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Aggregate Size and Shape Distributions in Amyloid-β Peptide Solutions

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A peptide with 42 amino acid residues (Aβ (1–42)) plays a key role in the pathogenesis of the Alzheimer's disease. Aggregation of this peptide leads to the formation of assemblies of different sizes and shapes, among which the amyloid fibril is the most prominent member. We used sedimentation velocity centrifugation to analyze Aβ (1–42) peptide solutions at different time points during aggregation regarding their hydrodynamic properties. New data evaluation software allowed us to determine not only sedimentation coefficients, but also at the same time the related frictional ratios of species present in a multicomponent system. Shortly after sample preparation, a sharp peak dominated the measured s-value distribution. Two-dimensional spectrum analysis assigned this species an s-value of $28 \cdot 10^{-13}$ s, a molecular weight of $1.23 \cdot 10^6$ and a frictional ratio of 1.44. Under the assumption of a rigid rod model this structural information is in good agreement with a fibril of 50 nm length build out of two neighboring β-sheets with about 110 monomers each, arranged in a parallel in-register fashion. Incubation for 1, 2 and 5 days causes further aggregation, which results in decreasing frictional ratios, implying a growth to more spherical-like particles.

1 Introduction

A peptide with 42 amino acid residues (Aβ (1–42)) plays a key role in the pathogenesis of the Alzheimer's disease. It is highly prone to self aggregation, leading to the formation of fibrils which are deposited in amyloid plaques in the brain of affected individuals[1]. In previous years increasing evidence arose that probably smaller oligomeric assemblies[2,3] play a more decisive role as neurotoxic agents than the mature fibril. Information about size and shape of Aβ peptide assemblies formed during aggregation is therefore of high relevance.

Analytical ultracentrifugation is a method for retrieving structural information about macromolecules by direct observation of their hydrodynamic properties in a centrifugal field. Advanced data analysis permits the determination of s-value, molecular weight and shape distributions for multicomponent systems.

Our objective is to evaluate the applicability of analytical ultracentrifugation in combination with the newly developed data evaluation software for generating size and shape distributions of solutions of the Aβ peptide. The method shall be used to monitor the effects of peptide concentration, solvent and added cosolutes upon aggregation.

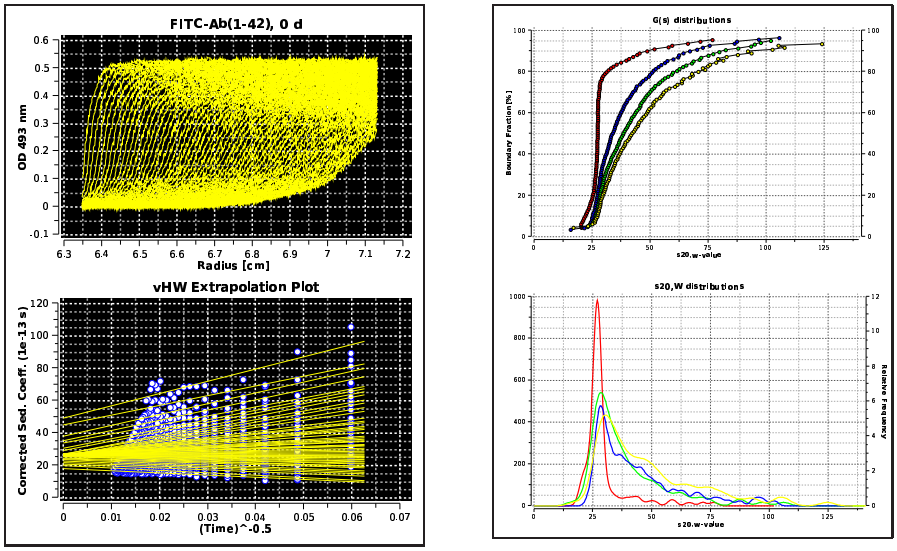

Figure 1. Van-Holde-Weischet Analysis. Left: Raw data (top) and corresponding vHW-analysis plot (bottom) for Aβ aggregation mixture 2 h after sample preparation. Sample volume was 300 μl. Right: Upper graph: G(s) distribution of sample after 0 d (red), 1 d (blue), 2 d (green), and 5 d (yellow) incubation at 20 ℃. Lower graph: s-value distribution of sample after 0 d (red), 1 d (green), 2 d (blue), and 5 d (yellow) incubation at 20 ℃.

2 Methods

The aggregation mixture contained 70 μM unlabeled and 14 μM FITC-labeled Aβ (1–42) in 10 mM sodium phosphate buffer, pH 7.4 with 6 % final DMSO concentration. Sedimentation velocity experiments were performed with an X-LA analytical ultracentrifuge (Beckman-Coulter), equipped with absorption optics. Samples were measured in standard double-sector aluminum cells at 20,000 rpm, 20 ℃. Radial step size was set to 0.001 cm.

Raw data from the analytical ultracentrifuge were processed and evaluated using the UltraScan software package[4] running on a 44 node AMD Opteron cluster under Linux. After determination of suitable start parameters by a van-Holde-Weischet analysis[5] the 2-dimensional spectrum analysis[6] (2DSA), implemented in the software package, was started. 2DSA solves the inverse problem of fitting sedimentation velocity data to a linear combination of finite element solutions of the Lamm equation. Each term of the linear combination reflects a solute in the 2-dimensional space over s and f/f_0. Finally Monte-Carlo simulations were used to identify statistically significant solutes.

3 Results

Data analysis of sedimentation velocity runs performed at 20,000 rpm, 20 ℃ at 4 time points during aggregation of Aβ (1–42) according to van-Holde-Weischet as depicted in Fig. 1 showed an increase of the mean s-value over the incubation time of 57 %. Aggregation of the amyloid-β peptide starts with a narrow distribution with an s_{avg}-value of 30 S. The distribution is dominated by a species of 28 S, a molecular weight of $1.22 \cdot 10^6$

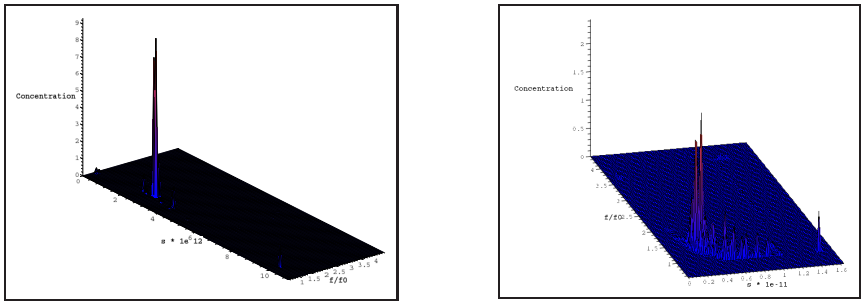

Figure 2. 3D-Plots of s-value *vs* f/f_0 distributions. Left: Aβ(1–42) after 2 h incubation at 20 °C. Right: Aβ(1–42) after 5 d incubation at 20 °C.

and a frictional ratio of 1.44, which comprises about 40 % of the sedimentation boundary (Fig. 2, left). This aggregate species is reproducibly detected in repeated experiments. It can be modeled by a rod-like particle with an axial ratio of 8.6. Under the assumption of a rigid rod model and molecule dimensions for β-strands this structural information is in very good agreement with a fibril of 50 nm length build out of two neighboring β-sheets with about 110 monomers each, arranged in a parallel in-register fashion, as proposed by[7].

Upon incubation, the measured distribution is asymmetrically broadening to higher s-values with a still distinguishable maximum at about 28 S. During the 5 d incubation of the sample, the f/f_0-value of detected solutes decreases from 1.6 to around 1 with s-values increasing from 28 S to 150 S. Particles are becoming more spherical during the incubation time within the observed growth range (Fig. 2, right). The method is a tool to study the action of drugs on the aggregation of Aβ (1–42) peptide.

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