granule where it is stored. Our studies have shown zinc, which is found at millimolar concentrations in the secretory granule, significantly inhibits hIAPP amyloid fibrillogenesis at concentrations similar to those found in the extracellular environment. We show here by ITC and PICUP cross-linking that zinc binds to a complex of several hIAPP peptides at micromolar concentrations similar to those found in the extracellular environment, and in the process, promotes the formation of small IAPP oligomers. Interestingly, this observed interaction is unique to the hIAPP as membrane disrupting peptides with similar sequences exhibit minimal interaction with zinc. By contrast, the fibrillar amyloid form of hIAPP has only low affinity for zinc. High-resolution NMR structures of hIAPP bound to zinc reveal changes in along residues that would be located along one face of the hIAPP alpha-helix proposed as an intermediate for amyloid formation. These changes occur on the hydrophilic side of the amphipathic alpha-helix, away from the proposed interface for amyloid nucleation on the hydrophobic side. Combined, these results suggest zinc promotes the formation of off-pathway oligomers while creating a thermodynamic barrier for the formation of amyloid fibers.

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Lipid Composition and Raft Domain Formation on the IAPP-Membrane Interaction: The Role of Cholesterol on the Inhibition of IAPP (Amylin) Fibrilization and the Reduction of Membrane Disruption in Model Liposomes and Mouse Pancreatic Islets

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The misfolding and aggregation of islet amyloid polypeptide (IAPP) is known to exhibit an important role in the etiology of type-two diabetes mellitus. While it has been believed that the peptide becomes toxic in its large fibrous form, recent studies suggest that pre-fibril oligomers are in fact the predominantly toxic species. Permiabilization of IAPP on membranes is thus biphasic in nature. The latter phase is correlated with fiber formation and reaches maximal leakage. The degree of leakage, and hence membrane disruption, of the initial phase is dependent on the peptide to lipid ratio and percent anionic lipid in the membrane. Studies of IAPP toxicity have focused on POPC/POPS(G) vesicle systems. The homogeneity and anionic nature of the membranes have been shown to increase aggregation and toxicity. However, cellular membranes are vastly more complex. In this study, we investigate the in vitro interaction between IAPP and non-homogenous, cholesterol-containing model membranes; extend previous observations of the role of anionic phospholipids in membrane-catalyzed IAPP fibrilogenesis; and show that the presence of cholesterol dramatically inhibits IAPP fribrilogenesis and decreases membrane disruption in large unilamellar vesicles. Additionally, we demonstrate that human IAPP strongly permiablilizes raft type membranes. Fluorescence microscopy of islet cells shows increased IAPP toxicity after the removal of cholesterol from the membrane. These findings demonstrate that the mode of IAPP membrane binding and permiablization is highly dependent on the fluidity and phase of the membrane. The differences in this behavior may have significant implications in the development of type-two diabetes, as the change in membrane composition is dependent on a number of factors also related to the disease.

2942-Pos Board B47

Nucleation of Hybrid Polymers in Sickle Cell Disease

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Upon deoxygenation, sickle hemoglobin (HbS) can polymerize into complex, 14 stranded polymers via a double nucleation process. While sickle cell hemoglobin nucleation has been well described, the description has focused on nucleation of a single species, deoxyHbS, with perhaps the presence of a crowding non-polymerizing species. However, there are three important cases of polymerization where hybrid polymers are created, and thus the nucleation process in such situations needs to be characterized and tested. First in vivo, polymerization always occurs in the presence of ligands. Second, polymerization is also possible in the presence of normal hemoglobin (HbA, eg. in sickle trait). Finally, antisickling drugs may not bind to all molecules and thus create a heterogeneous population with the opportunity for hybrid polymerization. Thus we have studied polymerization in the presence of HbA, as well as under cases of partial ligation (with CO, NO and O2). Homogeneous nucleation rates have been measured by laser photolysis of the CO-derivative and analysis of the stochastic fluctuations of onset-times. Heterogeneous nucleation is determined by following the exponential growth of light scattering. Existing models for nucleation have been successfully modified to account for the copolymerization probability of hybrid species, and revised models will also be presented. Incorporation of ligands is particularly challenging since it appears necessary to account for tertiary as well as quaternary effects, and these appear to differ depending on the ligands.

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Single Sickle Cell Occlusion and Manipulation in Microchannels

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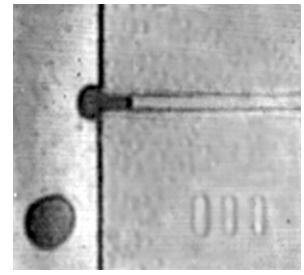
Sickle cell disease arises because a point mutation permits the hemoglobin in erythrocytes to form stiff polymers and obstruct the microcirculation. We have developed a microfluidic system to model vaso-occlusion in the smallest vessels to fill a critical gap in understanding the pathophysiology of sickle cell disease. Microfluidic methods heretofore have only studied far larger vessels, and could not observe events of speed comparable to physiologic transit times. We use microchannels of approximately rectangular cross section (3 μm x 1.5 μm). Because the cells fill the channel top-to-bottom, absorbance can determine intracellular concentration. Cells can be flowed, viewed statically, or stopped and restarted. By laser photolysis of COHbS, any desaturation of the cells can be created in milliseconds. Channels are constructed of PDMS, and cells are driven by hydrostatic pressure which can be controlled to stop the cell within the channel. The cell can then be photolyzed, and studied rheologically. Polymerized cells stick in the channels, but can be pushed through, with pressure that rises with the mass of the polymers formed. Cells sickled at the entry into such channels (see Figure) are found to maintain occlusion against much higher pressures.



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Elastic Light Scattering Measurements of Hemoglobin Oligomers

Yihua Wang, Alexey Aprelev, Frank A. Ferrone.

Protein solutions scatter light dominantly by the density fluctuations of the solute. However, the formation of small oligomers can create light scattering that will exceed this background. We are using such light scattering to probe the initial stages of sickle hemoglobin assembly. We employ a novel micro-method for measuring light scattering in which a small rectangular cell (0.2 x 4.0 x 30 mm) is filled with 24 μL of Hb solution. An optical fiber is sealed into the cell in contact with the solution, and scattering from a 785 nm, 1.5 mW laser diode is measured at 90°. Light is colllected by a microscope objective, detected via PMT. Temperature is controlled by a thermoelectric stage. We have measured scattering from deoxygenated sickle hemoglobin, which forms polymers above a readily accessible solubility (and have measured scattering below and above solubility). We have also measured light scattering from COHbS, COHbA, and deoxyHbA which do not form such polymers and differ from deoxyHbS by either a single amino acid (HbA) or by quaternary differences (COHbS). Fluctuation scattering is used to calibrate the relevant scattering volume, allowing for more precise calculation of the concentrations of oligomeric scatterers. As temperature is increased, we observe a relatively constant intensity, which then increases beyond some particular temperature. We interpret this as the appearance of oligomers of sufficient numbers to exceed the density fluctuation background. Whereas deoxyHbS creates more oligiomers at the same concentration and temperature as other derivatives, all derivatives can create equally large oligomeric concentrations with varied temperature and monomer concentration. Thus oligomer concentration is not diagnostic for polymerization. Analysis of this data will address two important questions: is there evidence for off-pathway aggregation in sickle hemoglobin, and must aggregates have the same structure as the polymers?

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Comparing the Folding and Misfolding Energy Landscapes of Phosphoglycerate Kinase

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Understanding the sequence specific partitioning of polypeptides between protein folding and amyloid formation is of outstanding physiological and pathological importance. Using yeast phosphoglycerate kinase as model, here we identify the features of the energy landscape that decide the fate of the protein: folding or amyloidogenesis. Structure formation was initiated from the acid-unfolded state by stopped-flow or manual mixing, and monitored by tryptophan and thioflavin T fluorescence from 1 ms to 10 days. Solvent conditions were gradually shifted between folding and amyloidogenesis and the properties of the energy landscape governing structure formation were reconstructed. A continuous transition of the energy landscape between folding and amyloid formation was observed. In the early steps of both folding and misfolding, the protein searches through a hierarchically structured energy landscape to form a molten globule on the seconds timescale. From this intermediate structure, folding to the native structure happens in a cross-barrier step in a few minutes, while