Abstracts

– Membrane Structure and Domains –

P-324

Unraveling Nystatin Molecular Action Mechanism: the Influence of Membrane Composition and Properties

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Nystatin (Nys), a naturally occurring polyene antibiotic, has strong antifungal activity, but elevated cytotoxicity towards mammalian cells. Nys targets the plasma membrane (PM) of sensitive organisms forming ion channels, possibly due to Nys-sterol interactions and/or preference for ordered membrane regions. The existence of ergosterol-free sphingolipid-enriched gel domains in yeast cells led us to hypothesize that Nys pore formation might be governed by the presence of gel phase in the PM of fungal cells. Fluorescence spectroscopy studies using liposomes composed of a fluid lipid and different gel-domain forming lipids (sphingomyelin (SM) or DPPC) show that Nys has stronger partition from the aqueous medium into gel-enriched membranes particularly containing SM. However, the distribution of membrane-associated Nys species among gel and fluid domains is markedly different in the presence of DPPC. Formation of Nys aggregates with long fluorescence lifetime (Nys active species) depends on the number of Nys molecules located within the gel phase. We conclude that Nys partition and aggregation depend on both lipid -type and -phase and are enhanced by the presence of DPPC gel phase.

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P-325

The role of lipid membrane in amyloid fibril formation and toxicity in Alzheimer's disease

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Alzheimer's disease is a progressive neurodegenerative disease associated with amyloid fibril formation in the brain. It is now accepted that the cytotoxicity is a result of the non-specific interaction of toxic soluble amyloid oligomers with the surface of plasma membrane. We used atomic force microscopy (AFM), atomic force spectroscopy (AFS), frequency modulated Kelvin probe microscopy (FM-KPFM), Langmuir-Blodgett monolayer technique and Surface Plasmon Resonance (SPR) to study effect of membrane structure and composition on binding of amyloid-β (1-42) peptide and fibril formation. We show that cholesterol induces electrostatic domains in lipid membrane which creates a target for amyloid binding. Hormone melatonin, which regulates and maintains the body's circadian rhythm, has been shown to be protective against AD, but molecular mechanism of this protection is not understood. We show that melatonin and cholesterol have the opposite effects of the lipid membrane properties which, in turn, affect amyloid binding to the lipid membrane.

E.Drolle, R.Gaikwad, Z.Leonenko, *Biophys. J.*, 2012, 103: L27-L29; F.Hane, E.Drolle, R.Gaikwad, E.Faught, Z.Leonenko, *J. of Alzheimer's Disease,* 2011, 26:485-494.

P-326

Effect of melatonin and cholesterol on the structure of DOPC and DPPC lipid membranes

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The cell membrane plays an important role in amyloid toxicity - amyloid oligomers can interact with lipid membrane, inducing various defects that are toxic to the cell. Membrane composition may affect its interaction with amyloid peptides. Both melatonin and cholesterol have been linked to amyloid toxicity, with melatonin having been shown to have a protective role against amyloid toxicity, however the underlying molecular mechanism of this protection is still not well understood. We used small-angle neutron diffraction from oriented lipid multilayers, small-angle neutron scattering from unilamellar vesicles and molecular dynamics simulation experiments to elucidate the structure of DOPC and DPPC model membranes determine the effects of melatonin and cholesterol. We find cholesterol and melatonin to have opposite effects on lipid membrane structure: the incorporation of melatonin results in membrane thinning, in stark contrast to the increase in membrane thickness induced by cholesterol. The fluidity and the state of disorder of the membrane are significantly increased in the presence of melatonin. These different effects of cholesterol and melatonin may help to understand their relation to amyloid toxicity.

P-327

Influence of ectoin on the structural organization of natural and artificial tear fluid lipid layer

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We investigate the influence of Ectoin on the structural organization of the natural and artificial tear fluid lipid layers (ATFLL) using surface activity analysis and topographical studies. The natural meibomian lipids exhibit a continuous pressure-area isotherm without any phase transitions. In the presence of ectoin, the isotherm is expanded towards higher area per molecule implying decreased interaction between the lipid molecules. The AFM scans show presence of fiber like structures in the natural meibomian lipid film. In the presence of ectoine, droplet-like structures are observed which are hypothesized to be triacyl glycerols excluded from the lipid film. ATFLL illustrate the fluidizing effect of ectoine on the lipid films where the pressure-area isotherms are expanded in the presence of ectoin. With the addition of a triacyl glycerol to the mixture of DPPC and Chol-Palmitate, we observed the formation of similar drop-like structures in the presence of ectoine as in the case of natural meibomian lipid films. Consequently, the hypothesis explaining the exclusion of tri/di acyl glycerol from the meibomian lipid film in the presence of ectoine in the subphase is confirmed which lead us to a model describing the fluidizing effect of ectoine on meibomian lipid films.