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Using a Peptide System to test the Coiled-Coil Model of Polyglutamine Aggregation

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Posters

Protein Assemblies & Aggregates II

1982-Pos Board B1
The Influence of Both Urea and 2,2,2-Trifluoroethanol in the Amyloid Fibril Formation of Bovine Serum Albumin
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The influence of external agents on proteins function and structure is essential to elucidate the unfolding pathways and self-assemble properties. The knowledge of the protein amyloid fibril formation process is important due to the fields that this subjected is related, in particular for the neurodegenerative disorders. In the present work we studied the influence of both urea and 2,2,2-Trifluoroethanol (TFE) and temperature on the structure and protein-protein interactions of Bovine Serum Albumin (BSA), by means of UV-Vis spectroscopy, static fluorescence and small angle X-ray scattering technique. The experiments were performed in samples composed by 10 and 3 mg/ml of BSA at pH 5.8, near the protein pI. First, Thioflavin-T fluorescence measurements indicated that urea, in the absence of TFE, was able to increase the amyloid fibril formation of BSA at 45oC and increasing the urea concentration the rate of amyloid fibril formation also increases. Concerning the presence of TFE, SAXS data suggest that BSA tridimensional structure is not altered by the presence of TFE 5% and 10% v/v in all studied protein concentrations. Interestingly, the presence of TFE on the urea-containing BSA also increases the rate of amyloid fibril formation, as compared to the TFE-free system, indicating that TFE can catalyze the amyloid-fibril formation. The presence of TFE 20% v/v, however, induces the formation of aggregates, but at this time we were not able to infer if such aggregates are amyloid-like or amorphous. Taking together, the results give support to infer that BSA can for fibrils in the presence of urea at 45oC and TFE can act as a stabilizer or as a denaturant agent for BSA.
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1983-Pos Board B2
Human Lysozyme Amyloidosis: MD Simulations Reveal Increased Structural Destabilization in Disease causing Mutants Compared to Wildtype
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D67H and I56T are the two known natural mutants of human lysozyme caused by autosomal dominant gene mutations. They both cause hereditary systemic nonneuropathic amyloidosis, a condition symptomatic with deposition of human lysozyme amyloid fibrils (sometimes in kilogram quantities) in the kidneys, gastrointestinal tract, lymph nodes, blood vessels, spleen and liver of patients [Pepys *et. al.*, *Nature*, 362, 553-557 (1993)]. The structural features of species formed early during the aggregation process, which trigger amyloidosis, appear important to investigate. In this study, we have compared the conformational dynamics of wild type and mutants of human lysozyme (D67H, I56T, and T70N) using all atom MD simulations under native conditions. All the four simulations (20 ns each) were performed using ff99SB Amber force field to investigate the conformational dynamics. We have analyzed the trajectories arising from these simulations to obtain insights on conformational features triggering amyloidosis. The analyses of backbone RMSD, B factor values, SASA, end to end chain distance, secondary structure content, distance matrix, S^2 order values, conformational entropy, water movement around residues in core domain and hydrophobic contacts all reveal greater structural destabilization in mutants in comparison to wild type human lysozyme. The higher β content in secondary structure, increased flexibility and disruption in hydrophobic contacts near the α/β domain interface and in β domain in I56T and D67H mutants perhaps leads to their amyloidogenicity. Our results also hint that Y38 residue at the hydrophobic core (near the α/β domain interface) to be the seed for fibril formation in mutants.

1984-Pos Board B3
Can Melatonin Help Prevent Alzheimer's Disease?
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In previous studies, the orthomolecular species melatonin has been found to have a tremendous impact on the β-amyloid peptide that causes Alzheimer's disease. Melatonin has been shown to inhibit oxidative stress and the death of neurons and neuroblastoma cells exposed to the peptide. The purpose of research on an orthomolecular species such as melatonin is to determine how the chemical substance reacts with a disease or abnormality by restoring proper levels of the chemical substance in the brain. The first step in the process of researching the effects of melatonin on the Alzheimer's β-amyloid peptide is to make melatonin water soluble. Studies suggest that melatonin powder can become water soluble when mixed with water at 20 °C or 50 °C. Studies have also shown that melatonin is soluble in ethanol. This study utilized all three techniques to create melatonin solutions. Melatonin in a fine powder was added to water at 20 °C , water at 50 °C , and ethanol at 20 °C. All three solutions were centrifuged to gather only the water soluble aspects of the melatonin solutions. The melatonin concentration of each solution was determined using a calibration curve. The calibration curve was created using a UV/vis machine to measure the absorbances of serially diluted solutions with known concentrations made from pure melatonin. The presence of melatonin in each solution was confirmed using ATR-IR spectroscopy by comparing spectra of each solution to a melatonin reference spectra. Each solution was combined with β-amyloid peptide and Congo red and measured in the UV/vis for a week. Each solution was combined with the β-amyloid peptide and measured with ATR-IR for a week. The data was then analyzed and compared to solutions with β-amyloid peptide and no melatonin.

1985-Pos Board B4
Effects of Silymarin on Aβ Fibrillization of Alzheimer's Disease
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In recent years, research has begun to be conducted on the effects that the compound Silymarin, a component of milk thistle and a current treatment for liver ailments, jaundice, and prostate cancer, has on the neurodegenerative disease of Alzheimer's. These studies have shown promising results with utilizing Silymarin as both a preventative and protecting agent against various neurodegenerative diseases. The aim of this current research is to further examine these possible effects on formation of fibrils in the brains of those afflicted by Alzheimer's. We aim to examine if this compound has any effect at either slowing or stopping the formation of fibrils all together and if so to what extent.

1986-Pos Board B5
Using a Peptide System to test the Coiled-Coil Model of Polyglutamine Aggregation
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We test a recent hypothesis from the literature that proposes a role for helical interactions in polyglutamine aggregation. We use as our model system a peptide sequence based on the GCN4-pLI parallel coiled-coil tetramer, with lysines at all e and c heptad positions, fused to a short polyglutamine of 25 residues. The coiled-coil sequence will bind preferentially to a second peptide, ecE, which contains glutamates at the same e and c heptad positions, to form a stable tetramer. using CD, FTIR, and DLS methods, we show that the lysine-containing peptide, ecK-Q25KK, when studied alone, shows a significant decrease in aggregation kinetics when compared to a control peptide, KKQ25KK. We show that ecK-Q25KK aggregates via a coiled-coil rich intermediate over a period of a week, and then transitions to a cross beta-sheet conformation after an additional week. Such a transition is not observed when ecE peptide is present, presumably due to increased stabilization of the coiled-coil conformation. We added a proline-containing linker between the coiled-coil sequence and the polyQ sequence to test whether inhibition of helix propagation would influence the kinetics of this assembly reaction. We found that the addition of the linker significantly slowed down the assembly kinetics. We also studied the morphology of the resultant fibrils, using AFM and TEM, and found that the fibrils are quite similar to those observed previously for the KKQ25KK model system. Our results are surprising since the natural N-terminal sequence from the huntingtin protein significantly accelerates beta-sheet aggregation, also in a model involving coiled-coil interactions. The difference in our results from these earlier experiments may lie in the stability of the coiled-coil interactions, with our coiled-coil sequence (28 residues) being longer than that found in the huntingtin sequence (17 residues).

1987-Pos Board B6
Structural Basis for Ternary Complex Formation between Tau, Hsp90, and FKBP51
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The normal function of the microtubule associated protein tau is to bind and stabilize microtubules (MT) via its C-terminal assembly domain. When tau dissociates from MTs it can be phosphorylated and targeted for proteasomal degradation. Hyperphosphorylated forms of tau can form aggregates that are thought to be toxic in Alzheimer's Disease. The molecular chaperone