We suggest that antiparallel β-sheet structure might represent a distinctive signature of amyloid oligomers (Cerf et al, Biochem J, 2009, 421:415-23) underlying their common pathogenic action.

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Amyloid Beta Peptide: The Influence of Intrinsic Factors on Fibril Formation

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Alzheimer's disease (AD) is the most common form of dementia. Studying the amyloid β-peptide (Aβ) involved in the pathogenesis and understanding the mechanism of the disease will hopefully lead to developing drug candidates that could cure or delay the onset of AD.

One way of approaching this is to inhibit the fibril formation. Mutating the peptide gives an insight on which amino acids are important in the aggregation mechanism. We have investigated the importance of the aromatic rings in the phenylalanine side chains in position 19 and 20 by substituting these with leucine. This way the hydrophobicity is preserved while aromatic stacking possibilities are removed. Single and double mutants were expressed in *E. coli* from synthetic genes. The kinetics of fibril formation was followed using a highly reproducible thioflavin T assay and show that Aβ(M1-42)F19L aggregates slower than the wild type, mainly observed as a longer lag time. Also the structural change, monitored by far-UV circular dichroism spectroscopy, differs between wild type and all mutants. This indicates that the phenylalanine rings have a role in the amyloid formation. With cryo-TEM it was confirmed that all peptides form fibrils with similar morphology.

The influence of the slower F19L mutant was further studied in co-aggregation experiments together with the wild type. Preliminary results show that the lag time of the mixture ends up in between those of the wild type and the F19L mutant, and that the lag time depends on the ratio of the two peptides as well as the total concentration. Further co-aggregation studies include a familial mutant peptide with faster aggregation kinetics than the wild type, as well as seeding experiments in which wild type is seeded with fibrils from F19L or the familial mutant.

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The Effect of a Membrane Mimicking Detergent on Alzheimer's Amyloid Peptide Aggregation Studied by EPR

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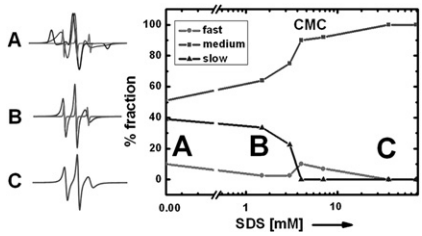
The aggregation of the β-Amyloid (Aβ) peptide into fibrils is the chief indicator of Alzheimer's disease. However, it seems that small, oligomeric aggregates, rather than fully formed fibrils could be the toxic species. Aβ -membrane interaction could also have an effect, so here effect of the membrane-mimicking detergent SDS is studied.

We measure, by spin-label-mobility EPR, Aβ aggregation at different SDS concentrations. It monitors all peptide in the sample and is not limited to particular aggregate sizes. The high-SDS form found (C) is consistent with the one-peptide/micelle model [1-2]. In the absence of SDS (A) the high concentration of Aβ (0.55 mM) most certainly enforces fibrils. At intermediate SDS (B), EPR reveals an increase in particle size suggestive of oligomer formation, eventually involving detergents. Thus a first glimpse into the behavior of SDS at sub-CMC (Critical Micelle Concentration) SDS concentrations, where high resolution techniques, such as NMR, fail because of particle size limitation, [1-2] are accessible.



[1] FEBS, Wahlström et al. (2008) 275: 5117-5128.
[2] J. Biomol NMR, Jüri et al. (2007) 39: 63-72.

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A Conventional and 2DCOS Infrared Study of Amyloid Fibril Formation

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Amyloidosis is characterized by the abnormal self-assembly and deposition of proteinaceous material into insoluble ordered aggregates. There are several pathologies associated with this aggregates, known as ''protein misfolding'' disorders. In many proteins, when heated at high temperature and low pH, a series of structural changes resulting in the formation of fibrillar structures are produced. Insulin is a model of fibril formation that has produced a wealth of biochemical and structural data. The time-course of fibril formation can be followed by infrared spectroscopy looking at the appearance of a characteristic band in the amide I region. The kinetics is triggered by temperature at 70 °C and pH at 2.3. The infrared spectrum shows, that after a lag time (concentration-dependent), α-helix and β-turns bands decrease first whereas the random coil component increases. Later, a band at 1626 cm-1, associated with extended chains, replaces the random coil component. Infrared 2D-COS has been applied to different stages of the process. Maps have been formed at different incubation times: before random coil formation and at different stages in the random coil-fibril exchange. The synchronous maps do not give in principle much information because they are dominated by the appereance of the band due to the fibril. In order to extract such information we have developed an approach in which we take a window and move it along the perturbation (2D-moving lapse). This, together with the asynchronous maps indicate a two-step process with a first stage associated with an opening of the protein driven by destabilization of β-turns, located in the outer part of the protein, and a second part where the fibril is formed.

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2239-Pos Board B9

Mechanism of Non-Specific Inhibitors of Amyloid Assembly: Interactions of Lacmoid with the Amyloid Beta Peptide

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Increasing evidence shows a strong link between the self-assembly of the amyloid beta peptide (Aβ) and the pathogenesis of Alzheimer's disease. Soluble oligomeric Aβ assemblies are thought to be the toxic species causing synaptic and neuronal injury in the patient's brain. Many inhibitors for the oligomerization and/or fibrillation process of neurodegenerative diseases have been reported, yet only little is known about the mechanistic details of these compounds. The present studies concern the interaction of one such inhibitor, lacmoid. We investigated this interaction by a broad biophysical approach revealing similar binding characteristics to Aβ as has been reported for detergents. Furthermore, we show that lacmoid has the ability to inhibit both oligomeric assembly and fibrillation of Aβ. Nuclear magnetic resonance experiments show an overall signal decrease upon addition of lacmoid while the chemical shifts only display small changes. High lacmoid concentration causes a loss of the major part of NMR signals including ^{1}H-^{15}N-HSQC, ^{1}H-^{15}N-TROSY and ^{1}H-^{13}C-HSQC cross-peaks. Circular Dichroism spectroscopy was applied to monitor the kinetic aggregation process of Aβ in presence of lacmoid. Low lacmoid concentrations slow down the conversion from a random coil-like towards a beta-sheet state while at high concentration lacmoid completely prohibits secondary structure changes.

Taken together, these findings provide the basis for a simple model which could explain how non-specific interactions with small molecules interfere with amyloid formation. Understanding the mechanistic details is potentially helpful for future drug design of small molecule therapeutics targeting amyloid disorders, such as Alzheimer's disease.

2240-Pos Board B10

Understanding Formation and Structure of Peptide Nanofibers via Steered MD Simulations

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Understanding the formation and structure of fibers formed via peptide aggregation is a major challenge for both prevention of diseases and also for utilising such structures for nanostructure templating. Here, we propose that single molecule pulling experiments can be used to characterise the mechanical and structural properties of these fibers. We demonstrate this idea via molecular dynamics simulations on fibers formed by a library of triblock peptides. In these oligomers, hydrophobic residues in the central block are replaced to control the resulting fibers. Our results suggest that, independent of the hydrophobic residue type, all these peptides yield an inter-dimer spacing of ~ 0.48 nm along the fiber axis. Hence, backbone hydrogen bonding alone dominates and the hydrophobic residues simply squeeze into the hydrophobic core of these fibers. We measure the relative strength of these fibers via steered MD simulations. Our results suggest that hydrophobic residues such as tyrosine and tryptophan not