Aggregate size and shape distributions in amyloid-β peptide solutions

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Summary

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 so-called amyloid plaques in the brain of affected individuals (1,2). During the last years increasing evidence arose that probably smaller oligomeric assemblies play a more decisive role as neurotoxic agents than the mature fibril (3,4). Information about size and shape of Aβ peptide assemblies formed during aggregation is therefore of high relevance.
We use sedimentation velocity (SV) centrifugation in an analytical ultracentrifuge to characterize Aβ (1–42) peptide solutions at different time points during aggregation. Aβ (1–42) aggregates in a reproducible manner to assemblies with s-values between 1 S and 100 S. Data evaluation via the 2-dimensional spectrum analysis (5) using linear combinations of finite element solutions of the Lamm equation combined with a Monte Carlo analysis as implemented in UltraScan (6) allowed us to determine s-value and shape distributions. Two to 4 hours after sample preparation, a very sharp peak dominates the measured s-value distribution. Two-dimensional spectrum analysis assigns 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 stick model and molecule dimensions for β-strands taken from (8,9) this structural information is in very well agreement with a fibril of 51 nm length build out of two neighboring β-sheets with about 110 monomers each, arranged in a parallel in-register fashion, as proposed by (8). Upon further aggregation for 1 d, 2 d and 5 d species with higher s-values appear with decreasing frictional ratios, implying a growth to more spherical-like particles.
Affirmed by these results we regard the method of analytical ultracentrifugation in combination with sophisticated data evaluation as a valuable tool to gain insights into the mechanism of protein aggregation. Different solvent conditions and cosolutes with regard to their effect on the measured size and shape distributions of the amyloid-β peptide will be studied soon.

Van-Holde-Weischet Analysis

At first raw data from sedimentation velocity runs are analyzed according to van Holde & Weischet (7) in a model independent manner to determine the degree of polydispersity of the studied solution. The observed range of s-values present in the solution is used as a constraint for further data evaluation via 2D-spectrum analysis.

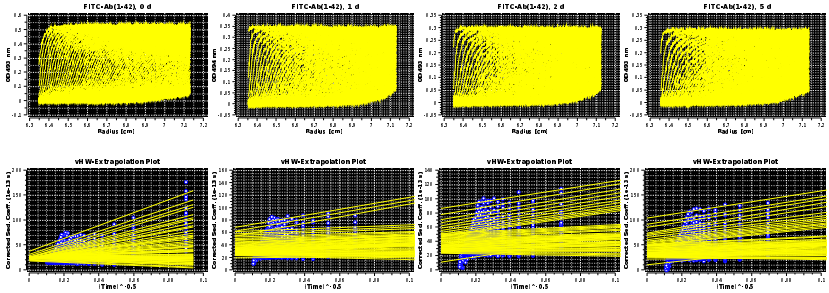


Fig. 1: Amyloid-β peptide aggregation. From left to right the measured raw data (first row) together with the corresponding vHW-analysis plot (second row) for a solution containing 70 μM Aβ (1-42) mixed with 14 μM FITC-labeled Aβ (1-42) in 10 mM sodium phosphate buffer, pH 7.4 and 6 % DMSO are shown for 0 d, 1 d, 2 d, and 5 d incubation at 20 °C. Sample volume was 300 μl. 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 °C. Radial step size was set to 0.001 cm, and only one cell per run was scanned at a 2-3 min time interval at 493 nm to collect as much data as possible.

Results

New data evaluation programs open up new vistas for the method of analytical ultracentrifugation. It is possible to extract from the raw data of a sedimentation velocity experiment not only the sedimentation-coefficient but also the relative quantity, ratio of frictional coefficients (f/f_0), and the molecular weight of each single solute sedimenting in a multicomponent system. We utilize this method to monitor the aggregational state of the Alzheimer's disease related amyloid-β peptide.
Data analysis of sedimentation velocity runs performed at 20,000 rpm, 20 °C at 4 time points during aggregation according to van-Holde-Weischet (Fig. 1 and table below) 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 measured distribution is dominated by a species of 28 S, a molecular weight of $1.22 \cdot 10^6$ and a frictional ratio of 1.44, which comprises at least 40 % of the sedimentation boundary. This aggregate species is reproducibly detectable in repeated experiments. It can be modeled by a rod-like particle with an axial ratio of 8.6.
Upon incubation at 20 °C 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.

	incubation time [d]			
	0	1	2	5
boundary height [OD_{493}]	0.54	0.35	0.30	0.30
average s-value [10^{-13} s]	30	39	44	48

Combined Van-Holde-Weischet Analysis

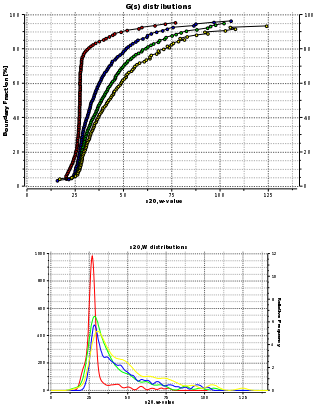


Figure 2: Amyloid-β peptide aggregation Upper graph: G(s) distribution of sample after 0 d (red), 1 d (blue), 2 d (green), and 5 d (yellow) incubation at 20 °C. Lower graph: s-value distribution of sample after 0 d (red), 1 d (green), 2 d (blue), and 5 d (yellow) incubation at 20 °C.

2D-SA with Monte Carlo Analysis: Aβ (1–42)-aggregation after 5 d incubation

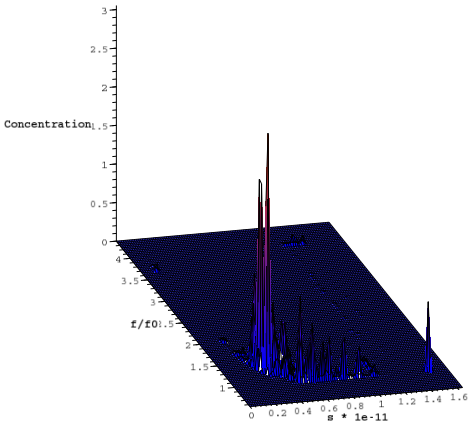
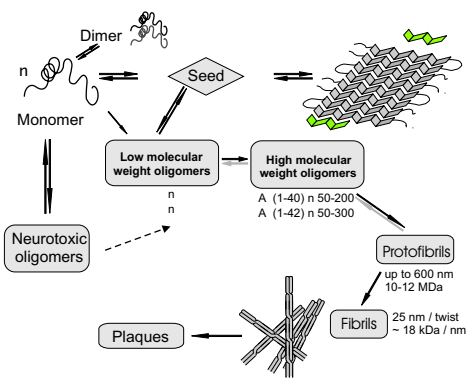


Figure 3: Aggregation of Aβ (1–42) after 5 d incubation. Evaluated SV data of 84 μM Aβ (1–42) in 10 mM sodium phosphate, pH 7.4, and 6 % DMSO. Centrifugation at 20,000 rpm, 20 °C. 50 Monte Carlo iterations.

Model for Aβ aggregation



2D-SA with Monte Carlo Analysis: Aβ (1–42)-aggregation after 2 h incubation

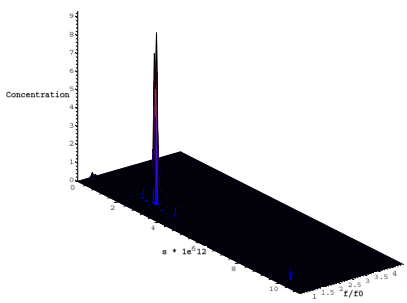


Figure 4: Aggregation of Aβ (1–42) after 2 h incubation. Evaluated SV data of 84 μM Aβ (1–42) in 10 mM sodium phosphate, pH 7.4, and 6 % DMSO. Centrifugation at 20,000 rpm, 20 °C. 50 Monte Carlo iterations

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