a previously proposed hypothesis that Met-35/Gly-33 can be oxidized[4] by Cu^{2+} to produce ROS by SSNMR.

2367-Pos

Detecting the Aggregation of Amyloid Peptides by Spin Label EPR

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Plaques containing aggregated β-Amyloid (Aβ) peptide in the brain are the main indication of Alzheimers disease. These plaques consist of Aβ fibrils. Oligomers of Aβ have been implicated as infective agents in the disease and may also be intermediates of fibril formation. Therefore, methods to study oligomers on the timescale of aggregation are sought. We show that by EPR the dynamics of spin-labeled Aβ in solutions in which fibrils are formed can be determined. The EPR experiments were performed on solutions of the Aβ peptide with 42 residues (1-42 Aβ) containing an N-terminal cysteine, which was spin labeled with the MTSL spin label (1-oxyl-2,2,5,5-tetramethyl-Δ-pyrroline-3-methyl]methanethiosulfonate) (SL- Aβ). For diamagnetic dilution, SL- Aβ was mixed with unlabeled Aβ. Fibril-formation in these solutions is shown by Congo-red binding and electron microscopy. Continuous wave, 9 GHz EPR reveals three fractions of different spin-label mobility, a fast one attributed to monomeric Aβ, one with a mobility that corresponds to a multimer of eight to 15 monomers, and a slow one due to larger aggregates or fibrils. The approach, in principle, allows detection of oligomers on the timescale of aggregation.

2368-Pos

Structural Characterization of Amyloids Comprised of Anchorless Prion Proteins

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Prion diseases are fatal, neurodegenerative diseases that afflict sheep, cows, and humans. The key event in prion diseases is the conversion of the α-helical, cellular isoform of the prion protein (PrP^{C}) to an insoluble, β-sheet-rich, infectious isoform (PrP^{Sc}). Host-encoded PrP^{C} is anchored to the cell membrane by a glycosylphosphatidyl inositol (GPI) anchor and converts to GPI-anchored PrP^{Sc} during prion infection. Here we describe prion infection in transgenic mice (Tg8015) that lack the signal sequence for attachment of the GPI anchor and therefore express non-anchored, free-floating, PrP^{C} (ΔGPI-PrP). The lack of the GPI anchor leads to incomplete modification of PrP, resulting in an unglycosylated molecule. Therefore, Tg8015 mice produce unglycosylated ΔGPI-PrP^{Sc} upon infection with the Rocky Mountain Laboratory (RML) prion strain. These mice did not show clinical signs of disease until >300 days post-infection (dpi), but their brains harbored ΔGPI-PrP^{Sc} and macroscopic amyloid deposits. In contrast, RML infection of wild-type (wt) mice resulted in clinical signs of disease within 120 dpi but no macroscopic amyloid formation. ΔGPI-PrP^{Sc} and wt PrP^{Sc} in the brains of Tg8015 and wt mice, respectively, were purified by two different protocols, both involving N-terminal truncation by proteinase K. The resulting, highly concentrated ΔGPI-PrP 27-30 and wt PrP 27-30 preparations were analyzed and compared by negative-stain electron microscopy to characterize possible differences in fibril morphology. Additional analyses were performed using Fourier transform infrared spectroscopy and X-ray fiber diffraction to compare the structures of ΔGPI-PrP 27-30 amyloid and wt PrP 27-30 amyloid preparations. Additionally, we tested whether ΔGPI-PrP^{Sc} could self-propagate *in vitro* by evaluating its seeding capacity in a prion-specific amyloid seeding assay and examined its biological activity *in vivo* using mouse-based bioassays.

2369-Pos

Oligomerization of Amphipathic Peptides in a Membrane Studied by Coarse-Grained Molecular Dynamics Simulations

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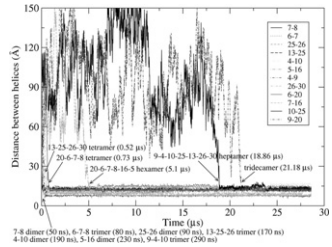
To gain insight into the aggregation and oligomerization of antimicrobial or amyloidogenic peptides, we carried out molecular dynamics simulations of a 26-residue amphipathic peptide at different concentrations (8, 16, and 32 copies) in a fully hydrated bilayer composed of 1600 POPC lipid molecules. With a coarse-grained representation of the molecules, >28 microseconds of simulations were accumulated for each system. Oligomers of various orders were observed to form. The system with 32 copies of the peptide was finally comprised of a 4-, 7-, 8-, and 13-mer. The final compositions of the systems with 16 and 8 copies of the peptide were 3-, 4-, and 9-mer for one and 3- and 5-mer for the other. Higher oligomers were formed by addition of monomers and by association of preformed lower oligomers (see Figure). Dissociation was not observed. In the lower oligomers (up to 4-mer) only the hydrophilic side of each copy was buried, but in the higer oligomers the hydrophobic sides of some copies were also buried. These simulations provide molecular insight into oligomerization of peptides inside membranes.



2370-Pos

Clues for the Mechanism of SEVI HIV Enhancement from Structural Studies of the SEVI Precursor Peptide $PAP_{248-286}$ in a Membrane Environment by NMR

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Despite the rapid progress of the AIDS pandemic, the HIV virus is a surprisingly weak pathogen *in vitro*. The large difference between *in vitro* and *in vivo* infection rates suggests that cofactors absent *in vitro* but essential for the natural transmission of the virus may be responsible for this discrepancy. A recently identified peptide in human semen, $PAP_{248-286}$, has emerged as a clear candidate for the missing cofactor as it dramatically enhances the infectivity of HIV by up to five orders of magnitude. $PAP_{248-286}$ appears to enhance HIV infection by forming amyloid fibers known as SEVI, which are believed to enhance the attachment of the virus by bridging interactions between virion and host-cell membranes. To understand the unique ability of SEVI to enhance HIV infection, we have solved the atomic-level resolution structure of the SEVI precursor $PAP_{248-286}$ using NMR spectroscopy in SDS micelles. In contrast to other toxic amyloid peptides that generally penetrate into the core of the membrane, non-toxic $PAP_{248-286}$ binds superficially to the surface of the micelle. Unlike most amyloid peptides that bind to the membrane in an α-helical state, $PAP_{248-286}$ is mostly disordered when bound to the surface of the micelle. The highly disordered nature of the SEVI peptide may explain the high ability of SEVI to enhance HIV infection, as partially disordered amyloid fibers will have a greater capture radius for the virus than more compact amyloid fibers. Two regions of nascent structure match the prediction of highly amyloidogenic sequences and may serve as nuclei for aggregation and amyloid fibril formation. NMR studies of the binding of $PAP_{248-286}$ to the anti-amyloid agent ECGC will also be presented.

2371-Pos

Formation of Toroidal Pores by Amyloid Proteins: Evidence of Lipid Transbilayer Exchange Induced by Islet Amyloid Polypeptide

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Recent computer simulations have indicated that amyloid peptides disrupt membranes by the formation of lipid-lined toroidal pores caused by excess membrane curvature, but experimental evidence for this mechanism has largely been lacking. A directly measurable consequence of this phenomenon is the significantly accelerated transbilayer exchange of lipids, which is a feature of the toroidal pore mechanism but not of other mechanisms of membrane disruption. Using vesicles asymmetrically labeled on the outer leaflet by pyrene-labeled lipids, we show that toxic versions of islet amyloid polypeptide, an amyloid peptide implicated in the pathogenesis of type II diabetes, induce rapid lipid flip-flop between bilayers. This manner is consistent with antimicrobial peptides known to disrupt membranes by the toroidal pore mechanism. We further demonstrate that a clear difference between toxic and non-toxic versions of IAPP can be observed in their binding to bicelles containing DMPC and the detergent DHPC, in which DHPC forms highly curved regions resembling toroidal pores. Using this model of a pre-constructed toroidal pore we show that toxic rat IAPP1-19 binds in the highly curved, pore-like DHPC enriched region while non-toxic rat IAPP1-37 binds to the flat lamellar DMPC enriched region away from the pore. Similarly, DSC indicates that toxic versions of the IAPP peptide strongly favor the formation of negative curvature in lipid bilayers, while the non-toxic rIAPP1-37 peptide does not. Further results on other amyloid peptides (including Aβ, calcitonin, and insulin) will also be presented as well as results from antimicrobial peptides.