and others form parallel β-sheet, but the conditions for the preferential formation of one over the other are unknown. Distinct splitting patterns within the amide I' band make FTIR a useful technique for distinguishing between antiparallel and parallel β-sheet (3). In this study, FTIR shows that H1 can also form parallel ß-sheet; H1 is thermostable in both forms (4). The A117L mutant also forms both ß-sheet organizations. Antiparallel A117L aggregates fully dissociate upon heating; the parallel configuration confers thermostability (4). The conversion from antiparallel to parallel ß-sheet is seen only for samples of high peptide concentration and is thermodynamically irreversible. We have proposed a high-concentration-dependent mechanism for the formation of parallel β-sheet aggregates and a structural model of fibril organization that accounts for their thermostability.

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473-Pos Board B352

Probing the effect of Heat Shock Protein 70 on the aggregation of α-Synuclein

Evan T. Spiegel[1], Phill Jones[2], Pamela McLean[2], Brad Hyman[2], Warren R. Zipfel[1].
[1]Cornell University, Ithaca, NY, USA, [2]Harvard Medical School, Charlestown, MA, USA.

The aggregation of α-Synuclein (αS) is crucial to the onset and progression of Parkinson's disease (PD). Recent studies on PD have demonstrated that while αS ultimately forms large dense intracellular plaques (Lewy bodies), the early oligomeric species contribute significantly to cell toxicity. In specific, molecular chaperones such as Heat Shock Protein 70 (HSP-70) have been shown to alter the aggregation properties of αS and are hypothesized to preferentially attack sub-populations of αS oligomers.

Traditionally, fluorescence correlation spectroscopy (FCS) is intended for the study of a narrow population of small diffusing molecules since the intensity fluctuations are proportional to the number of fluorophores and the speed at which they traverse the focal volume. With a solution of unknown size distribution, such as with αS oligomers, the presence of a few large species distorts the autocorrelation curve to a greater degree than the small species thereby impairing our ability to monitor changes in aggregate size. Thus we implemented a new technique, which segments out the large fluctuations and bins them in a burst histogram and autocorrelates the remaining background fluctuations. This technique allows for the concurrent quantification of the distribution of both small and large particles.

As a result of these new findings, we designed a flow chamber that permitted FCS measurements of cytosolic extracts taken from cells co-expressing αS and HSP-70. The presence of large bursts of photons confirmed that over time αS aggregates increase in size and quantity. Co-expression of HSP-70 significantly decreased the number of large aggregates in comparison to cells only expressing αS.

(Supported by NIH/NIBIB P41 RR04224 and NIH/NCI R01 CA116583 to WRZ.)

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The Effect of Cations on α-Synuclein Misfolding: Single Molecule AFM Force Spectroscopy Study

Junping Yu, Yuri L. Lyubchenko.
University of Nebraska Medical Center, Omaha, NE, USA.

Misfolding and aggregation of α-synuclein are important properties of this intrinsically disordered protein. There is plenty of evidence suggesting that environmental conditions such as metal cations are involved in α-synuclein misfolding and the disease development. Previously, we have applied AFM dynamic force spectroscopy (DFS) to show that at low pH the interaction between α-synucleins increases dramatically and the dimers formed by misfolded α-synuclein have enormously high stability. In this study, we applied the same approach to evaluate the effects of ionic strength and metal cations on α-synuclein misfolding. α-Synuclein was covalently attached to the substrate and probe through C-terminal cysteine. The interaction between α-synucleins was measured in the multiple approach-retraction steps at various locations over the surface. We studied the effect of ionic strength (from 10 mM to 250 mM) on the acidic pH induced α-synuclein misfolding. The DFS study revealed a weak effect of ionic strengths on the majority of DFS parameters. Importantly, the lifetime of the dimers only slightly increases then decreases dependent on ionic strengths and remains in second scale in the ionic strength range. These findings suggest that electrostatic interactions don't play major roles in the α-synuclein misfolding and dimerization. We also studied the effect of metal cations capable of promoting α-synuclein aggregation. We showed that at conditions without significant misfolding of α-synuclein (pH 7.0), the addition of zinc or aluminum cations leads to a dramatic increase of the misfolding events: the probability of events is 7.0% for aluminum and 3.9% for zinc vs. 0.7% in their absence in pH 7.0. Thus, aluminum and zinc cations increase the probability of α-synuclein misfolding explains the role of these cations on the α-synuclein aggregation.

Supported by grants from Nebraska Research Initiative (NRI) and DOE (DE-FG02-08ER64579)

475-Pos Board B354

A Single Mutation in the Non-Amyloidogenic Region of IAPP Greatly Reduces Toxicity

Kevin Hartman, **Jeffrey R. Brender**, Kendra R. Reid, Pieter E.S. Smith, Ravi P.R. Nanga, Marchello A. Cavitt, Edgar L. Lee, Duncan G. Steel, Ari Gafni, Robert T. Kennedy, Ayyalusamy Ramamoorthy.
University of Michigan, Ann Arbor, MI, USA.

While the disruption of cellular membranes by prefibrillar states of amyloid proteins is a likely cause of cell-death during amyloid-related diseases, research has been hampered by the complex nature of the aggregation process. The 1-19 fragment of IAPP, a peptide implicated in beta-cell death during type 2 diabetes, is particularly informative for mechanistic studies on amyloid prefibrillar states as it forms toxic oligomers when bound to the membrane but does not progress further to form amyloid fibers. Human $IAPP_{1-19}$ causes a rapid increase in beta-cell islet intracellular calcium levels indicative of a loss of beta-cell membrane integrity. The toxicity of IAPP may be linked to the induction of curvature in the membrane. Solid-state NMR and DSC show that toxic versions of IAPP stabilize negative curvature, while the non-toxic full-length rat IAPP peptide does not. Despite a difference of only one residue from $hIAPP_{1-19}$ (H18R substitution), the rat version of the $IAPP_{1-19}$ peptide is significantly less toxic both *in vitro* and *in vivo*. This difference is reduced at higher peptide to lipid ratios, suggesting that the self-association of $rIAPP_{1-19}$ within the membrane is impaired. The toxicity difference can be traced to the difference in charge at residue 18. At pH 6.0, membrane disruption by $hIAPP_{1-19}$ is significantly reduced and becomes equivalent to that of $rIAPP_{1-19}$. DSC shows that while $hIAPP_{1-19}$ has a minimal effect on the phase transition of lipid vesicles, $rIAPP_{1-19}$ has a strong effect, indicating a surface-associated topology for $rIAPP_{1-19}$ and a transmembrane topology for $hIAPP_{1-19}$; a result in agreement with NMR quenching studies. Our results indicate that the modulation of the peptide orientation in the membrane by His18 plays a key role in the toxicity of hIAPP by altering the interaction with the membrane.

476-Pos Board B355

Curcumin Inhibits The Formation Of Fibrils From Islet Amyloid Polypeptide

Gai Liu.
Clark University, Worcester, MA, USA.

Islet amyloid polypeptide (IAPP) forms assemblies that are toxic to the insulin-producing beta cells found in the pancreas. Inhibiting the formation of the toxic assemblies is therefore an attractive strategy for the development of anti-diabetes drugs. We are studying curcumin, a small molecule that is a component of curry spice, as a potential inhibitor of IAPP aggregation. Our preliminary results obtained by circular dichroism and electron microscopy show that curcumin works best if it is present at an inhibitor: IAPP ratio of 1:1. This suggests that the inhibition occurs at an early stage of the aggregation process. To elucidate the mechanism of inhibition, we are using limited proteolysis monitored by mass spectrometry and two-dimensional ^1H NMR spectroscopy. The results of our studies will be presented and discussed.

477-Pos Board B356

High-resolution Structures of Membrane-Bound IAPP Reveal Functional Implications of the Toxicity of Prefibrillar States of Amyloidogenic Proteins

Ravi P.R. Nanga, Jeffrey R. Brender, Kevin Hartman, Ayyalusamy Ramamoorthy.
University of Michigan, Ann Arbor, MI, USA.

Disruption of the cellular membrane by the amyloidogenic peptide IAPP (aka amylin) has been implicated in beta-cell death during type 2 diabetes. While the structure of the largely inert fibrillar form of IAPP has been investigated, the structural details of the highly toxic prefibrillar membrane-bound states of IAPP have been elusive. We have shown that a fragment of IAPP (residues 1-19)

induces membrane disruption to a similar extent as the full-length peptide. However, unlike the full-length IAPP peptide, IAPP1-19 is conformationally stable in a helical conformation when bound to the membrane. In vivo and in vitro measurements of membrane disruption indicate the rat version of IAPP1-19, despite differing from hIAPP1-19 by only by the single substitution of Arg18 for His18, is significantly less toxic than hIAPP1-19, in agreement with the low toxicity of the full-length rat IAPP peptide. To investigate the origin of this difference at the atomic level, we have solved the structures of the human and rat IAPP1-19 peptides in DPC micelles, as well as the completely non-toxic full-length rat and toxic full-length human peptide. While the structures of rat and human IAPP1-19 are similar, the charge at residue 18 plays a key role in controlling the toxicity of the peptide. At pH 7.3, the more toxic hIAPP1-19 peptide is buried deeper within the micelle, while both the less toxic rIAPP1-19 peptide and non-toxic full-length rIAPP peptide are located at the surface of the micelle. Deprotonating H18 in hIAPP1-19 moves the peptide to the surface of the micelle. This change in orientation is accompanied by a corresponding change in toxicity. At pH 6.0, the membrane disruption induced by hIAPP1-19 is significantly decreased and resembles that of the less toxic rIAPP1-19 peptide.

478-Pos Board B357

Amyloidogenic Propensity of ProIAPP and IAPP in the Presence of Negatively Charged Lipid Bilayers

Suman Jha.
TU Dortmund University, Dortmund, Germany.

The islet amyloid polypeptide (IAPP) is synthesized in the β-cells of the pancreas from its precursor, the proislet amyloid polypeptide (ProIAPP). ProIAPP is co-processed in the secretory granules, and then co-secreted to the extracellular matrix together with insulin. As indicated earlier, partially processed N-terminal ProIAPP is able to interact with negatively charged moieties, like heparan sulfate, which may lead to islet amyloid plaque formation. Amyloid plaques have been found both extracellularly and intracellularly in type II diabetic patients. Here, we studied the amyloidogenic propensity of native ProIAPP and compared it with that of IAPP in the absence and presence of negatively charged membranes. Our CD studies show that the secondary structure content of ProIAPP and IAPP is predominantly unordered with small amounts of ordered secondary structure elements as confirmed by ATR-FTIR spectroscopy. However, in the presence of anionic membranes, ProIAPP forms predominantly α-helices and loops that subsequently transform to intermolecular β-sheet structures. For comparison, IAPP forms intermolecular β-sheets largely via unordered and loop structures. The ATR-FTIR and fluorescence spectroscopy studies performed also reveal that ProIAPP has a higher amyloidogenic propensity in the presence of negatively charged membranes, but is still less amyloidogenic than IAPP. AFM studies have also been carried out which show that ProIAPP, at variance to IAPP, does not form long fibrils, but rather protofilaments or short fibrils, only. Hence, both, the presence of a small amount of unprocessed ProIAPP in β-cell secretion, or the interaction with negatively charged surfaces, like negatively charged lipid bilayers, may initiate islet amyloid plaque formation.

479-Pos Board B358

Structural Studies Of Islet Amyloid Polypeptide In The Presence Of Insulin And Lipid Membranes

Shuqiu Han.
Clark University, Worcester, MA, USA.

Previous work by others has shown that insulin inhibits the formation of fibrils from islet amyloid polypeptide. The mechanism for the inhibition is not known. To address this issue, we are studying IAPP in the presence of lipid membranes, with or without insulin. Circular dichroism spectra of IAPP alone in phospholipid membranes show that it undergoes a structural rearrangement from α-helix to a β-sheet conformation. In the presence of insulin, this transition is not observed, that is, IAPP remained a-helical. To explain this, we are using other biophysical methods including solution-state NMR, electron microscopy, SDS-PAGE and limited proteolysis monitored by mass spectrometry. Results from these studies will be presented and discussed in this poster.

480-Pos Board B359

Amyloid-like Misfolding Of Peptides By Membrane Mimicking Environments

Alex Perálvarez-Marín[1], Anna Wahlström[1], Jesper Lind[1], Loïc Hugonin[1], Jüri Jarvet[2], Lena Mäler[1], Andreas Barth[1], Astrid Gräslund[1].
[1]Stockholm University, Stockholm, Sweden, [2]The National Institute of Chemical Physics and Biophysics, Tallin, Estonia.

Sodium dodecyl sulfate has been proven as an amyloid-like misfolding agent [1-3]. Our study comprises the biophysical characterization of human peptides in the presence of submicellar and micellar concentrations of SDS. The prodynorphin derived peptides (Big dynorphin, dynorphin A and dynorphin B) [4], the amyloid β peptide [5], and the proinsulin derived C-peptide are our subject of study. As determined by CD and FTIR spectroscopy, the peptide structural transitions involve different secondary structures, such as random coil, β-sheet and α-helix. By means of NMR, dynamic light scattering, native-PAGE or ThT fluorescence, we have shown that all the peptides transit through a high molecular weight aggregated state at submicellar detergent concentrations. Finally, studies with model membranes with different charge composition have been carried out to relate the structural characterization of these peptides to their possible role in the cell and their action mechanisms in pathology.

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481-Pos Board B360

Modeling amyloid toxic ion channels

Hyunbum Jang[1], Ratnesh Lal[2], Ruth Nussinov[1].
[1]NCI-Frederick / SAIC-Frederick, Inc., Frederick, MD, USA, [2]University of Chicago, Chicago, IL, USA.

Non-amyloidogenic beta peptides, p3 ($A\beta_{17-42}$) and $A\beta_{11-42}$, resulting from α-secretase and BACE cleavage are often found in amyloid plaques. However, the biophysical properties and functional role of these non-amyloidogenic peptides are not understood. We present molecular dynamics (MD) simulations of channels consisting of the U-shaped *beta-strand-turn-beta-strand* peptides using available NMR-based coordinates of p3 and $A\beta_{9-42}$. Our results show that non-amyloidogenic p3 and $A\beta_{9-42}$ peptides form ion channel-like structures with loosely attached subunits. These channels are dynamic and are made of small peptide oligomers. The channels can conduct calcium and obtain shapes and dimensions consistent with Atomic Force Microscopy (AFM) images. All channels break into mobile subunits suggesting that membranes do not support intact β-sheet channels. We shall further present results of modeling both PG-1 and k3-β_2m channels, presenting a consistent general picture of toxic beta-sheet based channels. Funded in part by DHHS #N01-CO-12400.

482-Pos Board B361

Probing Tau-Vesicle Interactions

Shana Elbaum, Jocelyn Traina, David Eliezer, Elizabeth Rhoades.
Yale University, New Haven, CT, USA.

Tau is the major protein component of the neurofibrillary tangles that characterize a group of neurodegenerative diseases, including Alzheimers Disease. The transformation of tau from its native state where it functions as a microtubule-associated protein (MAP), to its pathological state as is found in patients suffering from any of the various associated diseases, is not well understood. Studies have shown that tau aggregation can be induced by anionic lipid vesicles, and that detergent micelles can induce folding of the microtubule binding domain of tau. Here we use fluorescence correlation spectroscopy (FCS) to monitor the interaction of tau with synthetic lipid vesicles, in order to investigate vesicle binding and aggregation. Studying several isoforms of tau, we find that solution pH plays a strong role in such interactions.

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An α-Helical Conformation of the SEVI Peptide, a Dramatic Enhancer of HIV Infectivity, Promotes Lipid Aggregation and Fusion

Jeffrey Brender, Kevin Hartman, Ravi P.R. Nanga, Stephanie V. Le Clair, Lindsey M. Gottler, Ayyalusamy Ramamoorthy.
University of Michigan, Ann Arbor, MI, USA.

A peptide ubiquitous in human seminal fluid has been recently described that dramatically enhances the infectivity of the HIV virus (3-5 orders of magnitude by some measures). Previous studies have shown that this peptide, a fragment of human Prostatic Acid Phosphatase ($PAP_{248-286}$) referred to as SEVI (Semen-derived Enhancer of Viral Infection), is amyloidogenic and the enhancement of viral infectivity is dependent on the aggregation state of the peptide. To complement these previous in vivo studies we have performed in vitro assays to investigate the physical mechanisms by which the $PAP_{248-286}$ promotes the interaction with lipid bilayers. Our results indicate a strong interaction of freshly dissolved $PAP_{248-286}$ with lipid bilayers but a weaker interaction with the amyloid form of $PAP_{248-286}$, as measured by the tendency of freshly dissolved $PAP_{248-286}$ to induce aggregation of lipid vesicles and membrane fusion. The