SAXS of the complex formed between the membrane protein Na,K-ATPase and the detergent C12E8 used to extract the protein from the natural membrane.

Objetivos:

The protein activity will be evaluated in the absence of irradiation as well as under low laser power irradiation (less than 50 mW) at different wavelenghts (green, red and infra-red lasers).

Metodos:

Several doses of irradiation will be investigated when applied to the enzyme reconstituted in model membranes in comparison to the enzyme imersed in natural membranes. SAXS data will be evaluated at same Na,K-ATPase concentration used for activity measurements to allow us correlating enzyme activity with structure of the formed complex.This project is focused on the structure determination by SAXS of the complex formed between the membrane protein Na,K-ATPase and the detergent C12E8 used to extract the protein from the natural membrane. The protein activity will be evaluated in the absence of irradiation as well as under low laser power irradiation (less than 50 mW) at different wavelenghts (green, red and infra-red lasers). Several doses of irradiation will be investigated when applied to the enzyme reconstituted in model membranes in comparison to the enzyme imersed in natural membranes. SAXS data will be evaluated at same Na,K-ATPase concentration used for activity measurements to allow us correlating enzyme activity with structure of the formed complex

Resultados:

Our data showed three differents regions of biomodulation of Na, K-ATPase in membrane fraction, from 0 to 7 J/cm2 is a region of inhibition of activity (up to -7%), using 12-32 J/cm2 we measured a biostimulation of the protein with maximal effect of 12% of increase, for doses higher then 35 J/cm2 it is another region of inhibition of the enzimatic activity. This pattern of doses and percentage of inhibition and stimulation occurs for the three lasers (green, red and infra-red lasers).

Conclusão:

Electromagnetic radiation from three differents lasers (green, red and infra-red lasers) interacts with Na, K-ATPase in membrane fraction, shows the same pattern of biomodulation, independently of

the wavelength, and the biostimulation or bioinhibition of the enzymatic activity depends of the dose used.

Apoio Financeiro:

Electromagnetic radiation from three differents lasers (green, red and infra-red lasers) interacts with Na, K-ATPase in membrane fraction, shows the same pattern of biomodulation, independently of the wavelength, and the biostimulation or bioinhibition of the enzymatic activity depends of the dose used.

Comitê de Ética:

26.020 Characterization of the store-operated calcium entry in Schistosoma mansoni

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Departamento de Fisica e Ciencia Interdisciplinar - IFSC

Introducao:

Schistosoma mansoni is one of the main causative agents of schistosomiasis, a neglected tropical disease affecting over 240 million people in developing and underdeveloped countries. Store-operated calcium entry (SOCE) is a conserved calcium pathway in eukaryotes although not yet studied in S. mansoni. The proteins STIM1 (calcium sensor) and Orai1 (calcium channel) are the central components of SOCE and the recently published genome of this parasite encodes orthologs of these two proteins, indicating that this Ca2+ pathway is present in S. mansoni.

Objetivos:

Study the SOCE pathway in S. mansoni through the expression of the recombinant proteins from this pathway.

Metodos:

Total mRNA was isolated from adult worms and used for reverse transcription. The resulting cDNA was used as template for PCRs reactions, amplicons of expected sizes were ligated into pGEM-T (Promega) and subcloned into pET SUMO expression vector. Integrity of the inserts was confirmed by nucleotide sequencing (3130 Genetic Analyzer, Applied Biosystems). Recombinant proteins were expressed in E. coli Rosetta (DE3) strain with expression induced by IPTG at 0.4 mM in LB medium for 16 h at 18°C with shaking.

Resultados:

Several constructs were designed comprising different domains of STIM and Orai. Due to the intrinsic difficulty of working with membrane proteins, some of these construct were designed to exclude the transmembrane domain. We were able to obtain three STIM constructs in the soluble form, comprising the domain predict to interact with Orai (named STIM_CAD) and two domains comprising the first coiled-coiled region that is not predicted to interact with Orai (STIM_1CC). The C-terminal domain of Orai was also obtained in the soluble form and purified successfully.

Conclusão:

The recombinant construct of the C-terminal domain of Orai was purified successfully, as well as three constructs of STIM; our current aim is to optimize the purification conditions form each construct and characterize the interaction of STIM and Orai in vitro.

Apoio Financeiro:

The recombinant construct of the C-terminal domain of Orai was purified successfully, as well as three constructs of STIM; our current aim is to optimize the purification conditions form each construct and characterize the interaction of STIM and Orai in vitro.

Comitê de Ética:

Nao foram utilizados animais neste trabalho.

26.021 IN VIVO ADMINISTRATION OF ANGIOTENSIN-(1-7) REDUCES CARDIAC ARRHYTHMIAS IN RATS

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Department of Morphology - UFMG

Introducao:

Angiotensin(Ang)-(1-7), a peptide of the renin-angiotensin system, has cardioprotective and antiarrhythmic effects in isolated rat hearts. However, the effects of this peptide on cardiac arrhythmias in vivo and details regarding its mechanism of actions are unknown.

Objetivos:

The aim of this study was to investigate the effects of Ang-(1-7) on cardiac arrhythmias in vivo and its mechanism of actions

Metodos:

Cardiac arrhythmias were induced in Wistar rats using a combination of halothane (1.5%) and epinephrine (5 to 20μg/kg, iv).The arrhythmogenic dose of epinephrine was defined as the smallest dose that produced 3 or more premature ventricular contractions within 30s. Electrocardiographic records were made by placing an electrode in each rat limb. A 10-minutes period of arrhythmias was followed by an additional 10-minutes period of arrhythmias in presence Ang-(1-7) (4nM). To analyze the mechanism of actions by which Ang-(1-7) plays its antiarrhythmogenic effects, we used A-779 (antagonist of Mas receptor), L-NAME (inhibitor of nitric oxide synthase) and DIZE (activator of angiotensin-converting enzyme 2). Blood pressure and in vivo heart rate were evaluated by a cannula inserted into the carotid artery. Statistical analysis was performed using the Wilcoxon test. All procedures received approval from the Ethics Committee on Animal Experimentation (CEUA/UFMG nº.3/2014).

Resultados:

Our data showed that Ang-(1-7) significantly reduced the arrhythmias when compared with control (epinephrine plus saline injection) [Control: 43±13 arrhythmic events and Ang-(1-7): 15±4 arrhythmic events]. This effect was blocked by A-779 [38±11 arrhythmic events] and by L-NAME [60±21 arrhythmic events]. DIZE injection also promoted a significant reduction in the arrhythmic events [12±8 arrhythmic events]. Saline, A-779 and L-NAME when injected individually did not affect the cardiac rhythm. Hemodynamic parameters did not change after Ang-(1-7) injection.

Conclusão:

We concluded that Ang-(1-7) reduces cardiac arrhythmias in vivo through activation of Mas and nitric oxide release. This effect was confirmed by DIZE. These data suggest that Ang-(1-7) might be a feasible therapeutic agent to prevent arrhythmogenic syndromes.

Apoio Financeiro:

We concluded that Ang-(1-7) reduces cardiac arrhythmias in vivo through activation of Mas and nitric oxide release. This effect was confirmed by DIZE. These data suggest that Ang-(1-7) might be a feasible therapeutic agent to prevent arrhythmogenic syndromes.

Comitê de Ética:

26.022 ELECTRICAL REMODELING OF THE HEART DURING ACUTE CHAGASIC CARDIOMYOPATHY: ROLE OF REACTIVE OXIGEN SPECIES

Campos, D. R. , Ribeiro, G. A. , Vieira, L. Q. , Cruz, J. S. , Cruz, J. S. ,
Departament of Biochemistry and Imunology - UFMG

Introducao:

The infection by Trypanosoma cruzi leads to the development of an intense inflammatory response. Among the consequences of this inflammation, high levels of reactive oxygen species (ROS), including superoxide anion, are produced and the overproduction of ROS may cause nonspecific tissue damage. In the heart, ROS has been found to regulate ion channels and many other proteins involved in the management of heart mechanical and electrical functions, and then, it seems reasonable to hypothesize that the ROS production has an important role in generating the cardiomyopathy related to Chagas’ disease.

Objetivos:

This study aimed to investigate the role of ROS over-production in the heart electrical remodeling during the acute phase of Chagas´ disease.

Metodos:

Wild-Type (WT) mice and NADPH phagocyte oxidase (phoxKO) deficient mice were infected or not with Y strain of T. cruzi. After 15±1 days of infection, the mice were sacrificed, and the hearts were taken off and mounted into a home-made Langendorff system. Cardiomyocytes were then isolated by perfusing a nutritive solution containing collagenase, trypsin and protease. The electrophysiological recordings were then obtained by using a Heka EPC9 patch-clamp amplifier, in whole-cell current clamp configuration for action potential (AP) measurements and whole-cell voltage clamp configuration for L-Type calcium current (ICa-L) and outward potassium current (IKo) measurements,

Resultados:

We found that when infected, phoxKO mice had no changes in AP duration at 90% of repolarization (96.87±11.12ms, n=10) compared with phoxKO non-infected (84.58±18.94ms, n=11) or WT (97.38±11.96ms, n=10) However, WT infected mice showed a significant AP prolongation (274.5±36.89ms, n=10) when compared to the other groups. Also, infected phoxKO mice did not show any alterations in ICa-L (-7.45±0.45pA/pF;n=19 at 0mV) and IKo (34.58±2.87pA/pF;n=16 at +70mV) peak current-density when compared to non-infected phoxKO (7.60±0.47pA/pF;n=26 for ICa and 37.70±3.84pA/pF;n=14 for IKo) or WT (8.48±0.28pA/pF;n=25 for ICa and 33.35±3.62pA/pF;n=22 for IKo), despite infected WT mice had a reduction in both ICa-L

(-4.92±0.25pA/pF;n=25) and IKo (23.70±1.48pA/pF;n=23).

Conclusão:

We conclude that ROS production directly modulates heart electrical remodeling during acute phase of chagasic cardiomyopathy.

Apoio Financeiro:

We conclude that ROS production directly modulates heart electrical remodeling during acute phase of chagasic cardiomyopathy.

Comitê de Ética:

26.023 The role of GPI anchored Alkaline Phosphatase on Membrane Dynamics, an Electron Spin Resonance Study.

Garcia, A. F. , Simão, A. M. S. , Bolean, M. , Ciancaglini, P. , Costa-filho, A. J. ,
Física - FFCLRP/USP
Química - FFCLRP/USP

Introducao:

Tissue-nonspecific alkaline phosphatase (TNAP) catalyzes the hydrolysis of phosphomonoesters. This enzyme is associated with a genetic disorder that can lead to failure of bone mineralization. The mechanisms used by TNAP to perform its function are still not completely understood. TNAP is localized at the external part of the plasma membrane by means of a GPI (glycosylphosphatidylinositol) anchor.

Objetivos:

Here, we propose to use our expertise in molecular biophysics to address the effects of the anchoring mechanism of this enzyme on the membrane surface.

Metodos:

TNAP was produced in a recombinant way in mammalian cells CHO-K1. Electronic spin resonance (ESR) experiments were done using lipids spin labeled in the acyl chain at different position (head group-DOPTC, 5-PCSL and 16-PCSL) in unilamellar vesicle of 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC).

Resultados:

5-PCSL ESR spectra showed drastic change with the shape of all lines affected by TNAP. The hyperfine splitting decreases due to reduction of system anisotropy. The acyl chain region is highly packed and the presence of TNAP changed the polarity of this environment, leading to an increase in the oscillation of nitroxide radical, which induced a narrowing in resonance lines making the system more isotropic. The same behavior was noted for probes. For 16-PCSL, the correlation time (tc) changed from 0.8 x 10-10 sec to 0.2 x 10-10 sec when TNAP was incorporated. Also the h-1/h0 parameter varied from 0.33 for control to 0.72 for TNAP confirming that the system is more dynamic. The change in the polarity along of the acyl chain was noticed and confirmed by ESR spectrum of 16-PCSL.

Conclusão:

All results together led us to conclude that TNAP probably layed down on the membrane surface, a result that can be interpreted as a way of modulating the enzymatic activity of TNAP.

Apoio Financeiro:

All results together led us to conclude that TNAP probably layed down on the membrane surface, a result that can be interpreted as a way of modulating the enzymatic activity of TNAP.

Comitê de Ética:

Não se aplica, no desenvolvimento do trabalho não foi utilizado nenhum tipo de cobaia.

26.024 FUSION PEPTIDE INFLUENCE IN ANIONIC LIPID BILAYERS

Schmidt, T. F. , Riske, K. A. ,
Biofísica - UNIFESP

Introducao:

Membrane fusion peptides are part of virus system serving as a gateway to the virus access into the cell. Particularly, the study of the process of peptide-induced membrane fusion is extremely important to better understand the mechanisms underlying virus entry in cells.

Objetivos:

Here we study the interaction of the dengue Flavivirus fusion peptide (FLAg) with model membranes composed of mixtures of anionic, POPG (palmitoyl-oleoyl-phosphatydil-glycerol) and zwitterionic, POPC (palmitoyl-oleoyl-phosphatydil-coline) phospholipids.

Metodos:

Both large (LUVs) and giant (GUVs) unilamellar vesicles were used here. Optical microscopy was used to assess peptide-induced vesicle aggregation and/or fusion between GUVs and GUVs-LUVs varying the POPC:POPG molar ratio and the FLAg concentration. Peptide-induced effects were monitored by two different methods: adding FLAg peptide to the external environment of the GUVs or by injecting a FLAg solution by micropipette technique. Isothermal Titration Calorimetry (ITC) was used to evaluate the interaction between FLAg and LUVs composed of different POPC:POPG molar ratios. Fluorescence resonance energy transfer (FRET) was employed to measure the degree of lipid mixing induced by the fusion peptide.

Resultados:

The results suggest that FLAg-induced aggregation/fusion depends on the model system used.

Conclusão:

The peptide interacts preferentially with POPG, evidencing the role of membrane charge in the fusion process.

Apoio Financeiro:

The peptide interacts preferentially with POPG, evidencing the role of membrane charge in the fusion process.

Comitê de Ética:

In this work we did not use any animal or human experimental methods.

26.025 Detergent-induced solubilization of liposomes of different membrane composition and phase

Mattei, B. , França, A. D. C. , Riske, K. A. ,
Biofísica - Unifesp

Introducao:

Detergents are amphiphilic molecules that exhibit the ability to interact and solubilize membranes due to their affinity with lipids. However, membranes with different compositions may not exhibit the same propensity to solubilization. Some compositions have been described as partially or completely insoluble. The resistance to detergent-induced solubilization has been associated with the liquid-ordered (Lo) phase, which in biological membranes is observed in regions rich in cholesterol and sphingomyelin.

Objetivos:

. In the present work we study the solubilization induced by Triton X-100 of membrane models (giant unilamellar vesicles, GUVs, and large unilamellar vesicles, LUVs) composed of different mixtures of palmitoyl oleoyl phophatidylcholine (POPC), egg sphingomyelin (SM) and cholesterol (cho). The compositions investigated were: POPC (liquid-disordered phase, Ld), POPC:cho (7:3) (Ld), SM (gel phase) and SM:cho (7:3) (Lo).

Metodos:

The solubilization process was assessed by optical microscopy of GUVs and measurements of LUVs turbidity during titration with Triton X-100.

Resultados:

Optical microscopy of GUVs shows that pure POPC and SM vesicles are completely solubilized by Triton X-100, whereas SM:cho is completely insoluble. GUVs composed of POPC:cho exhibit insoluble membrane fragments after partial solubilization. Incorporation of Triton X-100 in POPC and POPC:chol before the onset of solubilization causes increase in GUV surface area, which is higher for pure POPC membranes. Turbidity measurements show complete solubilization of POPC and SM LUVs, the latter occurring in a narrower detergent-to-lipid molar ratio range. LUVs composed of POPC:chol were only partially soluble, as judged by the residual turbidity at high detergent-to-lipid molar ratios.

Conclusão:

Our results show that cholesterol increases the solubilization resistance of membranes, which varies with the membrane phase, and confirms that Lo phase is virtually insoluble.

Apoio Financeiro:

Our results show that cholesterol increases the solubilization resistance of membranes, which varies with the membrane phase, and confirms that Lo phase is virtually insoluble.

Comitê de Ética:

Trabalho com modelos de membrana biológica, não com animais ou humanos

26.026 INSIGHTS ON INTERACTIONS BETWEEN LIPID MEMBRANES AND COPPER COMPLEXES

Freddi, P. , Facchin, G. , Torre, M. H. , Costa-Filho, A. J. ,
Departamento de Física - USP
Cátedra de Química Inorgânica - UdelaR

Introducao:

Sulfonamides and their different derivatives are extensively used in Medicine due to their pharmacological properties, such as antibacterial activity. Modified toxicological properties have been observed when those sulfonamides are administered in the form of metal complexes.

Objetivos:

In this work, we used the molecules sulfadimethoxine (4-p-aminobenzenesulfonamido-2,6-dimethoxypyrimidine) and sulfisoxazole (N (1) - (3,4-dimethyl-5-isoxazolyl) sulfanilamide) complexes with Cu(II) to assess their mechanism of interaction with lipid model membranes.

Metodos:

Differential Scanning Calorimetry (DSC) and Electron Paramagnetic Resonance (EPR) experiments were carried out to monitor the influence of the complexes on the lipid thermotropic behavior. Samples consisting of the multilamellar vesicles of dipalmitoylphosphatidylcholine (DDPC) and, in the case of EPR, spin labeled phospholipids were used as models for the membrane.

Resultados:

DSC results showed that the presence of the complexes affects both the pre-transition and the main phase transition of the lipids in the membrane. A disordering effect is evidenced by the decrease in Tm and Tp values. ESR results showed that the major alterations are detected in the lipid gel phase and for the labels positioned close to the lipid-water interface.

Conclusão:

Overall our results indicate that non-specific interactions between the complexes and the membrane might be a mechanism used by the drugs to overcome the physical barriers found in the cell.

Apoio Financeiro:

Overall our results indicate that non-specific interactions between the complexes and the membrane might be a mechanism used by the drugs to overcome the physical barriers found in the cell.

Comitê de Ética:

Não realizo experimentação animal e nem humana.

26.027 Myocardial Ischemia/Reperfusion: Evaluation of Cardioprotective Humoral Factors Released During Ischemic Preconditioning by Mass Spectrometry Technology

MACIEL, L. , Batista, G. , Costa, G. C. V. , Bisch, P. M. , Nascimento, J. H. M. ,
Biofisica - IBCCF

Introducao:

Brief periods of ischemia and reperfusion in the heart induce resistance to a sustained ischemia. This phenomenon was termed ischemic preconditioning (IPC). The IPC can be induced by regional ischemia in the heart or `at distance` in non-cardiac tissues, suggesting the release of an unknown humoral activator.

Objetivos:

The aim of this study was initially evaluate the protein content of humoral factors released during IPC by mass spectrometry techniques.

Metodos:

Perfused isolated rat hearts were submitted to an ischemia and reperfusion (I/R) protocol, consisting of 30 min. of ischemia followed by 60 min. of reperfusion. IPC consisted of 3 cycles of 5 min. ischemia and 5 min. reperfusion applied before I/R. The coronary effluent was collected during the IPC and fractionated in different molecular weight ranges by ultrafiltration. Total (Efl-ipc) or fractioned (<3kDa; 3-5 kDa; 5-10 kDa; 10-30 kDa; 30-50 kDa,>50 kDa) coronary effluent were perfused before I/R. 5-10 kDa fraction was also tested in the presence of blockers for ATP-sensitive K+ channels (KATP) glyburide (10 µM) and 5HD (100 µM), or JAK-STAT (10 μM AG490) and PKC (10 µM chelerythrine) pathway inhibitors We also analysed the humoral factors by in-solution digestion and LC-MS/MS, using a ESI-Q-Tof mass spectrometer.

Resultados:

The preliminary results show that Efl-ipc and IPC hearts had lower infarct area (IA), lower end diastolic pressure (LVEDP) and better recovery of left ventricular developed pressure (LVDP), compared to the control group (only I/R; $p < 0.001$). Only the fractions 5-10 kDa and <3 kDa were able to, reduce the IA and improve the postischemic recovery of LVDP and LVEDP ($p < 0.001$ vs. control). The cardioprotection induced by 5-10 kDa fraction was inhibited by glibenclamide and 5HD ($p < 0.05$ vs. control) and attenuated by chelerythrine and AG490 ($p < 0.05$ vs. Control and 5 -10 kDa fraction). The SDS-PAGE analysis showed proteins in different molecular weight ranges. The LC-MS/MS analysis identified 962 proteins, which 60 are known as cardioprotective. Five of these 60 molecules have molecular weight between 5 and 10 kDa.

Conclusão:

The cardioprotection exerted by 5-10 kDa fraction is sensitive to KATP channel blockers, and inhibitors of JAK-STAT and PKC pathways, suggesting the involvement of these pathways in their cardioprotection mechanism. Proteomic Analysis of Efl-pci showed the existence of proteins described by presenting cardioprotective activity

Apoio Financeiro:

The cardioprotection exerted by 5-10 kDa fraction is sensitive to KATP channel blockers, and inhibitors of JAK-STAT and PKC pathways, suggesting the involvement of these pathways in their cardioprotection mechanism. Proteomic Analysis of Efl-pci showed the existence of proteins described by presenting cardioprotective activity

Comitê de Ética:

IBCCF194-07/16

26.028 The additivity of electrostatic and hydrophobic interactions on the action of peptide L1A.

Viegas, T. G. , Ruggiero Neto, J.,
Department of Physics - Unesp

Introducao:

The selectivity of antimicrobial peptides to lipid bilayers has been attributed to the balance between electrostatic and hydrophobic interaction. Some authors relate these energetic contributions assuming their additivity, however this assumption is not always correct. We have explored the electrostatic and non-electrostatic contributions of the interaction of the synthetic peptide L1A (IDGLKAIWKKVADLLKNT-NH2) with anionic vesicles. L1A is a bioactive peptide that displays potent bactericide activity without being hemolytic.

Objetivos:

L1A was used to explore the additivity of electrostatic and hydrophobic interactions on the peptide adsorption to anionic POPC/POPG vesicles.

Metodos:

Large unilamellar vesicles (LUVs), obtained by extrusion, were composed of POPC/POPG with different amounts of POPG, from 20 to 80% in buffer solution of 1mM sodium citrate, 1mM sodium borate, 1 mM sodium phosphate and 150 mM NaF at pHs 4, 7 and 10. Adsorption isotherms were obtained by titrating peptide solution with vesicles, and by monitoring changes on peptide conformation by circular dicrhoism (CD).

Resultados:

Circular dichroism spectra indicate that in buffer the peptides already have reduced helical content. In the presence of LUVs the spectra were characteristic of helical structure, an indicative that the partition of the peptide to the bilayer is coupled with its folding.The CD titrations showed that the amount of helical conformation and increased with the increased fraction of POPG from 32 to 49% at pH 7 and from 35 to 59% at pH 10 while its behavior was not clear at pH 4.The observed partitioning free energy of the peptide into the bilayer changes linearly with the surface vesicle potentials, determined from zeta potential measurements or calculated with Gouy Chapman model.

Conclusão:

At pH 10, in the whole range of POPG fractions the electrostatic free energy was lower compared to the conformational free energy, but at pH 4 and 7 these energy are approximately the same. The results point also for non-additivity of these interactions, since the sum of electrostatic and hydrophobic contribuitions does not result in the total free energy.

Apoio Financeiro:

At pH 10, in the whole range of POPG fractions the electrostatic free energy was lower compared to the conformational free energy, but at pH 4 and 7 these energy are approximately the same. The results point also for non-additivity of these interactions, since the sum of electrostatic and hydrophobic contribuitions does not result in the total free energy.

Comitê de Ética:

These experiments did not involve animals.

26.029 Effect of gangliosides on liposomes constituted by DPPC:DPPE: differential scanning calorimetry approach

Rigos, C. F. , Yoneda, J. S. , Ciancaglini, P. ,
Departamento de Ciências Naturais/Centro Universitário Norte do Espírito Santo - UFES-CEUNES
Departamento de Química - FFCLRP-USP

Introducao:

Objetivos:

The aim of this work was to evaluate the effect of gangliosides (GM1) on the stability of liposomes constituted by DPPC:DPPE, since the focus was to study the biophysical behavior of lipids on the membrane, mainly the lipid composition of the caveolae, a specific region of the plasma membrane rich in cholesterol and sphingolipids. Experiments using techniques such as differential scanning calorimetry (DSC) and dynamic light scattering (DLS) were employed.

Metodos:

Liposomes were prepared by dissolve the lipids in chloroform:methanol (2:1); this mixture was dried in nitrogen flow, forming a lipid film, and it was desiccated overnight under vacuum for complete removal of the solvent. The film was suspended in Tris-HCl buffer, pH 7.0, by incubation for 1 h, at 70 °C. The mixture was extruded through a 100 nm polycarbonate membrane under hot air flow. The calorimetric study was performed with liposomes in a DPPC:DPPE proportion 1:1 at different mol/mol % (0, 10, 20, 30 and 40 %) of GM1.

Resultados:

The critical transition temperature (Tc) obtained from the thermograms of those liposomes compositions was, for the first heating cycle, 56.1°C for 10%; 55.6°C for 20%; 55.4°C for 30% and 51.3°C for 40%. The enthalpy values decreases from the addition of GM1 10, 20 and 30%, but there is a small increase for 40%. GM1 insertion in DPPC:DPPE-liposomes provides a broadening in the phase transition peak evidenced by an increase in Δt1/2 values.

Conclusão:

Since the liposome is an important tool to mimic the natural membrane, and the proportions on the lipids can be similar to the caveolae, it can be use to evaluate the thermodynamic parameters of those systems. These studies show that with an increase in GM1 proportion in the liposome, the Tc of the system decreases, occurring a phase segregation and an enthalpy increase for 40 % of GM1. Therefore, on the conditions presented in this study, the GM1 40% addition results in an increase on the stability of the system.

Apoio Financeiro:

Since the liposome is an important tool to mimic the natural membrane, and the proportions on the lipids can be similar to the caveolae, it can be use to evaluate the thermodynamic parameters of those systems. These studies show that with an increase in GM1 proportion in the liposome, the Tc of the system decreases, occurring a phase segregation and an enthalpy increase for 40 % of GM1. Therefore, on the conditions presented in this study, the GM1 40% addition results in an increase on the stability of the system.

Comitê de Ética:

Para este trabalho não foi necessário o pedido de uso de animais, visto que nenhum foi utilizado para este resumo.

26.030 MOLECULAR DYNAMICS OF THE METHYLENE BLUE AND 1,9-DIMETHYL METHYLENE BLUE COMPOUNDS IN POPC MEMBRANE MODEL

Siani, P. , Dias, L. G. ,
Química - USP

Introducao:

This work presents a molecular dynamics study of two cationic phenothiazinic dyes immersed in phospholipid bilayer. Among the phenothiazines, the methylene blue (MB) and a dye obtained by MB methylation at 1 and 9 positions, the 1,9-dimethyl methylene blue (DMMB), which has shown higher photocytotoxicity compared to the MB, were the chosen ones.

Objetivos:

The MB and DMMB molecular dynamics were analysed paying attention to the interactions with the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer, localization in the bilayer, as well as their neighborhood and spatial orientation. In addition, free energy differences were computed for the transfer process of a cationic dye from the aqueous medium to the bilayer specific regions, and decomposed (such decomposition sheds light on how different terms contribute to the solubilization process of similar dyes in the POPC bilayer) into electrostatic and non-electrostatic components, using the synergy between theoretical methods:

Metodos:

, TI (Termodynamic Integration) and FEP (Free Energy Perturbation).

Resultados:

In the region of phospholipid headgroups low density, the values for the transfer free energy were -6.9(±0.5) kcal/mol and -7.6(±0.6) kcal/mol, for MB and DMMB, respectively. In the region of high density, the values were -12.9(±0.4) kcal/mol for MB and -14.3(±0.5)kcal/mol for DMMB. For the last position, the center of phospholipid bilayer, were found the values of -3.3(±0.3) kcal/mol and -4.3(±0.4)kcal/mol, for MB and DMMB, respectively.

Conclusão:

In conclusion, the region of high density was the preferred one for both photosensitizers (also observed in the unrestricted molecular dynamics), due to the eletrostatic stabilization. The lesser mobility, higher insertion and stabilization of DMMB in the phospholipid bilayer must contribute to its higher photodynamical therapy efficiency compared to MB (Chem. Soc. Rev., 25(5); 351-359, 1996).

Apoio Financeiro:

In conclusion, the region of high density was the preferred one for both photosensitizers (also observed in the unrestricted molecular dynamics), due to the eletrostatic stabilization. The lesser

mobility, higher insertion and stabilization of DMMB in the phospholipid bilayer must contribute to its higher photodynamical therapy efficiency compared to MB (Chem. Soc. Rev., 25(5); 351-359, 1996).

Comitê de Ética:

ABF (Adaptive Biasing Force)

26.031 PERFIL LIPÍDICO E VIAS GLICÊMICAS HEPÁTICAS EM UM MODELO MURINO DIETÉTICO PARA A SÍNDROME METABÓLICA.

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Laboratório de Reatividade Cardiovascular - Ufal
Max Delbrück Center for Molecular Medicine - MDC
NanoBiofarmacêutica - INCT-NanoBiofar

Introducao:

A síndrome metabólica (SMet) é um agravo caracterizado pelo agrupamento de fatores de risco cardiovascular (Circulation.109,433-438,2004). A deposição de gordura no fígado é mais um componente desta síndrome (The Lancet.366,159-162,2005), no entanto, as vias que estão envolvidas nas modificações estruturais e funcionais hepáticas ainda não foram completamente esclarecidas.

Objetivos:

Analisar o perfil lipídico e as vias glicêmicas hepáticos em um modelo murinodietético para a SMet.

Metodos:

Utilizou-se camundongos C57BL/6 machos (10semanas), divididos em 2grupos: C57Bl/6alimentado com dieta “chow”, 10% Kcal provenientes dos lipídios (CT,n=6), e C57Bl/6 alimentado com dieta “high fat”, 45% Kcal provenientes dos lipídios durante 20-22 semanas (DHF, n=6). Avaliações metabólicas de peso corporal e depósito de gordura foram realizadas para monitoramento do fenótipo sindrômico. A deposição lipídica hepática foi avaliada pela técnica de Folch, hematoxilina e eosina e imunohistoquimica para adipofilina. Avaliou-se a gliconeogênese hepática e a atividade das enzimas alanina aminotransferase (ALT) e aspartatoaminotransferase (AST). A expressão gênica relativa no tecido hepático foi realizada pela técnica de RTqPCR e a expressão proteica pelo Western Blot.Os dados estão expressos como média ± EPM. Utilizou-se o teste tpara análise estatística. Valores de p< 0,05 foram considerados estatisticamente significativos.

Resultados:

Ao término da intervenção dietética os animais do grupo DHF apresentaram peso corporal significativamente maior (CT= 33,09 ± 1,028 n=6vsDHF=44,53 ± 1,022 n=6, p<0.0001), acompanhado pelo aumento do índice de adiposidade (CT=4,131 ± 0,8724 n=6vs DHF=8,526 ± 0,2703 n= 6, p<0.001).O peso relativo hepático não foi diferente entre os grupos. No entanto, a dosagem das enzimas ALT(CT=4,204 ± 0,6221 n=6vsDHF=9,227 ± 1,808 n=6, p<0,05) e AST(CT=8,648 ± 0,6499 n=5vsDHF=24,69 ± 3,560 n=6, p<0,005)mostrou que os animais DHF apresentaram prejuízo da função hepática. Além disso, o grupo DHF apresentou elevada de

posição hepática de lipídios totais (CT=0,3714 ± 0,02297 n=6vs DHF=0,5511 ± 0,04820 n=6, p<0,05), triglicerídeos (CT=116,0 ± 12,34 n=6vsDHF=637,6 ± 94,50 n=6, p<0,001)e ácidos graxos não esterificados (CT=1.553 ± 0.07606 n=6vs DHF=4.979 ± 0.8718 n=6, p<0,001). O acúmulo lipídico hepático nos DHF foi confirmado qualitativamente, constatando-se aumento na deposição lipídica micro e macrovesicular e de adipofilina. A gliconeogênese hepática não foi diferente entre os grupos. Entretanto, houve aumento significativo da expressão gênica hepática de glicose-6-fosfatase nos animais DHF (CT= 1.020 ± 0.09335 n=6 vs DHF=1.747 ± 0.2325 n=6, p<0,05). Os níveis protéicos do substrato do receptor da insulina 1, mas não de fosfoinositol-3quinase e AKT, foi significativamente menor no DHF (CT= 0,12 ± 0,02 n=6 vs DHF=0,06 ± 0,02n=5, p<0,05).

Conclusão:

Os resultados demonstraram que animais com SMet induzida por uma dieta “high fat” desenvolvem esteatosee dano hepático acompanhado de prejuízo nas vias glicêmica e gliconeogênica.

Apoio Financeiro:

Os resultados demonstraram que animais com SMet induzida por uma dieta “high fat” desenvolvem esteatosee dano hepático acompanhado de prejuízo nas vias glicêmica e gliconeogênica.

Comitê de Ética:

005480/2011-15

26.032 Effects of pH on the production of phosphate by proteoliposomes carrying alkaline phosphatase and nucleotide pyrophosphatase/phosphodiesterase-1

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Depto. Química - USP
Center for Molecular and Vascular Biology - Leuven/Belgium
Sanford Children?s Health Research Center - La Jolla/CA/USA

Introducao:

During endochondral bone formation, chondrocytes and osteoblasts synthesize and mineralize the extracellular matrix through a process that initiates within matrix vesicles (MVs) and ends with bone mineral propagation onto the collagenous scaffold. Tissue-nonspecific alkaline phosphatase (TNAP) degrades extracellular inorganic pyrophosphate (PPi), a mineralization inhibitor produced by ectonucleotide pyrophosphatase/phosphodiesterase-1 (NPP1), while contributing Pi from ATP to initiate mineralization. MVs have a nucleation core composed of amorphous calcium phosphate (ACP), complexed in part with phosphatidylserine (PS) to form the calcium-phosphate-lipid complexes (PS-CPLX) capable of inducing mineral formation when incubated in a synthetic cartilage lymph (SCL).

Objetivos:

We studied the hydrolysis of ATP, ADP, AMP and PPi by proteoliposomes harboring TNAP and/or NPP1 at pH 8 and 9 and compared our data with those obtained at physiological pH. We examined also the effects of ACP and PS-CPLX for their ability to modulate mineral formation induced by the proteoliposomes incubated in a SCL.

Metodos:

TNAP and/or NPP1 were incorporated into dipalmitoylphosphatidylcholine (DPPC) liposomes and the kinetic parameters for the hydrolysis of the substrates by the different proteoliposomes were calculated at pH 8 and 9.

Resultados:

While catalytic efficiencies in general were higher at alkaline pH, PPi hydrolysis was maximal at pH 8 and indicated a preferential utilization of PPi over ATP at pH 8 versus 9. In addition, all proteoliposomes induced mineral formation when incubated in a SCL containing 1 mM ATP and ACP or PS-CPLX. Propagation of mineralization was significantly more efficient at pH 7.5 and 8 than at pH 9.

Conclusão:

Since a slight pH elevation from 7.4 to 8 promotes considerably more hydrolysis of ATP, ADP, and AMP primarily by TNAP, this small pH change facilitates mineralization, especially via upregulated PPi hydrolysis by both NPP1 and TNAP, further elevating the Pi/PPi ratio, thus enhancing bone mineralization.

Apoio Financeiro:

Since a slight pH elevation from 7.4 to 8 promotes considerably more hydrolysis of ATP, ADP, and AMP primarily by TNAP, this small pH change facilitates mineralization, especially via upregulated PPi hydrolysis by both NPP1 and TNAP, further elevating the Pi/PPi ratio, thus enhancing bone mineralization.

Comitê de Ética:

Neste trabalho não foram utilizados animais.

26.033 Cholesterol x Cholestenone: influence of the lipid microenvironment in alkaline phosphatase activity incorporated into liposomes

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Química - FFCLRP-USP

Introducao:

Cholestenone (Achol) is a compound analogous to cholesterol (Chol), with the same thermodynamic characteristics. Tissue-nonspecific alkaline phosphatase (TNAP) is a phosphomonohydrolase that hydrolyses ATP and inorganic pyrophosphate (PPi) to generate phosphate for the precipitation of hydroxyapatite during the biomineralization process.

Objetivos:

Our aim was to evaluate the incorporation of TNAP into DPPC:Achol 36%, DPPC:Chol 36% and DOPC:Chol 36% (molar ratio)-liposomes and to compare the kinetic parameters of ATP and PPi hydrolysis by the different proteoliposomes.

Metodos:

TNAP was incorporated into the different liposomes and the kinetic parameters (maximum rate of hydrolysis (VM), affinity constant (K0.5), cooperativity (n) and catalytic efficiency (kcat/K0.5)) for the hydrolysis of ATP and PPi by the different proteoliposomes were determined and compared.

Resultados:

The concentration of TNAP incorporated varied from 0.074 mg/mL for DPPC:Achol 36% to 0.087 mg/mL for DPPC:Chol 36%. For DPPC:Achol 36% systems, ATP hydrolysis showed Vm of 453.5 U/mg, less than that obtained for the DPPC:Chol 36% system (1244.1 U/mg), but higher than that observed for DOPC:Chol 36% system (221.1 U/mg). The K0.5 value was 0.19 mM, resulting in a greater affinity for ATP than for the systems constituted of DPPC:Chol 36% (2.9 mM) and DOPC:Chol 36% (1.7 mM). Positive cooperativity was observed (n=2.0), similar to the behavior obtained for the systems constituted of DPPC:Chol 36% (n=2.9) and DOPC:Chol 36% (n=1.7). The catalytic efficiency was $4.8x10^3$ $M^{-1}.s^{-1}$, with similar values for the other systems. For the hydrolysis of PPi, DPPC:Achol 36% system presented Vm, K0.5 and kcat/K0.5 values very similar to those obtained for the systems composed of DPPC:Chol 36% and DOPC:Chol 36%. However, the cooperativity value was higher than for the other systems.

Conclusão:

The presence of Achol in the studied systems altered significantly the kinetic parameters for the hydrolysis of ATP only, which can be explained by thermodynamic factors, such as decreased enthalpy of transition, loss of pre-transition, the presence of surface charges from the polar heads of

phospholipids which can also cause conformational changes in TNAP structure resulting in different access of the substrate to the catalytic sites of the enzyme.

Apoio Financeiro:

The presence of Achol in the studied systems altered significantly the kinetic parameters for the hydrolysis of ATP only, which can be explained by thermodynamic factors, such as decreased enthalpy of transition, loss of pre-transition, the presence of surface charges from the polar heads of phospholipids which can also cause conformational changes in TNAP structure resulting in different access of the substrate to the catalytic sites of the enzyme.

Comitê de Ética:

Neste trabalho não foram utilizados animais.

26.034 Aggregation and Oligomerization of Na,K-ATPase detected by Dynamic Light Scattering and Analytical Ultracentrifugation

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Departamento de Quimica - FFCLRP - USP
Instituto de Fisica - IF - USP-Sao Paulo
Instituto de Quimica de São Carlos - IQ - USP-Sao Carlos

Introducao:

Na,K-ATPase is a membrane protein that uses the energy from ATP hydrolysis to transport Na+ and K+ across the plasma membrane. One of the discussions about Na,K-ATPase is on its oligomeric state. It is known that the minimal functional unit is the monomer. However, several experimental evidences show that the Na,K-ATPase associates in larger oligomers. The question that remains is whether these associations occur in vivo and what it would cause on the structure/function of the protein.

Objetivos:

The aim was to determine in which state oligomeric of the solubilized Na,K-ATPase is predominantly associated, using the techniques of Dynamic Light Scattering (DLS) and Analytical Ultracentrifugation (AUC).

Metodos:

Na, K -ATPase was solubilized with C12E8 and purified as described in Santos et al., 2002. The DLS experiments were performed using equipment from Beckman Coulter (model N5 Submicron Particle Size Analyser). AUC experiments were performed at the Laboratory of Spectroscopy and Calorimetry of LNBio-CNPEM in Campinas, using an ultracentrifuge from Beckman Coulter.

Resultados:

As soon as protein was eluted from the purification column, the sample was analyzed in DLS. The diameter obtained was 31.5±1.49nm, PI=0.564±0.144. When this sample was concentrated, the diameter was 65.3±2.3nm, PI=0.605±0.057. This increase indicated an aggregation process with concentration. The sample was filtered to try eliminating the aggregate. The diameter of filtered sample was 48.2±0.32nm, PI=0.263±0.018. It was observed that the aggregate is inactive. The AUC result showed a mixture of 7 populations of Na, K -ATPase after filtration. The monomer (αβ) and tetramer (αβ)4 were the main population.

Conclusão:

The results demonstrate that Na,K-ATPase solubilized assumes mainly its (αβ) form, but there are other oligomers in solution. Further investigations intend to find out why this association

happens and if this has influence on efficiency and/or stability of the pump.

Apoio Financeiro:

The results demonstrate that Na,K-ATPase solubilized assumes mainly its (αβ) form, but there are other oligomers in solution. Further investigations intend to find out why this association happens and if this has influence on efficiency and/or stability of the pump.

Comitê de Ética:

Não foram utilizados animais neste trabalho.

26.035 Cholesterol depletion by different cyclodextrins on synaptosomal membranes

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Depto de Bioquímica - Unicamp

Introducao:

Methyl-β-clycodextrin (MβCD), β-clyclodextrin (βCD) and hydroxypropyl-β-clyclodextrin (HPβCD) are cyclic oligosaccharides with a relative nonpolar cavity that can accommodate hydrophobic molecules, forming inclusion complexes. Membrane lipids, including cholesterol can be removed from the bilayers, after exposure to CD. In this way, cyclodextrins are widely employed in studies of cholesterol enriched membrane microdomains, detergent resistant membranes.

Objetivos:

Here we compared the effect of MβCD, βCD, and HPβCD on pig brain synaptosomal membranes. Synaptosomes are good membrane models for the study of lipid rafts since they are cholesterol-enriched and cholesterol is involved in the release/reuptake of neurotransmitters in presynaptic membrane.

Metodos:

Synaptosomal membranes were isolated from the cortex of pig brain. Cholesterol depletion was promoted by treating the membranes with different concentrations of MβCD, βCD and HPβCD, at 37 °C for 30 min. (and discarding the soluble cholesterol-cyclodextrin complex fraction by centrifugation), prior to synaptosome isolation. The synaptosomal membrane was then characterized regarding their cholesterol and protein content, as well as their fluidity, evaluated through electron paramagnetic resonance (EPR) with 5-doxyl stearate probe.

Resultados:

Synaptosomal membranes contained high cholesterol/protein ratio (0.8 μg/μg). The cholesterol depletion capacity profile was MβCD ? βCD = HPβCD since 50 mM MβCD, βCD or HPβCD promoted 50%, 20% and 10% depletion, respectively, of their total cholesterol. Moreover some protein depletion was also observed, in the order βCD ? HPβCD ? MβCD. EPR spectra revealed that membrane fluidity was significantly increased after cholesterol depletion by MβCD, but not after treatment with βCD and HPβCD (up to 50 mM).

Conclusão:

MβCD was found to be 5 times more efficient than the other cyclodextrins tested, being

suitable for the study of cholesterol-dependent cellular function in synaptosomal membranes.

Apoio Financeiro:

MβCD was found to be 5 times more efficient than the other cyclodextrins tested, being suitable for the study of cholesterol-dependent cellular function in synaptosomal membranes.

Comitê de Ética:

Os cérebros foram obtidos a partir de um abatedouro de suínos.

26.036 CHARACTERIZATION OF REMOTE-LOADING LIPOSOMES CONTAINING DIBUCAINE

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Departamento de Bioquimica e Biologia Tecidual - UNICAMP

Introducao:

Dibucaine(DBC) is an amino-amide local anesthetic. It has a unique chemical structure (quinoline ring with a butoxy group) that renders it very hydrophobic. Liposomes can be used as carriers for drug delivery. Low pH and anions inside the vesicles (remote loading) helped to entrap large amounts of actively loaded DBC+.

Objetivos:

The aim of this study was to develop special liposomes for DBC delivery, composed by hydrogenated soy phosphatidylcholine(HSPC) or egg phosphatidylcholine(EPC) plus cholesterol (chol) with an internal ionic gradient (protons, citrate, sulfate).

Metodos:

Multilamellar vesicles (LMV) composed by HSPC:Chol (2:1 mole%) or EPC:Chol (2:1.5 mole%) were produced by hydration of the lipid film with 300mM sulfate or citrate, at 50mM acetate buffer pH 5.5, as an internal ionic gradients. Extrusion trough polycarbonate membranes at 400 nm led to unilamellar vesicles (LUV) production, while multivesicular vesicles (LMVV) were prepared by extrusion through 100 nm pores, followed by 10 freeze-thawing cycles. The external pH was set to 7.4 after phase separation Hepes buffer. Vesicles size, zeta potential and polydispersity were measured by dynamic light scattering(DLS) during storage under refrigeration. Vesicle’s morphology was followed by transmission electron microscopy(TEM) and electron paramagnetic resonance(EPR) with spin probes were used to evaluate the fluidity (by the order parameter, S) of the bilayers.

Resultados:

LUV in the range of 500 nm and LMVV with 1000 nm were prepared; their zeta potentials were always negative(ca. -30 mV) for HSPC. Incorporation of DBC did not change the size, not the morphology of the liposomes(TEM) in good agreement with DLS results. EPR analysis showed that HSPC:chol(S = 0.85) are less fluid than EPC:chol membranes(S = 0.71).

Conclusão:

We successfully prepared ionic-gradient liposomes that improved DBC upload. HSPC:Chol liposomes showed a more rigid membrane than EPC:chol, qualifying them to retain the ionic gradient for a long time. Release kinetics experiments are under course to evaluate DBC release in

vitro.

Apoio Financeiro:

We successfully prepared ionic-gradient liposomes that improved DBC upload. HSPC:Chol liposomes showed a more rigid membrane than EPC:chol, qualifying them to retain the ionic gradient for a long time. Release kinetics experiments are under course to evaluate DBC release in vitro.

Comitê de Ética:

Nao foram utilizados animais nesse trabalho

26.037 Characterization of Liposomes and Proteoliposomes by Atomic Force Microscopy.

Bolean, M. , Borin, I. A. , Simao, A. M. S. , Trindade, L. F. , Hoylaerts, M. F. , Millan, J. L. , Ciancaglini, P. ,
Quimica - USP
Center for Molecular and Vascular Biology - Leuven, Belgium.
Sanford Childrens Health Research Center - La Jolla, CA, USA.

Introducao:

Proteoliposomes are systems that mimic lipid membranes in which proteins have been incorporated.

Objetivos:

We have explored here the differences in viscosity and elasticity properties presented by lipids and proteins employing the tapping mode Atomic force microscopy (AFM) technique to observe lipid-protein interaction in proteoliposomes.

Metodos:

The scan rate was adequately reduced to 0.2 – 0.3 Hz and the integral gain was obtained with the lowest possible value in order to prevent tip induced vesicles deformation. A Shimadzu SPM software was used to process and analyze the signals, two and three dimensional topography, height, acosD, asinD, amplitude, and phase imaging data were acquired simultaneously over a selected area.

Resultados:

AFM analysis for DPPC-liposomes showed intact and spherical vesicles, with a smooth surface and neither topographic irregularities nor significant visco-elastic alterations appeared. DPPC:DPPS 10%-liposomes (molar ratio) were also analyzed in order to evaluate if the presence of negative charges provide morphological changes when compared with DPPC-liposomes. The images obtained show spherical vesicles but with wrinkled surface, which could be observed in all analyzed signals. No distinct microdomains in both liposomes samples were observed. The presence of proteins alkaline phosphatase and annexin V into DPPC:DPPS 10%-proteoliposomes provides significantly changes in visco-elastic mechanical properties of proteoliposomes. In images obtained from acosD signal, lighter spots were observed (more rigid domains) in the vesicles surface indicating differences in elastic properties of certain regions. Using the phase mode, darker spots were observed in the proteoliposomes surface, which are domains with less visco-elasticity. When both proteins are present into the DPPC:DPPS 10%-proteoliposomes concomitantly, it can be observed that the microdomains with distinct phases are even bigger and more concentrated in the same region. Considering that proteins and lipids differ in their physical properties, the presence of these compounds can be detected as distinct visco-elastic domains.

Conclusão:

Thus, it can be suggested that these proteins induce the formation of these distinct microdomains with different mechanical properties and therefore it can be affirmed that they have different chemical composition. Such results encourage us to investigate even more the lipid-protein interactions once AFM is considered a very promising technique in topographic studies of nanometer vesicles.

Apoio Financeiro:

Thus, it can be suggested that these proteins induce the formation of these distinct microdomains with different mechanical properties and therefore it can be affirmed that they have different chemical composition. Such results encourage us to investigate even more the lipid-protein interactions once AFM is considered a very promising technique in topographic studies of nanometer vesicles.

Comitê de Ética:

No animals were used in this work.

26.038 A Comparative Studying of Proteins Conformational Changes by Site Direct Spin Label and Electronic Spin Resonance

Case, A. H. , Micheletto, M. C. , Garcia, A. F. , Costa, A. J. ,
Departamento de Física - USP

Introducao:

S100A12 belongs to the superfamily of calcium bind proteins (S100) having the presence of two EF-hand motifs consisting of helix-loop-helix elements with affinity for divalent ions Ca2+ and Zn2+. Upon ion binding, conformational changes occur in protein domain from `closed` to `open` conformation, exposing hydrophobic residues that will interact with target molecules.

Objetivos:

Our goal is to analyze physical and chemical factors that influence oligomerization, to investigate the influence of binding Ca2+ and Zn2+ on the conformational structure and to study the conformational change experienced by one specific helix of the EF-hand motif in both human (hS100A12) and porcine (pS100A12) S100A12 in the presence of Ca2+ and/or Zn2+ using SDSL-EPR techniques.

Metodos:

Mutants were expressed in E. coli (Rosetta) and purified by affinity and liquid chromatography in an Äkta Purifier 20 system. . All mutants were marked with MTSSL spin probe and measured by EPR in a JEOL JES-FA200 (X-band, continuous wave mode) spectrometer.

Resultados:

hS100A12 and pS100A12 mutants were expressed and purified. Each step of purification had aliquots collected from all mutants that were analysed on SDS-PAGE in tricine buffer. Results showed bands of expression with a high purity. After spin labeling mutants, EPR measurements were performed and dynamics of the helices forming the EF-hand motif were assessed for both proteins in absence and in presence of ions. Moreover, it is of our interest to check for potential protein-membrane interactions. Same mutants were used in EPR experiments in the presence of membrane model systems constituted by.DPPG and DPPC. For several mutants, spectral differences were observed upon membrane and/or ion binding and our results are analyzed together to find possible differences in binding mode for each case.

Conclusão:

SDSL-EPR results enabled us to monitor conformation changes upon ligand binding for both hS100A12 and p S100A12. A detailed comparison will allow us to discriminate molecular mechanisms underlying those bindings and to understand structural differences between the two calcium binding proteins.

Apoio Financeiro:

SDSL-EPR results enabled us to monitor conformation changes upon ligand binding for both hS100A12 and p S100A12. A detailed comparison will allow us to discriminate molecular mechanisms underlying those bindings and to understand structural differences between the two calcium binding proteins.

Comitê de Ética:

No animal or human experimentation.

26.039 The Psd2 defensin interacts with cardiolipin, an anionic lipid present in bacteria and mitochondria membranes

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Programa de Biologia Molecular e Estrutural - IBCCF
Instituto de Medicina Molecular - FMUL

Introducao:

Antimicrobial peptides (AMP) are part of the innate defense mechanism representing an emerging class of substances with therapeutic potential for infectious diseases and cancer. The complete mechanism of action of most AMP is still poorly understood. However, it has been shown that some of them interact with anionic lipids of membranes, as glycosphingolipids. The AMP Psd2, a defensin isolated from pea seeds, contains 46 aminoacids that are arranged by one α-helix and 3 β-sheets, stabilized by four disulfide bonds. It is active against several fungi, especially A. niger, C. albicans, N. crassa and C. musae with IC50 less than 5 mM.

Objetivos:

In order to better understand its mode of action the biophysical interaction of Psd2 with biomembrane systems that mimic the lipid composition of fungi, bacterial and mammalian was measured.

Metodos:

Fluorescence spectroscopy.

Resultados:

Pure Psd2 did not aggregated as followed by fluorescence of the hydrophobic probe ANS. Also, acrylamide fluorescence quenching data showed a linear profile indicating that the unique Trp42 was accessible to be used in partition assays in the presence of LUVs. No Psd2 partition was observed into LUVs containing POPC, POPC:Erg, POPC:CMH, POPC:PE (70:30 ratio). However, hyperbolic partition curves were obtained in the presence of POPC:Chol, POPC:PE:CL (60:30:10) and POPC:CL (70:30) (Kp x103 of 236, 130 and 767, respectively). The interaction of Psd2 with vesicles containing CL was higher than those composed of Chol and PE:CL. Acrylamide quenching studies suggested that Trp participated in this interaction, since no fluorescence decrease was observed in the presence of POPC:PE:CL. Studies of membrane permeabilization with carboxyfluorescein probe indicated that Psd2 did not alter the vesicle permeability or cause extravasations of internal contents of POPC or POPC:PE:CL (60:30:10) vesicles.

Conclusão:

Additional studies must be performed to clearly determine the nature of the interaction between Psd2 and vesicles composed with cardiolipin a component of bacterial and mitochondria membranes.

Apoio Financeiro:

Additional studies must be performed to clearly determine the nature of the interaction between Psd2 and vesicles composed with cardiolipin a component of bacterial and mitochondria membranes.

Comitê de Ética:

26.040 Cell viability of osteoblasts grown in the presence of graphene oxide.

Francisco, C. G. , Zancanela, D. C. , Simão, A. M. S. , Matsubara, E. Y. , Rosolen, J. M. , Ciancaglini, P. ,
Tecnologia - FCAV-UNESP
Quimica - FFCLRP-USP

Introducao:

Graphene oxide (GO), a single layer of sp2 bonded carbon atoms in a two-dimensional hexagonal structure, has attracted considerable attention as a potential biomaterial due to its physicochemical properties, including a large surface area, high dispersibility and hydrophilicity [1,2].

Objetivos:

However, for a new material be incorporated into biomedical applications, it is necessary a thorough assessment of possible damages that it might have on living organisms, being extremely necessary to study the toxicity and biocompatibility of the material in a biological environment.

Metodos:

. Thus, we characterized GO by SEM and EDS analysis. In addition, using a colorimetric assay, we analyzed cell viability of osteoblasts after 3 and 7 days of incubation with GO.

Resultados:

. SEM analysis revealed homogeneous GO sheets and by EDS analysis we observed the presence of small amounts of aluminum (0.25%) and sulfur (6.83%), possibly waste from the synthesis and/or purification processes. In the biological assays, with 3 days in the presence of GO (until 0.2 mg/mL), we observed an increase in cell viability of about 170%, but significant changes in cell viability were not observed from 0.2 to 0.4 mg/mL of GO. On the other hand, there was a decrease in cell viability at concentrations ?0.4 mg/mL. With 7 days of incubation, cell growth was greater than the control only with 0.025 mg/mL of GO, and with concentrations above 0.25 mg/mL a decrease of about 50% was observed in cell viability.

Conclusão:

Therefore, given the good cell viability results observed, we can proceed with our research to further investigate how the GO surface interfere with the biomineralization process, with the ultimate goal of developing a fully biocompatible system able to assist the events leading to the calcification process.

Apoio Financeiro:

Therefore, given the good cell viability results observed, we can proceed with our research to further investigate how the GO surface interfere with the biomineralization process, with the ultimate goal of developing a fully biocompatible system able to assist the events leading to the calcification process.

Comitê de Ética:

09.1.934.53.4

26.041 Effects of the detergent Triton X-100 on giant vesicles composed of raft-like ternary lipid mixtures

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Biofísica - Unifesp
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Introducao:

Detergents are widely used as solubilizing agents of biological membranes. However, biological membranes treated with detergents exhibit insoluble fragments called DRMs (detergent resistant membranes), rich in sphingolipids, cholesterol and certain proteins. Due to resemblances related to composition and characteristics, DRMs have been associated with rafts, which are functional membrane domains, and with the liquid-ordered (Lo) phase, obtained from mixtures of saturated lipids and cholesterol. The solubilization process of biological membranes depends on the interaction between detergents and lipids.

Objetivos:

Here, we use optical microscopy to study the effects of the detergent Triton X-100 on giant unilamellar vesicles composed of ternary lipids mixtures of palmitoil oleoyl phosphatidylcholine (POPC), sphingomyelin (SM) and cholesterol (chol). Our aim was to map the Lo/Ld (liquid-disordered) phase coexistence region of the phase diagram of POPC:SM:chol in the presence and absence of sub-solubilizing concentration of Triton X-100 (0.1 mM) and to correlate the extent of partial solubilization induced by Triton X-100 with the membrane composition.

Metodos:

GUVs containing 20, 30 and 40 mol% cholesterol with varying POPC:SM ratios were investigated. Fluorescence microscopy was used to observe the partition of the fluorescent probe DiIC18, which prefers the Ld phase.

Resultados:

All compositions were verified and we observed a predominance of Ld phase, characterized by the emergence of domains only after contact with the detergent. These compositions were rich in POPC. A small region, consisting of four compositions, rich in cholesterol and SM, showed characteristics of Lo phase. In this case, it was not possible to see domains under any condition. Finally, the portion of the diagram rich in SM showed domains before and after the contact with the TX-100. Injection of a concentrated Triton X-100 solution with a micropipette enabled observation of the solubilization process of GUVs. After contact with the detergent, part of the vesicle (mainly the Ld part) was removed and completely solubilized, leaving a smaller insoluble vesicle. Through the

vesicle diameters before and after solubilization, the extent of surface area solubilized was quantified.

Conclusão:

In the absence of Triton X-100, only a small Lo/Ld coexistence region close to the SM corner was observed. This region was clearly enlarged in the presence of Triton X-100. The results show that the extent of solubilization is reduced for compositions richer in cholesterol and SM. We conclude that the effects caused by Triton X-100 are largely modulated by the membrane composition.

Apoio Financeiro:

In the absence of Triton X-100, only a small Lo/Ld coexistence region close to the SM corner was observed. This region was clearly enlarged in the presence of Triton X-100. The results show that the extent of solubilization is reduced for compositions richer in cholesterol and SM. We conclude that the effects caused by Triton X-100 are largely modulated by the membrane composition.

Comitê de Ética:

This study does not involve humans or animals, just synthetic model membrane.

26.042 Interaction of the Rattlesnake Toxin Crotamine with model membrane

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Farmacologia - UNIFESP

Introducao:

Crotamine is one of the main constituents of the venom of the South American rattlesnake Crotalus durissus terrif icus. A common gene ancestry and structural similarity with the antimicrobial β-defensins (identical disulfide bond pattern and highly positive net charge) suggested potential antimicrobial activities for this snake toxin. Although crotamine demonstrated low activity against both Gram-positive and Gram-negative bacteria, a pronounced antifungal activity was observed against Candida spp., Trichosporon spp., and Cryptococcus neoformans. Crotamine’s selective antimicrobial properties, with no observable hemolytic activity, stimulated us to evaluate the potential applications of this polypeptide as an antiyeast or candicidal agent for medical and industrial application.

Objetivos:

Aiming to understand the mechanism(s) of action underlying crotamine antimicrobial activity and its selectivity for fungi, we present herein studies using membrane model systems (i.e., large unilamellar vesicles, LUVs, and giant unilamellar vesicles, GUVs), with different phospholipid compositions

Metodos:

The GUVs were prepared by the electroformation method. Briefly, 20 μL of lipid in chloroform solution [2 mg/mL] was spread on the surfaces of two conductive glasses coated with fluor tin oxide, which were then placed with their conductive sides facing each other and separated by a 2 mm thick Teflon frame. This electro-swelling chamber was filled with 0.2 M sucrose solution, and it was connected to an alternating current of 2 V with a 10 Hz frequency for 2 h. The vesicle suspension was removed from the chamber and diluted into a 0.2 M glucose solution containing the desired amount of crotamine. The osmolarity of the sucrose and glucose solutions was measured with a Gonotec 030 cryoscopic osmometer (Osmomat, Berlin, Germany) before use, and they were carefully matched to avoid osmotic pressure effects. The vesicles were then immediately placed on the observation chamber. Due to the differences in density between sucrose and glucose solutions, the vesicles were stabilized by gravity at the bottom of the observation chamber. Vesicles were observed by using an inverted microscope Axiovert 200 (Carl Zeiss; Jena, Germany) in the phase contrast mode (Plan Neo-Fluar 63× Ph2 objective (NA 0.75) and A-plan 10 × Ph1 (NA 0.25)) and

fluorescence mode (103W Hg lamp, HXP 120, Kubler, Carl Zeiss, Jena, Germany). A Zeiss 43 HE filter set (excitation at 538−563 nm and emission at 570−640 nm) was used to observe the fluorescent dye Cy3 bound to crotamine. Images were recorded with an AxioCam HSm digital camera (Carl Zeiss).

Resultados:

We show here that crotamine presents a higher lytic activity on negatively charged membranes compared with neutral membranes, with or without cholesterol or ergosterol content. The vesicle burst was not preceded by membrane permeabilization as is generally observed for pore forming peptides.

Conclusão:

GUV experiments showed that, in a first step, crotamine is homogeneously distributed over the entire vesicle surface. We may hypothesize that positively charged crotamine initially bound to the lipid bilayer exerts anionic lipid clustering in the membrane surface; this interaction, by turn, may favor the accumulation of crotamine on a limited membrane region, causing its destabilization that ends in membrane rupture. The assemblage may create a macropore that compromises the membrane integrity, leading to its rupture and vesicle bursting.

Apoio Financeiro:

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Comitê de Ética:

26.043 BIOPHYSICAL STUDY OF STRUCTURE/FUNCTION OF THE CYTOCHROME P450 from Streptomyces clavuligerus.

Cravo, H. L. P. , Garcia, A. F. , Araujo, A. P. U. , Nascimento, O. R. , Nonato, M. C. , Filho, A. J. C. ,
Departamento de Física - FFCLRP - USP
Departamento de Física - IFSC - USP
Departamento de Física - FCFRP - USP

Introducao:

The cytochrome P450 from Streptomyces clavuligerus (P450CLA) is a hemoprotein with a Fe+3 ion bound to a porphyrin group. Streptomyces clavuligerus is a gram-positive bacterium used on an industrial scale for the production of clavulanic acid, a potent inhibitor of the β-lactamase enzymes. The synthesis of this compound has been well characterized over the years, however, many routes remain partially understood, especially one with participation of enzyme P450CLA. Studies indicate that the P450 family proteins are flexible and undergo conformational changes when interacting with membranes or with their substrates. This suggests these changes are correlated with activity/function performed by the protein.

Objetivos:

Study through biophysical techniques possible conformational changes of P450CLA as result of its interaction with its substrate and membrane models, in order to contribute to the understanding of its role in the clavulanic acid biosynthesis.

Metodos:

Spectroscopic studies of structural integrity by CD. Determination of oligomeric state by Size Exclusion Chromatography. Integrity check porphyrin group and the binding with Fe+3 by EPR and UV-Vis. Thermal denaturation monitored by CD and DSC. Analysis of the interaction protein-membrane by EPR and DSC. Assays to determine the crystallographic structure of the protein for production of the mutants which provide structural change information.

Resultados:

CD spectra show a structured sample with α-helix profile. Thermal stability by CD and DSC presented results consistent, with denaturation temperature close to 40°C. Oligomerization assay allowed to estimate the mass and confirm its monomeric state. EPR spectrum was characteristic to others P450s in ferric state (Fe+3, S=1/2), low spin and orthorhombic symmetry, indicating the incorporation of the ion in the sample. This result was also observed by Soret band and the Q bands in the UV-vis spectrum. Studies with model membrane suggest a protein-membrane interaction, in which one observes the destabilization of the membrane, both by EPR and DSC. Different

crystallization conditions were tested and seem to be favorable for obtaining the crystal.

Conclusão:

Preliminary results suggest good conditions for continuing the crystallization trials. Studies by EPR and DSC indicated a probable protein-membrane interaction, as has been described for other cytochromes. However, it requires more study in order to clarify the evidence collected.

Apoio Financeiro:

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Comitê de Ética:

Não utilizo experimentos com seres humanos ou animais.

26.044 ANALYSIS OF TERMITES MICROBIOTA GROWN IN DIFFERENT CULTURE MEDIA AND SUBSTRATES.

Gonçalves, L. C. , Veiga, H. C. , Santos, E. , Souza, W. , Grieco, M. A. B. ,
DIMAV - INMETRO

Introducao:

Termites are important cellulose degraders in nature, being this polysaccharide the most abundant polymer in plant biomass. The gut of termites has great diversity of microorganisms, figuring among these protists, bacteria and archaea inhabitants. Many of these are potentially able to degrade cellulose and thus are of interest to study in the field of second generation bioethanol technology. However, little is known about the symbiosis between termites and gut flora, and because of that, this relationship has been of interest for basic and applied research.

Objetivos:

Having thus as our goal to isolate and identify bacteria in the intestine of cellulose-degrading termites.

Metodos:

For the investigation of the bacterial symbionts in the gut of the termites, Synthermes sp and Cornitermes sp (Family Termitidae) were collected in the field. Workers and soldiers were selected, the guts were dissected and the total homogenate (separated by species and castes) was divided in two parts: one was plated on solid culture media and another one incubated in liquid culture media for observation of culturable bacteria in the different conditions. The media used for growing were: Minimum Medium with carboxymethylcellulose (CMC), Minimum Medium and Tryptone soy broth (TSB). After isolation, the bacterial colonies were stained with Congo Red for observation of degraded cellulose. Aliquots from bacterial consortia grown in liquid medium were collected daily, during one week for further proteomic and metagenomic analyses.

Resultados:

The results show a large amount of bacteria in the intestines of termites are able to degrade cellulose, identified in stained by Congo Red plates. After separating and selecting the positive colonies, genomic DNA was extracted; its quality was assessed, and their purified DNA is in the process of preparation for sequencing.

Conclusão:

There is a large amount of bacteria in the intestines of termites that are able to degrade cellulose. We leave now for sequencing these samples.

Apoio Financeiro:

There is a large amount of bacteria in the intestines of termites that are able to degrade cellulose. We leave now for sequencing these samples.

Comitê de Ética:

There was no need for the approval of the ethics of animal use committee.

26.045 Studying the mode of action of the antimicrobial peptide Esculentin 1b (1-18) using liposomes: the role of negatively charged membranes

Moreira-Silva, I., Goncalves, L. C. S. , Arcisio-Miranda, M., Juliano, M. A. , Riske, K. A. , Perez, K. R. ,

Biofísica - Unifesp

Introducao:

Antimicrobial peptides are part of the immune system of flora and fauna, and they have shown a broad spectrum of action against microorganisms. Esculentin 1b is a 46-amino acid peptide, but only the peptide region comprising the first 18 amino acids (GIFSKLAGKKLKNLLISG-NH2) exhibits antimicrobial activity without hemolysis.

Objetivos:

The aim of this work was to study the mode of action of Esculentin 1b (1-18) on liposomes composed of different molar ratios of a neutral (POPC) and a negatively charged (POPG) phospholipid.

Metodos:

For this purpose, several techniques were employed: optical microscopy of giant unilamellar vesicles (GUVs), fluorescence measurements of the leakage of carboxyfluorescein entrapped in vesicles, isothermal titration calorimetry (ITC), electric measurements with planar lipid bilayers, static and dynamic light scattering, zeta potential and circular dichroism (CD).

Resultados:

Esculentin 1b (1-18) induced permeabilization of GUVs and ion passage through planar lipid bilayers. Leakage of carboxyfluorescein occurs predominantly through a very fast process, followed by a much slower content release. Complete leakage was obtained at 0.16 and 0.08 P/L, for POPG and POPC:POPG 1:1 vesicles, respectively. For this P/L range, no peptide-induced vesicle aggregation was detected with light scattering measurements. The ITC study showed an endothermic ΔH for the interaction of the peptide with the vesicles (1.0 and 1.4 kcal/mol of peptide, for POPG and POPC:POPG 1:1, respectively). Analysis of ITC data reveals a direct interaction of Esculentin 1b (1-18) with POPG, where each peptide interacts with 2.5 POPG molecules, leading to a final charge balance of 0.5 negative charge for each peptide positive one. The CD spectra showed that the peptide is in α-helix conformation in the presence of POPC:POPG 1:1 and POPG vesicles.

Conclusão:

We can conclude that the mode of action of Esculentin 1b (1-18) is mainly via pore formation in

POPG-containing membranes, increasing the permeability of negatively charged membranes.

Apoio Financeiro:

We can conclude that the mode of action of Esculentin 1b (1-18) is mainly via pore formation in POPG-containing membranes, increasing the permeability of negatively charged membranes.

Comitê de Ética:

26.046

Influence of both urea and glycerol on the kinetic formation of amyloid-like structures from model proteins

Barbosa, L. N. , Barbosa, L. R. S. ,
Física Geral - IFUSP

Introducao:

The influence of external agents on proteins function and structure is essential to elucidate its unfolding pathways as well as the protein self-assemble properties. Besides, the knowledge of the protein amyloid fibril formation process is important due to the fields that this subjected is related, in particular for the neurodegenerative disorders as Parkinson`s and Alzheimer`s diseases.

Objetivos:

In the present study we evidenced the influence of urea in the amyloid fibril formation of Bovine Serum Albumin (BSA), and lysozyme

Metodos:

In order to get such information,we used spectroscopic techniques like UV-Vis, Static Fluorescence and small angle X-ray scattering (SAXS).

Resultados:

Concerning BSA, the presence of urea (in concentrations < 2 M) was able to induce the formation of amyloid fibrils at 328 K and increasing urea concentration the amount of protein in the amyloid form also increases. Such effect was evidenced to be larger on BSA as compared to Lysozyme, where the fibril formation was shown to be much slower. SAXS results indicate that the earlier stages of protein aggregation are probable dominated by partially unfolded monomer co-existing with dimers. Furthermore, GndHCl, was able to induce the formation of amyloid fibrils in BSA, but in a much smaller content as compared with urea, whereas glycerol was able to decrease the amyloid fibril formation kinetic of BSA even at small concentrations as 5% v/v.

Conclusão:

Taking together, the findings observed in the present study indicate that the hydration shell, where the denaturant agents act, can play an important role in the amyloid aggregation process.

Apoio Financeiro:

Taking together, the findings observed in the present study indicate that the hydration shell, where the denaturant agents act, can play an important role in the amyloid aggregation process.

Comitê de Ética:

26.047 Physical damage in membranes by photosensitization processes

Rosa, R. , Itri, R. ,
Departamento de Física Aplicada - USP

Introducao:

Photodynamic therapy is a promising therapeutic modality in the treatment of diseases that involve abnormal and uncontrolled growth of tissue or infection by micro - organisms and viruses. Its application has been demonstrated in many areas of health. Understanding the mechanisms involved in photo - induced cell death is of paramount importance in order to suggest improved clinical protocols , and damage to membranes are fundamental to understand the processes of cell death.

Objetivos:

In this work, we investigate the response of liposomes , biologically important nanostructures as model membranes , to the action of photosensitizers under irradiation . We are particularly interested in investigating the biophysical changes induced in the membrane by photo-oxidation , such as area increase, changes in fluidity and membrane permeability , which culminate in disruption and / or rupture of the membrane .

Metodos:

The experimental techniques used are small angle X-Ray scattering (SAXS) that provides information about the structure of the lipid bilayer and electron paramagnetic resonance (EPR), which allow us inferring about membrane fluidity .

Resultados:

Conclusão:

Apoio Financeiro:

Comitê de Ética:

No animal experimentation were performed

26.048 The role of entropic mechanisms in the formation of complexes between lamellar phase of non-cationic lipids and DNA.

Gerbelli, B. B. , Silva, E. R. , Oliveira, E. A. ,
FEP - IFUSP

Introducao:

Lamellar phases can be used for the encapsulation of macromolecules such as DNA and proteins, since they have a well-defined aqueous layer which can be controlled in the preparation of lamellar phase (by changing, for example, the quantity of water in the system or composition of the membrane).

Objetivos:

In this work the aim was to understand the role of the membrane in the formation of such complexes, from the determination of structural parameters of complex compounds of membrane and DNA fragments of 150 bp. The membrane used is made of soya lecithin and commercial co-surfactant Simulsol. We studied two compositions of membrane containing 30/70 and 10/90 of lecithin and Simulsol mass, respectively.

Metodos:

For this study we used optical microscopy (polarized and fluorescent light) and small angle x-ray scattering techniques.

Resultados:

We observed a large polymorphism on the mesophases due to the confinement of the DNA fragments. In general there were phase transitions, where the host phase became a fluid lamellar phase (result obtained by WAXS), but the DNA fragments having a hexagonal structure à centered rectangularà nematic according to a decreasing water quantity.

Conclusão:

Apoio Financeiro:

Comitê de Ética:

26.049 Allosteric regulation: from Lumry and Eyring to the novel concept of Productive Induced Metastability (PIM).

Montes de Oca JM, Rodriguez-Fris JA, Appignanesi GA,
Appignanesi GA - INQUISUR-UNS-CONICET
Departamento de Química, Universidad Nacional del Sur - INQUISUR-UNS-CONICET

Introducao:

Already in the middle of the last century, Lumry R., Eyring, Biltonen and others reported that allosteric signaling is an activated process that requires the presence of structural or mobile defects to change the free energy of the protein and promote the ligated state. In particular, Lumry refers to the distortions of the H-bonds and local structures as the driving forces of `fluctuating enzymes`[1,2]. In this study, we elucidate the allosteric binding modality by unraveling a local structural motif that promotes association with the ligand. We specifically show that allosteric modulators promote a local metastable state that is stabilized upon association. The induced conformational change generates a local enrichment of the protein in the so-called dehydrons[3], which are solvent exposed backbone hydrogen bonds. These structural deficiencies that are inherently sticky are not present in the Apo form and constitute a local metastable state that promotes the association with the ligand. In other words, we find that the allosterically activated conformation of the enzyme could not prevail in the ensemble of conformations of the Apo form of the enzyme because of the inherent instability of the packing defects (the Lumry `mobile defects`). Therefore the allosteric ligand acts protecting these exposed interaction from water, thus turning the active state into the most stable configuration. This productive induced metastability[4] (PIM) is likely to translate into a general molecular design concept. 1. Lumry R & Eyring H (1954) Conformational changes in proteins, J. Phys. Chem. 58, 110-120. 2. Lumry, R., The new paradigm for protein research, in Gregory R B (ed.), Protein-solvent interactions, Marcel Dekker, Inc. New York (1995). 3. Fernández A, Transformative Concepts for Drug Design: Target Wrapping (Springer, Heidelberg, 2010). 4. Montes de Oca J., Rodriguez-Fris A., Appignanesi G. and Fernández A. Productive induced metastability in allosteric modulation of kinase function, FEBS J ,10.1111/febs.12844 (2014).

Objetivos:

The main goal of this work is to elucidate an unified mechanism of allosteric regulation, focused on the structural requirement of a metastable intermediate state, functioning as a hinge between active and inactive conformations of the enzyme. Therefore in this study we were looking for evidences of

how the allosteric ligand solves this dilemma of stability, allowing to one of the states prevail over the other.

Metodos:

All de data of this work was collected from protein coordinates deposited in the Protein Data Bank (PDB) and processed using an ad hock in-home developed software, and also in some cases we perform molecular dynamics simulation using The AMBER Molecular Dynamics Package.

Resultados:

in summary in this work we found that in every one of the enzymatic systems studied, the allosteric transition takes place from a relatively stable structure (with few structural defects -dehydrons- exposed to the solvent) passing through a more defective one, that is finally corrected by means of the association with the ligand.

Conclusão:

This work provides a structural interpretation of allosteric modulation of kinase activity based on induced folding. We demonstrate that the induced folded state of the target kinase is actually metastable, more specifically, it is a locally defective structure in which the backbone is incompletely shielded from water attack. This metastable state is different from the more stable apo form. However, the metastable state detected within the protein–ligand complex behaves as an affinity enhancer because the ligand stabilizes the induced defective fold in a productive way by adding protection to the protein backbone. In contrast with previous studies that highlight the importance of wrapping interactions in protein–ligand associations, we show that a partial induced destabilization of the pro tein structure is actually required for the ligand to become an effective binder. This finding heralds a novel mode of structural adaptation generically termed productive induced metastability (PIM), be it the induced–degradation–stabilization binding mode. The result may appear counterintuitive because it makes degradation of the apo structure compulsory. However, the paradox is removed once we take into account that a metastable state can be inherently sticky because of the added stabilization brought about by the association with the ligand.

Apoio Financeiro:

This work provides a structural interpretation of allosteric modulation of kinase activity based on induced folding. We demonstrate that the induced folded state of the target kinase is actually metastable, more specifically, it is a locally defective structure in which the backbone is incompletely shielded from water attack. This metastable state is different from the more stable apo form. However, the metastable state detected within the protein–ligand complex behaves as an affinity enhancer because the ligand stabilizes the induced defective fold in a productive way by adding protection to the protein backbone. In contrast with previous studies that highlight the

importance of wrapping interactions in protein–ligand associations, we show that a partial induced destabilization of the pro tein structure is actually required for the ligand to become an effective binder. This finding heralds a novel mode of structural adaptation generically termed productive induced metastability (PIM), be it the induced–degradation–stabilization binding mode. The result may appear counterintuitive because it makes degradation of the apo structure compulsory. However, the paradox is removed once we take into account that a metastable state can be inherently sticky because of the added stabilization brought about by the association with the ligand.

Comitê de Ética:

Does not apply: the entire investigation is based on the thorough analysis of crystallized protein structures deposited in the Protein Data Bank (PDB).

26.050 Biophysical membranes proprieties under oxidative stress and the action of the pore formation toxines

Bonome, B. A. , Junqueira, H. C. , Itri, R. , Gomide, A. B. ,
Fisica Aplicada - USP
Fisica Aplicada - Unianchieta
bioquimica - USP

Introducao:

Experimental evidence shows that the mechanism of pore formation by sticholysins I (StI), actinoporins from the sea anemone Stichodactyla helianthus, is a multistep process involving binding of the water-soluble monomer to the membrane and subsequent oligomerization on the membrane surface leading to the formation of a functional pore. The main purpose of this work was to investigate comparatively the permeabilizing activity of StI on giant unilamellar vesicles (GUVs) of different lipid composition as model membranes. GUVs were composed of dioleoylphosphatidylcholine (DOPC), POPC-oxidized, egg sphingomyelin (SM), and cholesterol (Chol).

Objetivos:

On this project of Scientific Initiation we investigate the action of pore-forming toxins in terms of activity in membranes containing photo-oxidized areas (composed of oxidized DOPC, SM and cholesterol) and evaluate changes in biophysical properties of the membrane caused by oxidative stress and response the action of the toxin.

Metodos:

The GUVs were prepared by the electroformation method. Briefly, 20 μL of lipid in chloroform solution [2 mg/mL] was spread on the surfaces of two conductive glasses coated with fluor tin oxide, which were then placed with their conductive sides facing each other and separated by a 2 mm thick Teflon frame. This electro-swelling chamber was filled with 0.2 M sucrose solution, and it was connected to an alternating current of 2 V with a 10 Hz frequency for 2 h. The vesicle suspension was removed from the chamber and diluted into a 0.2 M glucose solution containing the desired amount of StI. The osmolarity of the sucrose and glucose solutions was measured with a Gonotec 030 cryoscopic osmometer (Osmomat, Berlin, Germany) before use, and they were carefully matched to avoid osmotic pressure effects. The vesicles were then immediately placed on the observation chamber. Due to the differences in density between sucrose and glucose solutions, the vesicles were stabilized by gravity at the bottom of the observation chamber. Vesicles were observed by using an inverted microscope Axiovert 200 (Carl Zeiss; Jena, Germany) in the phase contrast mode (Plan Neo-Fluar 63× Ph2 objective (NA 0.75) and A-plan 10 × Ph1 (NA 0.25))

and fluorescence mode (103W Hg lamp, HXP 120, Kubler, Carl Zeiss, Jena, Germany).

Resultados:

Conclusão:

Apoio Financeiro:

Comitê de Ética:

26.051 Interaction of the Alkylphospholipids with model membranes at the room and physiological temperature

Ferlini, J. H. A. , Itri, R. , Gomide, A. B. ,
Biofísica - USP
Biofísica - Unianchieta

Introducao:

The interaction of alkylphospholipids (ALPs) with membranes can lead to cell death, and the mechanism of action differs from classical chemotherapeutics that act on DNA. The mechanism proposed relates lipid domain as the gateway of ALPs, favoring cell internalization for subsequent inhibition of phosphatidylcholine synthesis at different levels. Lipid domains exhibit a coexistence of liquid-ordered (Lo) and liquid-disordered (Ld) phases of the order of hundreds of nanometers in living cells. They also can be observed as microscopic domains in isolated membranes systems. Indeed, molecules that affect the separation of lipid phase can have important effects on physiological responses, such as promoting (dis) association of important proteins in cellular signaling processes. Recently, alkylphospholipid 10 - (octyloxy) decyl-2-(trimethylammonium) ethyl phosphate, ODPC, showed cytotoxic in some cancer cell lines comparable to other ALPs effects, but with a lower haemolytic activity and cytotoxicity.

Objetivos:

Investigate the action of ALPs, in particular ODPC in model membranes of different lipid compositions, in order to understand how the presence of Ld-Lo phase (lipid domains) influences the mechanism of action of this molecule varying temperature and composition of GUV`s using the optical microscopy technique of phase contrast and fluorescence.

Metodos:

The GUVs were prepared by the electroformation method. Briefly, 20 μL of lipid solution was spread on the surfaces of two conductive glasses coated with fluor tin oxide, which were then placed with their conductive sides facing each other and separated by a 2 mm thick Teflon frame. This electro-swelling chamber wasfilled with 0.2 M sucrose solution, and it was connected to an alternating current of 2 V with a 10 Hz frequency for 2 h. The vesicle suspension was removed from the chamber and diluted into a 0.2 M glucose solution containing the desired amount of StI. The osmolarity of the sucrose and glucose solutions was measured with a Gonotec 030 cryoscopic osmometer (Osmomat, Berlin, Germany) before use, and they were carefully matched to avoid osmotic pressure effects. The vesicles were then immediately placed on the observation chamber. Due to the differences in density between sucrose and glucose solutions, the vesicles were

stabilized by gravity at the bottom of the observation chamber. Vesicles were observed by using an inverted microscope Axiovert 200 (Carl Zeiss; Jena, Germany) in the phase contrast mode (Plan Neo-Fluar 63× Ph2 objective (NA 0.75) and A-plan 10 × Ph1 (NA 0.25)) andfluorescence mode (103W Hg lamp, HXP 120, Kubler, Carl Zeiss, Jena, Germany).

Resultados:

Conclusão:

Apoio Financeiro:

Comitê de Ética:

26.052 Indirect measurements of flexibility of lipid membranes: the role of a cosurfactant

Rubim, R. L. , Bougis, K. , Gerbelli, B. B. , Oliveira, C. L. P. , Navailles, L. , Nallet, F. , Oliveira, E. A. ,

Física Experimental - Universidade de São Paulo

Matière Molle: Structure et Dynamique - Université de Bordeaux

Introducao:

Lipid membranes are present in many structures of living cells, such as the plasma membrane, the Golgi complex and other organelles. Changes in the composition of the membranes can modify their mechanical properties and structure, like the rigidity, which plays a key role in their three dimensional spatial organization.

Objetivos:

In this work, we investigate the flexibility of lipid membranes and the effect of incorporation of ethoxylated fatty acids on this parameter.

Metodos:

The lamellar system is composed by a mixture of soy lecithin and a commercial molecule known as Simulsol in different proportions. This system is then inserted in a polymer solution, in order to apply an osmotic pressure to the bilayers, and the intensity of the pressure (P) is controlled by the concentration of polyvinylpyrrolidone (PVP) in solution. Small Angle X-ray Scattering (SAXS) experiments are carried out on the lamellar phases in equilibrium with the polymer solution, allowing us to obtain P as function of the lamellar period (D) of the system.

Resultados:

From this curve it is possible to obtain the behaviour of the compression modulus (B) of the lamellar system, and for a given osmotic pressure, we observe that the lamellar period is shifted to higher values upon incorporation of fatty acids, indicating that repulsive forces are enhanced. With fittings of SAXS curves we may obtain structural information of the membranes. We may than observe the behavior of Caillé parameter, which is related to the bending rigidity (κ) of the bilayer and B. Combining the information obtained from both methods it is possible to characterize the elastic parameters of the membrane, such as its flexibility.

Conclusão:

We observed that these parameters are sensible to the incorporation of fatty acids, and these findings reveal the great potential of this methodology for studies of membranes of biological interest.

Apoio Financeiro:

We observed that these parameters are sensible to the incorporation of fatty acids, and these findings reveal the great potential of this methodology for studies of membranes of biological interest.

Comitê de Ética:

In this work we did not used experiments neither in humans or animals.

26.053 Structural characterization of membrane-induced GAPDH prefibrillar species and its implications in Parkinson disease

Torres-bugeau, C. M. , Avila, C. L. , Lizárraga, F. G. , Vera, C. C. , Barbosa, L. R. S. , Itri, R. , Chehin, R. N. ,
Química Biológica - INSIBIO, CONICET-UNT
Física Aplicada - IFUSP

Introducao:

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multifunctional enzyme that has been associated to neurodegenerative diseases. GAPDH colocalizes with α -synuclein in amyloid aggregates in post-mortem tissue of patients with sporadic Parkinson disease and promotes the formation of Lewy body-like inclusions in cell culture. In a previous work, we showed that in vitro heparin can trigger GAPDH amyloid aggregation and that prefibrillar species present during the aggregation process can modify the α-synuclein aggregation kinetic. The heparin-induced GAPDH prefibrillar species are capable to abolish the toxicity of α-synuclein species in cell cultures. The GAPDH prefibrillar species responsible for this activity were structurally characterized as protofibrils. We have also demonstrated that acidic membranes can trigger the GAPDH amyloid aggregation. However, structural and functional features of membrane-induced GAPDH prefibrillar species were not studied, and thus, its effectiveness on α-synuclein aggregation kinetic is unknown.

Objetivos:

The aim of the present work is to perform a structural and functional characterization of membrane-induced GAPDH prefibrillar species, and to compare them to heparin-induced GAPDH prefibrillar species.

Metodos:

Structural characterization of GAPDH prefibrillar species was performed using Fourier transform (FT-IR) together with Small Angle X-Ray Scattering (SAXS). Biocomputational techniques were used to obtain a model of GAPDH-membrane interaction.

Resultados:

In this work we demonstrated that membrane-induced and heparin-induced GAPDH prefibrillar species are structurally different. This suggest that aggregation evolves through different pathways. Aggregation was seen to be dependent on membrane composition, curvature and fluidity. Using biocomputational techniques we modeled GAPDH:membrane interaction. We observed that both

hydrophobic and electrostatic forces drive the aggregation process induced by membranes . On the contrary, heparin induced aggregation was driven mainly by electrostatic forces. These differences arising during the first steps of aggregation, could explain the differences in the aggregation pathway.

Conclusão:

Since neuronal membrane composition changes during aging, studying the impact of these changes on protein aggregation becomes mandatory. The data reported herein sheds light into the impact of membrane composition and physical state on protein aggregation. It also helps understand some mechanisms involved in the first steps of this process. The model provided herein might prove useful in the development of therapeutic strategies against synucleinopathies like Parkinson’s disease.

Apoio Financeiro:

Since neuronal membrane composition changes during aging, studying the impact of these changes on protein aggregation becomes mandatory. The data reported herein sheds light into the impact of membrane composition and physical state on protein aggregation. It also helps understand some mechanisms involved in the first steps of this process. The model provided herein might prove useful in the development of therapeutic strategies against synucleinopathies like Parkinson’s disease.

Comitê de Ética:

The work does not involve animal or human experiments.

26.054 Annotation and Modeling of a diacetylchitobiose-6-phosphate hydrolase from giant snail Achatina fulica gastric acid metagenome

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Introducao:

Lignocellulosic biomass has become one of the main targets on the search for second generation fuels, due to it’s potential to become feedstock of bioethanol production. This kind of biomass is largely available in Brazil, mainly as residue from first generation ethanol production. So, studying organisms and/or enzymes with potentiality to degrade lignocellulosic biomas and extract fermentable sugars from large polymers, has become a reliable research target and has been presented promising results.

Objetivos:

On this work, we analyze and model one of the enzymes obtained from the gastric acid metagenome of Achatina fulica using bioinformatics and molecular modelling tools.

Metodos:

For the annotation part we used subsequent BLASTp searches using the atarget sequence (named GH4p), and several databases in order to achieve vital informations regarding it`s names, functions and metabolic pathways in which the enzyme is present, aswell as a enzyme structure to be used as template for the Comparative Modeling. Thereafter we used PSIPRED for secondary structure prediction, SignalP for signal peptide sequence presence prediction, and PROMALS and ClustalW in order to analyse the alaginments obtained.In order to build a model, we used the template obtained previously and software MODELLER. Building initially a hundred candidates, and validating them using DOPEscore and GA341 (both score from MODELLER), Ramachandran plots (PROCHECK) and RMSD (from structural alignments between template and candidates using PyMol).

Resultados:

We could successfully identificate the target (querry) sequence as a diacetylchitobiose-6-phosphate hydrolase enzyme, member of Glycosil Hydrolase 4 family, participant of the chitin degradation pathway on Citrobacter sp and could obtain a good amount of information regarding conserved domains present on the enzyme.As template, we could found a 6-phospho-beta-glucosidase from Geobacillus stearothermophilus (PDBID 1S6Y), which alignment with the querry displayed 51% identity and 96% cover. Following what is proposed in the literature, we built a homotetrameric structure using 1S6y`s monomer`s and the arrangement of another 6-phospho-beta-glucosidase,

from Thermotoga maritma (PDBID 1UP7). We have chosen model candidate 56, for it displayed 91% amino acid residues in most favored and 0.8% in disallowed zones of the Ramachandran plot, DOPEscore of -213679.21875 and RMSD model x template of 1.452 angström.

Conclusão:

With the high quality homotetrameric model built, we can proceed to further studies in Molecular Dynamics in order to check the homotetrameric conformation stability given the conditions observed on the literature. As well as performing studies of molecular docking comparing the activity with diacetylchitobiose-6-phosphate and different 6-phospho-beta-D-glucosides.

Apoio Financeiro:

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Comitê de Ética:

This work is exclusively on the computational biology area, therefore was realized using only in silico aproaches and experiments, and did not required any animal subjects.

26.055 INFLAMMATORY AND ADIPOGENIC POTENTIAL OF ADIPOSE-DERIVED STROMAL/STEM CELLS FROM OBESE SUBJECTS

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Introducao:

Adipose tissue has gained importance in recent years, being now considered an endocrine regulator center. New knowledge about the physiology and sub??populations of cells are revolutionizing concepts for the understanding of pathophysiological processes, particularly obesity. The aim of this study is to investigate cellular mechanisms involved in mass increase of three depots of white adipose tissue - subcutaneous (SC), visceral (VC) and preperitoneal (PP) - all from morbid obese patients. Adipogenic and inflammatory potential at tissue and cellular levels were analysed.

Objetivos:

Evaluate and compare physiological and morphological traits of adipose tissue and functional activity of this tissue stem cell/stromal in morbidly obese individuals and between different fat depots of obese individuals in order to investigate the contribution of these stromal stem cells for tissue physiology and to the genesis of obesity.

Metodos:

Fragments of SC, VC and PP adipose tissues from non-diabetic morbid obese female (BMI = 47.5± 5.6Kg/m2) were obtained under approval by the Research Ethics Committee of the University Hospital Clementino Fraga Filho (Protocolo 076/10). After embedded in paraffin, 3 histological sections of 5mm in thickness with 100mm with interval between them were stained by hematoxilin and eosin to determine the area occupied by adipocytes in each tissue depot. Quantification of 900 adipocytes among 15 high-powered images of randomly selected areas of each paraffin section (200x) revealed that the SC adipocytes occupies the highest area (kruskal-wallis test, $p<0,0001$). To evaluate the adipogenic potential of Adipose-Derived Stromal Cells (ASC) of the three adipose depots, cells were cultivated in adipogenic medium for 3 weeks (n=3). Quantification of cytoplasmic lipid accumulation was determined by the absorbance of Oil Red O captured in droplets, revealing that ASC from VC adipose tissue has the lowest adipogenic potential (ANOVA test – $p<0,001$).

Resultados:

The cytokine secretion profile of ASC was evaluated under control medium or lipopolysaccharide

(LPS) 0.5 mg/ml incubation for 24 hours, through of the detection of cytokines secreted in the supernatant by multiplex immunoassay technique. After LPS stimulus, ASC from SC adipose tissue secreted five times more IL-6 (Interleukin-6) and 23 times more MCP-1 (monocyte chemotactic protein-1) than control (non-stimulated cells).

Conclusão:

Differences in the area occupied by adipocytes and the potential to generate them by ASC from three adipose depots reveal distinct contributions for adipose mass increase in morbid obese patients. Moreover, ASC may have a role in the inflammatory scenario, by secreting pro-inflammatory cytokines.

Apoio Financeiro:

Differences in the area occupied by adipocytes and the potential to generate them by ASC from three adipose depots reveal distinct contributions for adipose mass increase in morbid obese patients. Moreover, ASC may have a role in the inflammatory scenario, by secreting pro-inflammatory cytokines.

Comitê de Ética:

The present study aims to evaluate cellular aspects of white adipose tissue, focusing on the composition of the SVF and functional characteristics of stem cell / stromal derived from subcutaneous white adipose tissue of obese individuals móbidos as well as deposits of pre-peritoneal adipose tissue and visceral morbidly obese, for a better understanding of obesity and development of new therapeutic approaches to prevent or treat this disease and its complications.

26.056 RESCUING CFTR IN TMD1 AND 2 MUTATIONS BY SMALL MOLECULE CORRECTORS

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Introducao:

Cystic fibrosis (CF) is a recessive autosomal disorder caused by mutation in CFTR (cystic fibrosis transmembrane conductance regulator). The main clinical manifestations are lung inflammation and fibrosis, pancreatic insufficiency and male infertility. So far, there is no therapy to overcome the basic defect in CFTR mutated protein. Correction of the processing of F508del-CFTR by small molecules is a major goal in the development of new therapies for CF. Recently, a clinical trial in patients carrying F508del-CFTR showed some promise effects with the corrector VX-809.

Objetivos:

The aim of this study was to determine whether correctors can rescue TMD1 and TMD2 mutations and understand the mechanism of action of the best corrector.

Metodos:

Cos-7 cells were transfected with G85E, E92K, R1070P, L1077P and M1101K mutations and treated with increasing doses of correctors for 16h. Also, cells were treated with proteasome, aggresome and lysosome inhibitors to understand for which pathway these mutations are degraded.

Resultados:

G85E, E92K, L1077P, M1101K are degraded by proteasome, mainly, and aggresome. CFTR was rescued in all these mutation with CFFT-002, CFFT-003, C3, C4 and C18 treatment. The combination of C18 and C4 presented the best effect on TMD1 and TMD2 mutations and was able to rescue CFTR on the surface. G85E, E92K, L1077P, M1101K stable transfected in HEK-293 cells showed quickly degradation of CFTR by inhibition of protein synthesis. However, the treatment of these mutations with C18+C4 presented increase of the half-life. The immunopreciptation assay showed that treatment with C18+C4 reduce the binding of CFTR with Hsp40 and increasing with Hsp90.

Conclusão:

Small molecule correctors presented beneficial effects in different mutations in TMD1 and TMD2, rescuing CFTR. Furthermore, the association of C18 and C4 showed the best result, reducing the degradation and rescuing CFTR on the surface. These correctors may be a feasible alternative for

future clinical application.

Apoio Financeiro:

Small molecule correctors presented beneficial effects in different mutations in TMD1 and TMD2, rescuing CFTR. Furthermore, the association of C18 and C4 showed the best result, reducing the degradation and rescuing CFTR on the surface. These correctors may be a feasible alternative for future clinical application.

Comitê de Ética:

26.057 Computational evaluation of the first oligomers formed in the auto-aggregation process of 33-mer peptide.

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Departamento de Física - IFISUR
Departamento de Química - INQUISUR

Introducao:

Coeliac disease is an autoimmune disorder that produces lesions in the small intestine. It is triggered by the ingestion of gluten, a protein complex found in wheat, rye and barley. It has been published that the 33-mer peptide, a fragment found after partial degradation of the protein α2-gliadin, is highly resistant to digestion and shows three T-cell epitopes previously identified in patients. It has been proposed that this peptide, LQLQPF(PQPQLPY)3PQPQPF, initiates the inflammation process that leads to the damage of the epithelial cells of the small intestine. However, the molecular bases of the process are not yet fully understood. We are particularly interested in studying its ability of auto-aggregation into more complex structures, since different superstructures have been observed in vitro. Molecular modelling methods represent essential tools to undertake the study of biological systems, providing a better understanding of both their structure and functionality. In this work we applied molecular dynamics simulations and electrostatic calculations to analyse the initial steps of auto-aggregation of 33-mer peptide. As a starting point we used GROMACS package to carry out a molecular dynamics simulation of the monomer, in water for 50 ns, to assess its behaviour. In order to evaluate the aggregation process we first performed an electrostatic energy calculation, using APBS, on different configurations that might lead to the formation of a dimer and a trimer. For both oligomers, we calculated the electrostatic energy of interaction and chose the systems with the most negative energy to perform molecular dynamics simulations (again of 50 ns each). We found that the formation of dimers and trimers is statistically possible, and its structural characteristics are consistent with experimental data available.

Objetivos:

Metodos:

Resultados:

Conclusão:

Apoio Financeiro:

Comitê de Ética:

26.058 Analysis of the five-fold symmetry axis of icosahedral virus capsids

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Unidad de Biofísica - EHU-UPV

Introducao:

Viral capsids play a crucial role in the process of viral infection, nevertheless, very little is known of this process. Better understanding of the structural properties, which are related with the virus functionality, it is important to comprehend this mechanism. In this work we study the structure of different viral capsids and compare their tridimensional configuration as well as their aminoacids composition and properties. In particular we focus our study in Triatoma Virus and compare it with other viruses from the same viral family, dicistroviridae, and viruses with similar structure. The reason for studing TrV is because it infects several species of triatomine insects, which are the vectors for human trypanosiomiasis, commonly known as Chagas Disease. Because of this TrV is proposed as a biological control against this kind of insects. For this reason it is important to better understand its structure. To this end, we use Molecular Dynamics to do computational simulations of the different systems. These simulations are done with the program Gromacs. Since the number of particles that form the viral capsids is very large, we focus our attention to the pentameric subunit form around the five-fold symmetry axis. Finally, we carry out a comparison analysis of all the structures.

Objetivos:

Metodos:

Resultados:

Conclusão:

Apoio Financeiro:

Comitê de Ética:

26.059 On the self-assembly properties of the Growth Hormone Releasing Hexapeptide

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Department of Pharmaceutical Technology - CGEB
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Department of Molecular Nanoscience and Organic Materials - ICMAB
Department of Biochemical and Pharmaceutical Sciences - SPC-USP

Introducao:

Growth hormone releasing peptide, GHRP-6, is a synthetic hexapeptide (His-(D-Trp)-Ala-Trp-(D-Phe)-Lys-NH2,). It belongs to a class of synthetic growth hormone secretagogues, which stimulate growth hormone secretion from somatotrophs in several species including humans. Even though its use for the treatment of several human diseases is being explored, there is also an increased interest in its self-assembly properties and its application in the field of nanotechnology.

Objetivos:

In this study, we shed light into the self-assembling properties of GHRP-6 using a combination of biophysical tools

Metodos:

To study the process of self-assembly we use small angle X-ray scattering, transmission electronic microscopy (TEM), and molecular dynamics simulation.

Resultados:

The combined results demonstrated that GHRP-6 at 20 mg/mL in phosphate buffer self-assembles into very long nanotubes, with inner and outer cross-sections of 7(1) and 13(1) nm. At concentrations > 30 mg/ml, these nanotubes form bundles with hexagonal arrangement, with center-to-center distance of circa 15 nm. Within the nanotube, the peptides self-assemble in a partially interdigitated structure with the amino termini at the peptide water interface. While the carboxi termini remains buried, the long side chain of Lys-6 stretch out of the hydrophobic core to the cylinder surface.

Conclusão:

Interestingly, such arrangement is quite similar to that observed on cationic lipidic vesicles. Nevertheless, the cross-section dimension of GHRP-6 nano-assemble is rather small as compared

to others surfactant-like peptides, a fact that could be exploited in the design of new nanomaterials.

Apoio Financeiro:

Interestingly, such arrangement is quite similar to that observed on cationic lipidic vesicles. Nevertheless, the cross-section dimension of GHRP-6 nano-assemble is rather small as compared to others surfactant-like peptides, a fact that could be exploited in the design of new nanomaterials.

Comitê de Ética:

The experiment does not include experimentation with humans or animals.

26.060 ANALYSIS OF LIPID MEMBRANES ERYTHROCYTE PACKED RED BLOOD CELLS AFTER IRRADIATION WITH GAMMA RAYS

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Introducao:

The use of irradiation of blood products using γ-irradiation, dormant T cells, which cells are responsible for graft versus host disease, thus preventing the development of pathology. Despite the importance of irradiation as a preventive measure, previous studies in the literature have shown that irradiation of red blood cell induces an increase in plasma K+ concentration (Ann Hematol. 87:113,2008). Thus, it is interesting to understand the structural changes induced by irradiation, as well as ensure the quality of blood products.

Objetivos:

To investigate possible changes in the human erythrocyte membrane after exposure to irradiation. The lipid analyzes were performed on different days after irradiation and 3, 5, 7, 9,11,14, 21 and 28 days

Metodos:

Blood bags(control and irradiated with 25 Gy) assigned by Hemominas Divinópolis were used. The blood bags were stored at 4 ° C in the Laboratory of Cellular Biochemistry UFSJ for 28 days. The lipid extract was obtained according to the methods described by Rose (J Lipid Res, 6:428,1965). and Vokurkova (Life Sci,77:1452,2005) .The total cholesterol content was determined according to the method of Higgins (Oxford.1:104,1987),the quantification of phospholipid by the method of Chen (Anal.Chem.28:1756,1956), the lipid peroxidation was determined by the concentration of thiobarbituric acid by the method described by Buege (Methods Enzymol,52:302,1978). The results were analyzed using the GraphPadPrism program 5. Using analysis of variance (ANOVA) followed by Tukey`s multiple comparison test and compare the two groups, Student`s t test was performed. Significance was set at p value <0.05.

Resultados:

On day 3rd of the irradiated samples compared with the control occurred a significant decrease of 50.0% of total phospholipids (0,5 ± 0,06nmol/µL). For samples control, an initial decrease in the total content was observed, about 40.1% on the by the 5th day and 46.5% on the by the 9th day. In the irradiated samples, the phospholipid content was reduced by approximately 35% on day 7 of storage and increased 60.9% on the 11th day(0,6 ± 0,15nmol/µL). After day 14, the phospholipid content decreased and maintained at the same level until day 28 (n=4). On the 3rd day post-irradiation, a 36.0% decrease was observed in cholesterol levels in the irradiated samples

compared with the non-irradiated. On days 5 and 9, the total cholesterol of irradiated samples increase 43.0 (0,2 ± 0,01 µg/µL) and 77.0%(0,17 ± 0,03 µg/µL)respectively (n=4). The significant decrease in the total cholesterol was observed in the non-irradiated samples, using day 3 as a control. The increase in levels of thiobarbituric acid reactive substances were significant on day 5th post-irradiation (n=3)

Conclusão:

The effects of gamma irradiation on red blood cells showed a possible reorganization of the lipid content of membrane. The use of gamma irradiation for preventing TAGVHD is require. Because of the changes in the lipid composition of erythrocyte membrane observed in this study, is important to evaluate the shelf life of blood bags currently used in the clinic, after irradiation.

Apoio Financeiro:

The effects of gamma irradiation on red blood cells showed a possible reorganization of the lipid content of membrane. The use of gamma irradiation for preventing TAGVHD is require. Because of the changes in the lipid composition of erythrocyte membrane observed in this study, is important to evaluate the shelf life of blood bags currently used in the clinic, after irradiation.

Comitê de Ética:

The study showed no need for review by committee ethics (parecer emitido pelo CEP Hemominas em 2009).