Final Scientific Report
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1. Epitaxial assembly dynamics of mutant amyloid ß25-35_N27C fibrils explored with time-resolved scanning force microscopy

Amyloid ß25-35 (Aß25-35) is a toxic fragment of Alzheimer's beta peptide. We explored the kinetics of epitaxial assembly of the mutant fibrils at different peptide and KCl concentrations by using *in situ* time-resolved AFM. We measured the length of Aß25-35_N27C fibrils as a function of time. Increasing free peptide concentration enhanced fibril growth rate, and the critical peptide concentration of fibril assembly was 3.92 μM. Increasing KCl concentration decreased the number of fibrils bound to the mica surface, and above 20 mM KCl fibril formation was completely abolished even at high peptide concentrations. By modulating peptide and KCl concentrations in the optimal ranges established here the complexity of the Aß25-35_N27C network can be finely tuned. A paper was in the international journal Biophysical Chemistry.

2. Comparison of epitaxially and solution-grown amyloid ß25-35 fibrils

We compared the structure and dynamics of solution- versus surface-grown Aß25-35 fibrils with atomic force microscopy, force spectroscopy and Fourier transform infrared (FTIR) spectroscopy methods. The nanomechanical behavior of Aß25-35 fibrils was characterized by the appearance of force staircases which correspond to the force-driven unzipping and dissociation of protofilaments. Both fibrils showed similar nanomechanical responses, the elementary plateau force was 30 pN. The fibrils showed clear differences in their morphology and formation kinetics. We used FTIR spectroscopic method to study secondary structural composition of fibrils. The IR spectrum showed an intense peak for both fibrils in the range indicative of beta-sheet structure: 1630 cm^{-1} for epitaxially-grown Aß2535 and 1623 cm^{-1} for fibrils assembled in solution. The shift in the amide I band is sensitive to the hydrogen bond distances, smaller wavenumbers corresponding to more compact structures. Based on our results we conclude that the structure of the epitaxially grown respective in solution grown fibrils is slightly different: solution grown fibrils are more compact structures, the interaction between the mica surface and Aß2535 peptides loosens the interfibrillar structure. In contrast to the presumably helical structure the fibrils grown in solution the epitaxially grown fibrils form a linear, sheet-like structure. A paper was published in BBA Proteins and Proteomics.

3. Highly oriented, functionalized nanoarray based on epitaxially grown amyloid beta 25-35 fibrils

To functionalize Aß25-35_N27C fibrils for nanotechnology applications, we used either maleimido-nanogold (1.4 nm), or maleimido-NTA followed by GFP-His6 binding. The structure of the functionalized network was investigated with AFM. In the nanogold-labeled amyloid network spherical particles of 1.4-nm diameter lined up along the oriented fibrils. In the GFP-functionalized network the surface of the fibrils was covered with spheroid partices of approximately 4-nm topographical height. The density of the particles could be adjusted by varying the ratio of the wild-type and mutant peptides. The functionality of the amyloid-bound GFP was tested with epifluorescence microscopy. We could demonstrate that the fluorescence properties of GFP were retained in the amyloid network. Thus, the oriented amyloid network may be used towards the construction of functional biomolecular nanoarrays. In further experiments we began labeling the mutant amyloid nanoarray with Dictyostelium discoideum myosin motor domain molecules. The motor proteins show up in the AFM images as globules along the mica-adsorbed fibrils. We are currenetly invetigting the motile functionality of the myosin molecules in the in vitro motility assay. Several conference abstracts were published on the above topics in Biophysical Journal.

4. Measuring and modeling the atomistic detail in amyloid binding to mica.

The oriented amyloid network formed on mica suggests that the fibrils follow the lattice structure of mica during the formation of fibrils. This so called epitaxial growth is determined by the underlying crystal structure and is influenced by the properties of the peptide. Mica has a hexagonal surface lattice structure which explains the trigonal orientation of the filaments. However, it is unclear which symmetry axis of the hexagon is followed by the fibil growth axis. There are two possibilities for this: the fibril axis is parallel either with the vertex-to-vertex or side-to-side axes of the crystal-lattice hexagon. To resolve this matter experimentally, atomic resolution imaging is required while at the same time being able to image the amyloid fibrils. We recently acquired an atomic resolution AFM which enables to image te fibrils and the mica surface at unprecedented resolution. Our results indicate that the fibril axis is parallel to the axis drawn between opposite sides, rather than the vertices, of the Si-atom-based hexagon. Molecular-dynamics simulation has supported this finding. Thus, the Aß25-35 peptides attach to the mica surface with their Lys28 side chain and form a fibril the axis of which runs along the side-to-side axis of the Si-atom hexagon. A manuscript is in preparation on this work, and it is anticipated to be submitted within a few weeks.

5. Development of composite systems with carbon nanotubes

In a collaborative effort we have been attaching the photosynthetic reaction center protein to carbon nanotubes so as to generate functional nanostructures. In this model system the oriented amyloid nanoarray is essentially replaced by a random array of conductive carbon nanotubes. These experiments shed light on the possibility of incorporating optically reactive and possibly switchable protein systems into a composite environment in such a way that the protein functionality is retained. We believe that these results will be pivotal in advancing the integration of optically switchable protein systems into the amyloid-based nanoarrays systems. Three papers were published on these results in the international journal Phys. Status Solidi B.

6. Development of microfluidic systems for the analysis of biomolecular and polymer gel systems.

The possiblity of carrying out detailed and precise analytics in combination with our self-assembled biomolecular systems or other gel-based systems is pivotal. For this purpose we have been exploring the combination of custom-built microfluidic devices into which various biomolecular systems can be conveniently integrated. We managed to construct such devices in a variety of forms. Furthermore, these microfluidic devices were successfully incorporated into soophisticated microscopic systems such as bright-fielf video microscopy, optical tweezers and epiflurescence. Two papers were published on our results in the international journals Entropy, and Sensors and Actuators B.

7. Exploration of self-asembling filamentous biomolecular polymer systems

In conjunction to our amyloid-based self-assembling systems we have been investigating other self-assembling biomolecular polymer systems as well. The exploration of such systems improve the understanding of the biomolecular logic behind the mechanisms of self-assembly, and facilitate the possible exploitation of the observations. We have made original observations on the giant filamentous protein titin, the intermediate filament desmin and the exracellular polymer system of fibrin. In titin we discovered domains which apparently are able to unfold and refold at low, physiologically relevant force levels. Furthermore, we developed an AFM-based screening system that allows us to monitor the spatial location of the mechanicaly unfolded domains along the molecule. In desmin we uncovered a novel depolymerization intermediate. Finally, we developed an AFM-based nanothrombelastographic method for monitoring the microscopic mechanisms of fibdin assembly ad disassembly. Our research results were published in several prime international journals: J. Cell Sci, PPLoS ONE, J. Struct Biol.

Budapest, 12. February, 2015. Miklós Kellermayer, PI