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NETIC RESONANCE TECHNIQUE TO QUANTIFY THE PROPORTION OF PHOSPOLIPIDS IN LIQUID DISORDERED PHASE IN AN EXOGENOUS PULMONARY SURFACTANT

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Pulmonary surfactant (PS) is a mixture of phospholipids (PL), neutral lipids (mainly Cho) and at least four specific proteins. The particular lipid composition of surfactant induces segregation of liquid-ordered phase (Lo) and liquid-disordered phase (Ld) in surfactant membranes and films at physiological temperatures. This coexistence of phases would be crucial for the surfactant activity, and the role of Cho in this organization has been extensively researched. Currently the proportion of phases is determined by qualitative methods. Although the role of the proportion of these phases in the surfactant function is unknown, it would be of great relevance elucidate it, since the essential proteins of the PS are in the Ld phase.

Objective: To design and standardize an electronic spin resonance (ESR) spectroscopic technique that allows the quantification of the proportion of Lo/Ld phases present in an exogenous pulmonary surfactant (EPS).

Methods: EPS with or without extra Cho added was labeled with 5DE and TEMPO for its study by ESR. The original technique developed by McConnell (1972) was adapted to quantify Ld proportion in this EPS. The order parameter of the EPS for each sample in the different experimental conditions was also evaluated. PL were determined by Stewart method (1980) and Cho by enzymatic method.

Results: We found that at 50°C all PL of EPS were in Ld phase. At this temperature, the TEMPO partition coefficient obtained for this EPS system was 0.81±0.03. The order changes in EPS membranes did not affect the TEMPO partition coefficient, nor did the Cho concentration. The results obtained with the different batches of EPS showed no significant difference in the phase proportion values. For this EPS, the reference value of the Ld/Lo ratio, calculated at 25°C is 0.30±0.02.

The relevance of this technique is that allows the quantification of the proportion of Ld/Lo phases evaluating if changes in the lateral structure affects the EPS physiological properties.

Keywords: exogenous pulmonary surfactant, Ld/Lo phases, ESR

(1275) **HEMOLYSIS AND ANTIHEMOLYSIS INDUCED BY ARGININE-BASED SURFACTANTS**

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Two novel arginine-based surfactants, Bz-Arg-NHC_{10} and Bz-Arg-NHC_{12}, were characterized in terms of surface properties and interaction with human red blood cells (HRBC) membranes. *CMC* values, Γ_{max} (maximum surfactant adsorption at the air/liquid interface) and A_{min} (area per molecule) revealed better surface properties for Bz-Arg-NHC_{12}. The observation of cylindrical worm-like aggregates of Bz-Arg-NHC_n through atomic force microscopy (AFM) supported the predictions based on the surfactant packing parameter value (SPP). Erythrocyte membrane solubilization was induced by surfactant aggregates, since cell lysis was only evidenced at surfactant concentrations above *CMC*. Changes in HRBC shape observed at different surfactant concentrations allowed to conclude that a slow mechanism based on the insertion of surfactant monomers into the HRBC membrane, followed by shedding of microvesicles is responsible for the hemolysis produced by both surfactants at the lower concentrations tested. On the other hand, the extraction of membrane lipids upon collisions between HRBC and surfactant aggregates competes and prevents the microvesicles release for the higher concentrations assayed.

Moreover, we study the interaction of Bz-Arg-NHC12 with sheep red blood cells (SRBC) and HRBC due to their different membrane protein/lipid composition. SRBC is a little more resistant than HRBC to the hemolytic effect of surfactant, but in both cases, the micellar form of the surfactant is the entity responsible of the hemolytic effect. As HC_{50}, cAH_{max} value was higher than the CMC value. Thus, a biphasic behavior was observed for the surfactant studied, showing a wide range of protective concentrations when HRBC were tested, while for SRBC, the degree of protection of Bz-Arg-NHC_{12} was about 50% lower than for HBRC. However, only for SRBC treated with Bz-Arg-NHC_{12} a remarkable volume expansion was evidence, although no correlation with the antihemolytic potency was found.

Key words: Arginine based surfactants; hemolysis; antihemolysis; lipid composition; microvesicles

(390) **INTERACTION OF AMPHOTERICIN B WITH A SYNTHETIC GLYCOLIPID IN LANGMUIR MONOLAYERS**

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Amphotericin B (AmB) is an amphipathic polyene antibiotic used to treat systemic fungal infections. The therapeutic action as well as the toxic side effects of AmB depends directly on the molecular organization of the drug. AmB posses very low solubility in aqueous media and new alternatives for its administration are needed. An interesting strategy is the use of surfactants to transport hydrophobic drugs. In previous work we have synthesized a glycolipid formed by β-cyclodextrin and an alkyl chain (βCD_{anf}). The surface behavior at the air-water interface of βCD_{anf}, alone and in the presence of other amphiphiles, is well known.[1]

As this glycolipid could offer an alternative to transport AmB our aim was to get inside about the interactions established among them. For this purpose, Langmuir monolayers of films formed by AmB and AmB/βCD_{anf} were studied at the air-water interface and their topography accessed by Brewster angle microscopy (BAM). Also spectroscopic techniques such as NMR, circular dichroism and FT-IR were performed to elucidate the interactions. Our study indicates that strong interactions take place among AmB/βCD_{anf} mainly through the sugar moieties. Also, the organization acquired by AmB at the interface varies considerable due the presence of βCD_{anf}. The presence of βCD_{anf} favors the monomeric form of the drug which highly desirably for clinical use.

Keywords: Amphotericn B, synthetic glicolipid, cyclodextrin, monolayer, nuclear magnetic resonance.

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(551) **DIACYLGLICEROL LIPASE ACTIVITY IN ROD OUTER SEGMENTS DEPENDS ON THE ILLUMINATION STATE OF THE RETINA**

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The aim of the present research was to determine how the synthesis of endocannabinoid 2-arachidonoylglycerol (2-AG) for diacylglycerol lipase (DAGL) enzyme is modulated by the illumination state of the retina. DAGL activity was analyzed in purified rod outer segments (ROS) obtained from dark or light adapted retinas. Retinas were dissected from the eyes after dark or light adaptation. Dark-adapted bovine ROS (DROS) and bleached ROS (LROS) were purified by a discontinuous gradient of sucrose from retinas whose optic cup was either maintained under dim red light or ex-

posed to light (3000 luxes) for 30 min. This activity was assayed using [^{3}H] glycerol-DAG as substrate and determined by [^{3}H]-MAG production. MAG was partially metabolized to glycerol by MAGL action. DAGL activity in LROS was higher than in DROS under all conditions assayed. When the enzyme activity was assayed in a range between 100 μg and 200 μg of ROS protein, [^{3}H]-MAG production was increased in LROS ($p<0.01$). However, the light effect on DAGL activity disappeared when 300 μg of protein were used. It was also observed that light increased DAGL activity at 1 and 2 hours of incubation. Interestingly, endocannabinoid production increased at 2 hours in DROS ($p<0.001$) and LROS ($p<0.01$) with respect to 1 hour of incubation. Additionally, it was observed that MAGL associated to DAGL activity was stimulated by light ($p<0.01$). The expression of cannabinoid receptors (CB1 and CB2) was also increased under light conditions. The data was analyzed using Student t-test, two way-ANOVA and Bonferroni test to compare different conditions. These results suggest a light effect on DAGL, the principal enzyme involved in 2-AG synthesis, as well as on the receptors to which this endocannabinoid binds to, thus indicating a potential role of 2-AG in phototransduction processes.

Keywords: Retina, Photoreceptors, 2- arachidonoylglycerol, diacylglycerol lipase

(609) **AMYLOIDOGENIC TENDECY OF N-TERMINAL VARIANTS OF HUMAN APOLIPOPROTEIN A-I**

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Amyloidosis is a heterogeneous disease involving protein´s misfolding. Among more than 20 described natural variants, some N-terminal mutated apoA-I (as Leu60Arg (L60R) and Trp50Arg (W50R) are involved in renal amyloid protein deposition.

Previous studies with other mutants (Gly26Arg, Lys107-0 and Arg-173Pro) suggested that structural conformational shifts determine the involvement of apoA-I in this pathology. In the present study we extended our knowledge and compared protein stability, aggregation tendency and susceptibility to proteolysis of the protein with the native sequence (Wt), with the N-terminal variants W50R and L60R.

Structural parameters were analyzed under pH 7.4 and 5.0 by fluorescence, following protein chemical denaturalization, Trp arrangement by the quenching with acrilamide and hydrophobic pockets by the binding of BisAns. The aggregation tendency was evaluated measuring Thioflavin T associated fluorescence following incubation at 37°C in the presence and absence of heparin (as a glucosaminoglycan model). Susceptibly to Trypsin-induced proteolysis was determined at pH 7.4.

Our results show that both mutants are less stable than Wt, especially L60R (p 0.05) and showed a higher quenching of Trp residues (p 0.001); this variant evidenced a loss of hydrophobic pockets. While the aggregation tendency of both variants was similar than Wt at pH 5.0, W50R showed higher ThT binding than Wt or L60R under this condition. No aggregation of the variants tested was detected at pH 7.4, either pure or combined with heparin. Susceptibly to proteolysis was increased for L60R (p 0.05) respect Wt and W50R.

We suggest that structural unstability of the variants induce misfolding that could expose binding sites of ligands and cleavage sites for proteases in the protein which could either increase protein catabolism or to favor an aggregation-prone conformation. Maybe induced under a pro inflammatory microenvironment.

Keywords: misfolding, aggregation

(1746) **OXIDATIVE STRESS AND PEROXISOMAL BIOGENESIS IN MICROSCLEROTIA PRODUCED BY THE ENTOMOPATHOGENIC FUNGI *BEAUVERIA BASSIANA* AND *METARHIZIUM ROBERTSII***

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Insect pathogenic fungi are able to produce resistance structures called microsclerotia (MS), which are potential candidates for use in biological control programs. Although oxidative stress was reported to be involved in MS differentiation in some plant pathogenic fungi, there is no information available for entomopathogenic fungi.The main goal of this study was to compare the microsclerotial growth in *Beauveria bassiana* strain GHA and *Metarhizium robertsii* strain ARSEF 2575, and to characterize the expression pattern of genes involved in oxidative stress responses and peroxisomal biogenesis. Fungi were cultured in agitated (250 rpm) complete liquid medium with optimal carbon/nitrogen ratio for MS production. Daily aliquots were collected and examined by both optical and transmission electron microscopy (TEM) after staining with the peroxidase activity marker 3,3-diaminobenzidine (DAB). Samples were also used for qPCR analysis to study the expression pattern of superoxide dismutase genes (*sod*), catalase genes (*cat*), and peroxins (*pex*) involved in peroxisome biogenesis. DAB staining showed high peroxidase activity in MS for both strains, with lower staining in hypha close to the borders of the structure. TEM images also showed higher peroxidase activity in mitochondria and peroxisomes. Although *pex* genes were induced in both strains, *Bbpex7* was more induced in *B. bassiana*, whereas *Mrpex19* showed higher expression levels in *M. robertsii.* At least one of each oxidative stress marker family was also induced in both strains. We conclude that an oxidative stress scenario is triggered in MS producing fungi, including proliferation of peroxisome-like organelles and high peroxidase activity. More studies are need to be carried out to elucidate the relationship between MS formation, oxidative stress and peroxisomal biogenesis to better understand the similarities and differences found in microsclerotial metamorphosis of entomopathogenic fungi.

(1234) **STRUCTURAL STABILITY STUDIES OF HUMAN GLYCOGENIN-1 MUTANT ALA16PRO ASSOCIATED WITH GLYCOGEN STORAGE DISEASE XV**

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Abstract: Glycogenin is a glycosyltransferase that catalyzes the transfer of glucose residues from UDP-glucose to itself, creating a linear polymer of approximately 12 glucose units bound by α-1,4-glycosidic linkages. This oligosaccharide chain serves as the primer for the combined action of glycogen synthase and glycogen branching enzyme that will complete the polysaccharide synthesis. In humans, there are two forms of glycogenin, glycogenin-1 and glycogenin-2. Glycogenin-1 (HGN1) is mainly expressed in skeletal muscle and heart and to a lesser extent in lung, kidney, brain, pancreas, and placenta. Instead, glycogenin-2 is primarily expressed in the liver.

Glycogen storage disease (GSD) XV is a rare metabolic disorder caused by mutations in the GYG1 gene, which encodes HGN1. To date, eight point mutations have been described in GSD XV patients. One of them was homozygous for an N-terminal missense variant (c.46G>C, p.Ala16Pro) of the protein and exhibited skeletal myopathy with storage of polyglucosan in muscle fibers. The mutation was confirmed at the RNA level but the mutant protein was not detected in the skeletal muscle biopsy of the patient.

Since human and rabbit glycogenin amino acid sequences are 93% identical, we have introduced Ala16Pro mutation into rabbit enzyme, the most studied member of the family, and expressed the mutant in *E. coli.* We have previously described that Ala16Pro mutant was inactive for auto- and transglucosylation and has a diminished substrate binding affinity, probably due to a conformational change. In order to explain the absence of the protein in the patient muscle tissue, in this work we have analyzed its stability and oligomerization state by different *in vitro* techniques. Here we show that, in contrast to wild type HGN1, which exists as a dimer, the Ala16Pro variant forms soluble high molecular weight oligomers. Besides, our results suggest that the mutant has a less stable conformation, more prone to proteolytic digestion.

Keywords: glycogen, metabolic disorder, conformational stability

(1242) **S-NITROSYLATION OF HUMAN TRIOSEPHOS-**