1311-Pos

Molecular Basis for the Catalysis of B-Sheet Formation by Water-Nonpolar Interfaces

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Recent studies suggest that the toxicity of several neurodegenerative pathologies such as Alzheimer's and Parkinson's diseases involves the interaction of oligomeric aggregates of amyloidogenic proteins with the neuronal membrane. We examine the physical and structural basis of peptide adsorption and aggregation in a model membrane using molecular dynamics simulations. Blocked amphipathic octapeptides with simple, repetitive sequences, (Gly-Ala)₄ and (Gly-Val)₄, are used as models of β-sheet-forming polypeptide chains found in the core of amyloid fibrils. Placed in aqueous solution in the presence of an n-octane phase mimicking the nonpolar core of lipid membranes, the peptides spontaneously partition at the octane-water interface. The adsorption of nonpolar sidechains displaces the conformational equilibrium of the peptides from a heterogeneous ensemble characterized by a high degree of structural disorder towards a more ordered ensemble favoring β -hairpins and elongated β strands. Once adsorbed at the interface, peptides spontaneously aggregate and rapidly evolve β-sheet structure on a 10-to100-ns time scale, while aggregates of the same peptides in water remain amorphous. The catalysis of β -sheet formation at the water-membrane interface results from the combination of the hydrophobic effect and of reduced conformational entropy of the polypeptide chain. While the former drives interfacial partition and displaces the conformational equilibrium of monomeric peptides, the planar interface further facilitates β-sheet organization by increasing peptide concentration and reducing the dimensionality of self-assembly from three to two dimensions. These findings have general implications to the formation of β-sheets on the surface of globular proteins and to amyloid self-organization on the surface of biological membranes.

1312-Pos

Oligomerisation, Fibrillation and Activity of Hen Lysozyme in Alkaline Medium: A Concentration Dependent Investigation

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Oligomerisation and fibrillation are hallmarks of protein misfolding diseases like Alzheimer's, Prion, Parkinson's, systemic amyloidosis and so on. Hen eggwhite lysozyme (HEWL) which spontaneously aggregates at pH 12.2 under room temperature as shown by us previously is an excellent model protein for aggregation studies. The present work was focussed on investigating the role of protein monomer concentration on the size and morphology of aggregates. For this purpose we employed HEWL concentrations ranging from 120 µM to 300 nM. Our findings reveal that A) FRET efficiency between dansyl labelled HEWL and dabcyl labelled HEWL in the aggregate, monitored over 12 hours was inversely proportional to HEWL monomer concentration B) Size exclusion chromatography after 12 hours showed that while all aggregates of 50 & 120 uM HEWL eluted much later, a small population of aggregates from 10 uM HEWL eluted early through the void volume C) Binding of ANS revealed fluorescence spectra that were gradually blue shifted and more intense, over 12h, in the following order with aggregates of 120 > 50 > 3 uM, while for 300 nM, these spectra were fairly invariant in emission wavelength and intensity over 12 hours. D) HEWL enzymatic activity decreased almost uniformly with time in alkaline pH for all concentrations suggesting a concentration independent early unfolding step E) AFM images showed extensive fibrils in 3 & 0.3 µM HEWL within 12 hours but predominantly large globular aggregates with 120 μM and 50 μM HEWL samples in the same time period. The above results clearly suggest that size and morphology of HEWL aggregates at alkaline pH are critically dependent on the initial monomer concentration.

1313-Pos

The Addition of an Osmolyte, Trimethylamine N-Oxide Promotes Secondary Structure Heterogeneity Among Amyloid Fibrils Melissa J. Paulite, Nikhil Gunari, Gilbert C. Walker.

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We demonstrated that secondary structure heterogeneity was observed among mature amyloid fibrils synthesized by the addition of an osmolyte, called trimethylamine N-oxide (TMAO) by promoting the formation of anti-parallel β -sheet structure along with parallel β -sheet structure. In regards to amyloid fibrils formed in the absence of TMAO, only parallel β -sheet secondary structure was observed. This structural effect was observed regardless of pH. The amyloid fibrils were investigated via Fourier-transform infrared spectroscopy and near-field microscopy, and the characterized by circular dichroism and

UV-Vis spectroscopy. Using FTIR spectroscopy, the peak observed at $1630\,\mathrm{cm}^{-1}$ indicates the presence of parallel and/or anti-parallel β -sheet structure and a peak at $1692~\mathrm{cm}^{-1}$ denotes the presence of anti-parallel β -sheet structure. This latter peak, which appears only in the TMAO-containing fibril solutions, indicates that TMAO promotes anti-parallel β -sheet fibrils in solution.

1314-Pos

Structural and Functional Analysis of Amyloid Fibril Formation by Two Closely Related Light Chains

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Light chain amyloidosis (AL) is a hematological disorder in which a clonal population of B cells expands and secretes enormous amounts of immunoglobulin light chain protein. These light chains misfold and aggregate into amyloid fibrils, leading to organ dysfunction and death. In AL the sequence of the light chain is unique for each individual, giving rise to a highly variable course of disease. We are studying two proteins, designated AL09 and AL103, derived from the κ I O18:O8 germline that are highly similar in sequence, yet had significant differences in the disease phenotype. We have studied the *in vitro* kinetics of fibril formation and the structure of the resulting fibrils in order to explain this phenomenon.

We have begun by undertaking a systematic study of different solution properties and co-solutes that may affect fibril formation in these two proteins. We find that even though the proteins have similar thermodynamic properties, their fibril formation behavior is very different. AL09 readily forms fibrils under virtually every condition studied, while AL103 forms fibrils both more slowly and under fewer conditions. We have also explored the potential role of different glycosaminoglycans (GAGs) in the fibril formation process, specifically looking at the role of the size and charge of the GAG molecules. Furthermore, we have analyzed fibrils formed by these two disease proteins using limited proteolysis and mass spectrometry and have determined that in spite of their different phenotype the fibrils share a significant portion of their amyloid-forming core residues. Further structural studies are ongoing to determine how proteins with such different fibril formation kinetics can share a common amyloid structure.

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1315-Pos

Instantaneous Fibril Formation of α -Synuclein by Lateral Association of the Preformed Oligomeric Granules

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Elucidation of underlying mechanism of amyloidogenesis is a central issue not only for developing prophylactic and therapeutic means against amyloid-related diseases including Parkinson's and Alzheimer's diseases, but also for utilizing protein fibrils as nanomaterials for future nanobiotechnology. While the nucleation-dependent fibrillation is the prevailing notion to illustrate the amyloid fibril formation, we demonstrate an alternative mechanism of amyloidogenesis with α-synuclein, a pathological component of Parkinson's disease, in which the amyloid fibrils are formed via lateral association of the preformed oligomeric species of α-synuclein by acting as an assembling unit. Homogeneous oligomeric granules of α-synuclein were isolated in the middle of the lag period during the fibrillation kinetics. In the presence of an organic solvent of hexane, the granules instantaneously turned into the fibrillar structures which are indistinguishable from the amyloid fibrils obtained without the hexane treatment. Moreover, accelerated fibril formation of α-synuclein was also observed by imposing shear force on the granular structures by either centrifugal filtration or rheometer. Both organic solvent and physical influence might cause granules to experience structural rearrangement, resulting in the granular assembly into amyloid fibrils. As consequence, we propose a double-concerted fibrillation model to explain the *in vitro* fibrillation of α -synuclein, in which two consecutive associations of monomers and subsequent oligomeric granules are responsible for the eventual amyloid fibril formation.

1316-Pos

New Fluorescent Probe for Continuous Monitoring of Alpha-Synuclein Aggregation

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The aggregation of the presynaptic protein alpha-synuclein (AS), associated with Parkinson's disease, results in fibrils with a cross-beta-amyloid structure. Thioflavin T (ThT) is widely used for the identification and quantification of