peptide. This peptide can be present as monomers with soluble disordered structure, which change conformation into partially folded intermediates and further assemble to form dimers, trimers, oligomers, protofibrils and larger fibrils. Earlier studies have suggested that neurotoxicity of the disease lies not in the insoluble fibrils but in the formation of soluble oligomers, which impair and alter neurotransmision. Thus, the solution conformation of Aβ is of significant interest for understanding the molecular mechanism of Aβ fibrillogenesis. In the present study we have investigated the process of fibril formation in the 25 residue Aβ(10-35) which has a similar fibril structure as $A\beta_{40}$ and $A\beta_{42}$. In addition, Aβ(10-35) contains the central hydrophobic cluster (residues 17-21) suspected to initiate folding.

We have employed fluorescence, circular dichroism (CD) and time-dependent UVRR spectroscopic methods to explore the fibril formation process in vitro. Thioflavin-T induced fluorescence and CD studies indicate a conformational transition from an unfolded to β-sheet structure. UVRR studies conducted with 215 nm and 230 nm excitation facilitate characterization of the two phenylalanine and one tyrosine residues in the peptide, respectively. It is observed that formation of soluble Aβ oligomers is accompanied by a decrease in the intensities of the Phe vibrational mode, v8a (1606 cm^{-1}) and the Tyr vibrational mode, v8a (1617 cm^{-1}) over a 150 min incubation period. These results indicate that fibril formation proceeds via phenylalanine and tyrosine π-stacking interactions, which stabilize parallel β-sheets and reduce solvent exposure. Based on these results we have proposed a probable mechanism of fibril formation in Aβ(10-35).

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Amyloid Beta Oligomer Formation Analysis by Photon Counting Histogram

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Amyloid beta (Ab) is 4 kDa peptide which is thought to form aggregates such as oligomers and fibrils, and to cause Alzheimer disease (AD). Recently, it has been suggested that soluble Ab oligomers are the causative agent of AD since such oligomers are more cytotoxic than fibrils. It was also suggested that Ab oligomers affect not only cell death but also early stage of cell dysfunction and cause memory loss.

However, the mechanism how soluble oligomers are produced is still unknown. In this study, we analyzed formation of Ab oligomers in vitro at a single molecule level using photon counting histogram (PCH)(Chen, Y. et al. (1999) *Biophys. J.* 77, 553-67; Terada, N. et al. (2007) *Biophys. J.* 92, 2162-71.). Using PCH method, the number of protomers in oligomers and concentrations are obtained from histograms of photons from fluorescent molecules. Combination of a conforcal optics and a photon counting sensor enables us to catch the fluorescence from molecules diffusing through the conforcal volume at PCH. The concentration distribution of oligomers can be calculated from histograms.

Fluorescent intensity of fluorescein-labeled Ab monomer (FL-Ab) was evaluated using PCH. After 30 min incubation of FL-Ab in buffer solution, dimer fraction was successfully observed with PCH, assuming that fluorescent intensity is in proportion to the number of protomers in oligomers. Detailed analysis of formation of Ab oligomers such as amyloid-beta derived diffusible ligands (ADDLs) using these techniques is in progress.

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The Aggregation of Aβ 16-22 Probed by Circular Dichroism and Infrared Spectroscopies

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The misfolding of certain proteins into β-sheets and their ensuing aggregation into fibrous networks that accumulate to form insoluble plaques has been found to be related to several neurodegenerative disorders such as Alzheimer's Disease, Huntington's Disease, and the spongiform encephalopathies. The structural details of these protein aggregates and their mechanisms of aggregation, however, have not yet been fully understood. This research analyzes the structure and misfolding processes of a fragment of the β-amyloid (Aβ) polypeptide comprising residues 16 - 22 (KLVFFAE). Aβ 16-22 aggregates by forming intermolecular antiparallel β-sheets; lamination of these sheets results in formation of fibrils or micro-crystallite structures. We have probed the conformation of Aβ 16-22 as a function of concentration, temperature, and solvent using circular dichroism (CD) and infrared (IR) spectroscopies. The data show that, as the concentration increases, the conformation of Aβ 16-22 changes from a random coil to a β-sheet to higher-order structures; these higher order structures have a unique CD signature, with enhancement of the positive feature at ~200 nm. Addition of hexafluoroisopropanol (HFIP) disrupts the β-sheet structure, and the kinetics of this change can be observed spectroscopically. Overall, these observations allow us to construct a model of the mechanism of Aβ 16-22 aggregation.

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Conformational Change Induced In A Random Coil Peptide By Prion Peptide Aggregates

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The *in vivo* aggregation of many different polypeptides into β-sheet-rich amyloid fibers is associated with a range of diseases. The mechanism of aggregation for many of these proteins is still unknown; small polypeptides are useful models for exploring this process by both physical and computational methods. H1, a peptide derived from residues 109-122 of the Syrian hamster prion peptide (Ac-MKHMAGAAAAGAVV-NH_2) forms antiparallel β-sheet aggregates in solution. As determined by isotope-edited infrared (IR) spectroscopy, the H1 β-sheets have three overhanging residues at the N-terminus, with residue 117 aligned in all strands (1). The A117G mutant, however, exists nearly entirely as monomer as a result of backbone flexibility and the inability to align across residue 117 (2). In this study, the results of mixing wild-type H1 and A117G were monitored by Fourier Transform infrared spectroscopy (FTIR). Regardless of their relative concentrations, combining H1 and A117G resulted in the spontaneous, irreversible conversion of A117G from its unordered form to β-sheet by a mechanism in which the H1 peptides are proposed to serve as a nucleating template. Isotope-edited IR studies suggest that the β-sheet formed after mixing H1 and A117G is composed of alternating strands from each peptide variant. This process occurs spontaneously over time, and its rate is accelerated with increasing temperature. This work suggests a model for the templating of an unordered polypeptide into a β-sheet fibril.

(1) Silva, R.A.G.D., W. Barber-Armstrong and Decatur, S.M. (2003) *J. Am. Chem. Soc.* **125**: 13674.
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Successful de novo conversion from [psi−] to [PSI+] Saccharomyces cerevisiae

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Prion protein disorders, such as transmissible spongiform encephalopathies (TSEs) and Creutzfeldt-Jacob Disease (CJD), are caused by amyloid aggregation within a cell. The amyloid aggregates are proteinaceous, rich in β-pleated sheets, and characterized by protease resistance. *Saccharomyces cerevisiae* naturally expresses Sup35, a protein that spontaneously aggregates and forms intracellular amyloid fibers. Sup35 exhibits multiple prion states: [*psi*$^-$], strong [*PSI*$^+$], and weak [*PSI*$^+$] phenotypes. Prior studies have induced multiple [*PSI*$^+$] phenotypes by using *in vitro* aggregation reactions of bacterially expressed Sup35NM at varying temperatures and then in some instances transforming *S. cerevisiae* with these aggregate ''seeds.'' By implementing a unique color assay, the different phenotypes may be visually identified as red, pink, and white colonies for [*psi*$^-$], weak [*PSI*$^+$], and strong [*PSI*$^+$], respectively. Here our research focused on inducing strong [*PSI*$^+$] and weak [*PSI*$^+$] prion states from [*psi*$^-$] cells without the use of preformed prion seeds. We have found that exposure of mid-exponential phase cultures of [*psi*$^-$] 74-D694 yeast to a 4°C environment for an extended period of time induced *de novo* an array of [*PSI*$^+$] phenotypes. Our current focus is on purifying the amyloids of these phenotypic variants and analyzing their secondary structure and conformations using a variety of techniques, including transmission electron microscopy and X-ray diffraction. We will also compare the structural data from the *de novo* amyloid with those of amyloid fibers that formed *in vitro* from bacterially-expressed Sup35NM.

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Conversion Of Antiparallel β-sheet To Parallel β-sheet In A Prion Peptide Aggregate

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Residues 109-122 of the Syrian hamster prion peptide (Ac-MKHMAGAA AAGAVV-NH_2) make up a conserved amyloidogenic portion of a protein associated with several neurodegenerative diseases. In previous studies using Fourier Transform infrared spectroscopy (FTIR) and isotope-edited infrared spectroscopy, H1 has been determined to form antiparallel β-sheets in solution with three overhanging residues at the N-terminus and alignment of residues 117 in all strands (1, 2). Some amyloidogenic proteins form antiparallel β-sheet