2362-Pos

X-Ray Fiber Diffraction Reveals Major Structural Differences Between Brain-Derived Prions and Recombinant Prion Protein Amyloid

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X-ray fiber diffraction was used to study the structure of brain-derived prions and recombinant prion protein amyloid. Partially oriented, dried fibers were prepared from brain-derived PrP 27-30 and recombinant PrP amyloid. Fiber diffraction patterns were analyzed and used to interrogate models for the structure of the infectious prion.

Fiber diffraction patterns of recombinant PrP amyloid displayed characteristic, meridional reflections at ~4.8 Å and equatorial reflections at ~10 Å. These patterns were similar to those of other amyloids and are consistent with a basic cross-β architecture. In contrast, diffraction patterns from brain-derived PrP 27-30 displayed meridional reflections at ~9.6, ~6.4, and ~4.8 Å, which correspond to the 2nd, 3rd, and 4th order of a ~19.2-Å repeating unit, suggesting that PrP 27-30 contains four β-strands in a cross-β architecture. Furthermore, PrP 27-30 lacked the typical, equatorial reflection at ~10 Å, but instead produced equatorial reflections characterizing the diameter of the amyloid fiber and of individual protofilaments. Therefore, PrP 27-30 seems to have a structure consistent with a β-helix or β-solenoid, not unlike the model that was proposed earlier (Govaerts et al., 2004). This interpretation was also supported by extensive modeling, simulation of diffraction, electron microscopy, and FTIR.

In a previous study, recombinant PrP amyloid induced a transmissible prion disease in transgenic mice overexpressing PrP, and was thus termed a "synthetic prion" (Legname et al., 2004). Serially transmitted, synthetic prions were purified from mouse brains and analyzed by fiber diffraction. These brain-derived, synthetic prions showed the same structural characteristics as natural prion isolates and not those of its recombinant protein precursor. The relationship between structural differences and prion infectivity can be explained by several hypotheses. It remains to be determined which one, if any, is correct.

2363-Pos

Mutant Huntingtin Fragments Form Oligomers in a Polyglutamine Length-Dependent Manner

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A feature of many neurodegenerative diseases is the rearrangement of a specific protein to a non-native conformation, promoting aggregation, amyloid fibril formation, and deposition within tissues or cellular compartments. Such diseases include Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Huntington's disease (HD) is caused by an expansion above 35-40 polyglutamine (polyQ) repeats in the huntingtin (htt) protein and results in accumulation of inclusion bodies that contain fibrillar deposits of mutant htt fragments. Intriguingly, polyQ length is directly proportional to the propensity for htt to form fibrils and to the severity of HD, and is inversely correlated to the age of onset. Although the structural basis for htt toxicity is unclear, the formation, abundance and/or persistence of toxic conformers that mediate neuronal dysfunction and degeneration in HD must also be polyQ length-dependent. Here we used atomic force microscopy (AFM) to show that mutant htt fragments and synthetic polyQ peptides form oligomers in a polyQ length-dependent manner. Time-lapse AFM shows oligomers form before fibrils, are transient in nature, and are occasionally direct precursors to fibrils. However, the vast majority of fibrils appear to form by monomer addition that coincides with the disappearance of oligomers. Thus, oligomers must undergo a major structural transition that precedes fibril formation. These results demonstrate that oligomer formation by a mutant htt fragment is strongly polyQ length-dependent, consistent with a causative role for these structures in HD pathogenesis.

2364-Pos

Polymorphism in Alzheimer Aβ Amyloid Organization: Insight into Aβ Aggregation

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Alzheimer disease (AD) is a progressive neurodegenerative disease associated with accumulation of aggregated $A\beta_{1-40}/A\beta_{1-42}$ peptides in the brain. Ordered aggregates can extend into β-strand enriched fibrils, regardless of their initial native conformational states. To date, the self-assembly mechanism leading to ordered fibril formation is not fully understood. Understanding the mechanisms and the range of structural features of the aggregates are of crucial importance for effective drug design to reduce aggregate formation. The polymorphism of $A\beta_{1-42}$ based on ssNMR, EM, 2D hydrogen exchange and mutational studies, was investigated using all-atom molecular dynamics simulations with explicit solvent. Open questions relate to (1) how the monomeric peptides assemble into oligomers; (2) which segments of a long peptide constitute the recognition motifs and as such playing key roles in amyloid fibril formation; (3) how the β-strands arrange relative to one another; (3) is there a favored organization between the β-sheets and if so as one would expect (4) what is it and what are the intermolecular interactions between the layers that stabilize the favored amyloid fibril organization(s) are discussed. This project has been funded in whole or in part with Federal funds from the NCI, NIH, under contract number HHSN261200800001E.

2365-Pos

Structural Determinants of Amyloid B-Protein Oligomerization

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The oligomerization of amyloid β-protein (Aβ) is a seminal event in the neurodegenerative process in Alzheimer's disease (AD). Aβ40 and Aβ42, the two predominant forms of Aβ, display different aggregation behavior which underlies the special pathogenetic significance of Aβ42. Previous computational studies have revealed that a turn-like structure exists at the C-terminal of Aβ42 that is not observed in Aβ40. We report here results of studies to define the structure of this turn and to establish its role in Aβ assembly. We used molecular dynamics to simulate the structure of the Aβ C-terminus (Gly31-Val40/Ala42) and discovered that the dihedral angles of residues 36 and 37 tend to be locked into a region of Ramachanran space consistent with type-II β-turns. Aβ(31-42) predominantly formed a β-hairpin-like structure that was stabilized by hydrogen bonds and hydrophobic interactions between residues 31-35 and residues 38-42. In contrast, Aβ(31-40) appeared relatively unstructured. To investigate the possible role of this peptide-specific, β-hairpin-like structure in Aβ assembly, we synthesized a number of Aβ "mutants" containing amino acid substitutions that we postulated would stabilize or destabilize the hairpin. The stabilizing substitutions facilitated hexamer and dodecamer formation by Aβ42, abolishing formation of fibrils. Interestingly, compared to wild type Aβ42, these substituted peptides were equally toxic. When these substitutions were incorporated into Aβ40, the modified Aβ40 oligomerized like Aβ42, instead of an "Aβ40-like" distribution. In addition, the modified Aβ40 was significantly more toxic than wild type Aβ40. Substitutions in Aβ42 that were predicted to destabilize the turn abolished hexamer and dodecamer formation and resulted in an Aβ42 oligomer size distribution similar to that of Aβ40. Our experiments appear to define the structural determinant that "makes Aβ42 Aβ42." If true, this structure would be an exceptionally important therapeutic target.

2366-Pos

A Solid-State NMR Study Reveals Structure and Dynamics in Copper(ii)-Binding to Alzheimer's Beta-Amyloid Fibrils

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β-amyloid (Aβ) peptide associated with Alzheimer's diseases exhibit neural toxicity upon aggregation. One of the most widespread hypotheses on the origin of the toxicity of Aβ aggregates is the binding of Cu^{2+} ions to Aβ fibrils and subsequent generation of reactive oxygen species (ROS) such as H_2O_2 by Cu^{2+}-bound Aβ. Although a variety of studies have been performed on Cu^{2+}-binding to Aβ, the proposed binding sites or models have been controversial partly because non-crystalline and insoluble nature of Aβ fibrils have limited access to site-specific structure and dynamic properties on Cu^{2+}-bound Aβ fibrils. Here, we examine the effect of Cu^{2+} binding to amyloid fibrils of 40-residue Aβ(1-40) by UV-VIS spectroscopy and solid-state NMR (SSNMR). Specifically, we will answer the following questions (i) Is the Cu^{2+} binding is site specific? (ii) If so, which sites are involved in binding? (iii) Are there any major structural changes introduced by Cu^{2+} binding or oxidization due to Cu^{2+}?

UV-VIS spectroscopy showed that Cu^{2+} binds to Aβ(1-40) fibrils almost completely when the ratio of Cu^{2+} to Aβ(1-40) is less than 1. Based on the result, we performed high-resolution SSNMR experiments on Cu^{2+}-bound Aβ(1-40) fibrils. First, the ^{13}C T_1 paramagnetic relaxation enhancement (PRE) due to Cu^{2+} binding on Aβ was measured for different residues; the PRE data highlight possible binding sites, where the relaxation enhancements are notable. The analysis indicates that the binding is specific, and Cu^{2+} most likely binds to His-13/14 and His-6. Second, the comparison of 2D $^{13}C/^{13}C$ correlation spectra of Aβ fibrils with and without Cu^{2+} revealed that the secondary structure of Aβ(1-40) fibrils is largely unaltered by Cu^{2+} binding. Third, we tested

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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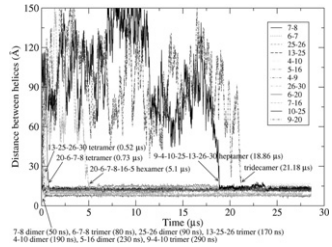
To gain insight into the aggregation and oligomerization of antimicrobial or amyloidogenic peptides, we carried out molecular dynamics simulations of a 26-residue amphipathic peptide at different concentrations (8, 16, and 32 copies) in a fully hydrated bilayer composed of 1600 POPC lipid molecules. 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.