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Modulation of Aggregate Size and Shape Distributions of Amyloid-β Peptide Solutions by a Designed β-Sheet Breaker

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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. 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. In our study we established a method to analyze the aggregation behavior of the amyloid-β peptide with a combination of sedimentation velocity centrifugation and enhanced data evaluation software as implemented in the software package Ultrascan. Important information which becomes accessible by this methodology is the s-value distribution and concomitantly also the shape-distribution of the peptide aggregates generated in the process of self-association. These informations get especially valuable upon evaluating the properties of potential aggregation inhibitors. With this method we characterized the aggregation modifying effect of a small organic molecule, designed as a β-sheet breaker. This compound is built from three head-to-tail connected aminopyrazole moieties and represents a derivative of the already described Tripyrazole. The compound showed reduction of aggregate formation measured by FCS and decreased amyloid formation as measured by Thioflavin T measurements. By addition of this compound to a solution of the Aβ(1–42) peptide the maximum of the s-value distribution calculated for the formed amyloid-β aggregates experienced a clear shift to smaller s-values as compared to solutions where only the vehicle DMSO was added. This shift to smaller s-values was stable for at least 5 days. It could be shown that the strength of the shift was related to the amount of the added compound. The results will be discussed in terms of their significance regarding the mechanism by which the compound interferes with the fibril formation of the Aβ peptide.

1 Introduction

Protein misfolding diseases pose a major health problem not only because of their increasing incidence but especially because they still have to be regarded as incurable[1]. One of the major targets for therapy under study are the formed protein aggregates themself, whether by enhancing their clearance or the inhibition of their formation. In the case of Alzheimer's disease the misfolded component is a protein fragment generated by proteolytic cleavage of the amyloid precursor protein and consists out of 39 to 43 amino acids.

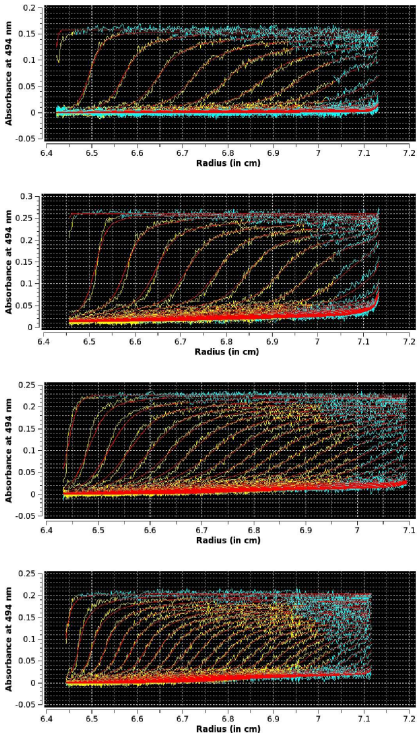

Figure 1. Raw data (yellow, blue) obtained from sedimentation velocity centrifugation of 21 μM Aβ-42/Aβ-42-OG at 20,000 rpm, 20 ℃ in 10 mM sodium-phosphate buffer, pH 7.4, 4 % DMSO. Fitted data from 2D-SA are overlaid in red. Prior to centrifugation samples were incubated slightly agitated at room temperature for 5 d. The effect of 0, 50, 150 and 200 μM compound (top to bottom) on aggregate formation is shown.

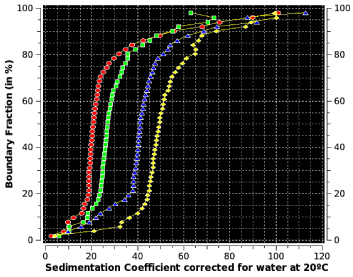

Figure 2. G(S)-distributions obtained by Van-Holde-Weischet Analysis. Yellow: 21 μM Aβ-42/Aβ-OG after 5 d incubation at room temperature without compound, with 50 μM (blue), with 150 μM (green) and with 200 μM compound (red).

The most prominent peptide is the Aβ(1–42). It is highly prone to self-association leading to different kinds of aggregates from which the mature amyloid fibril was long thought to be solely responsible for the neurodegenerative processes as observed during the course of the disease[2]. Our objectives are the development of aggregation inhibitors and the characterization of their properties *in vitro*. In previous years increasing evidence arose that probably smaller oligomeric assemblies[3,4] 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 an absolute 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[5]. In contrast to methods quantifying only the amyloid content of a sample, as f. e. Thioflavin T or Congo Red based fluorescence measurements, it will be possible to detect all aggregate species present in solution, from monomers to multimers consisting of several thousands units. On this account we believe that the method[6] is especially helpful in determining the effects of potential aggregation modulators. Here we present the results for a small organic compound, which is a derivative of the previously described β-sheet binder molecules consisting of aminopyrazole building blocks[7].

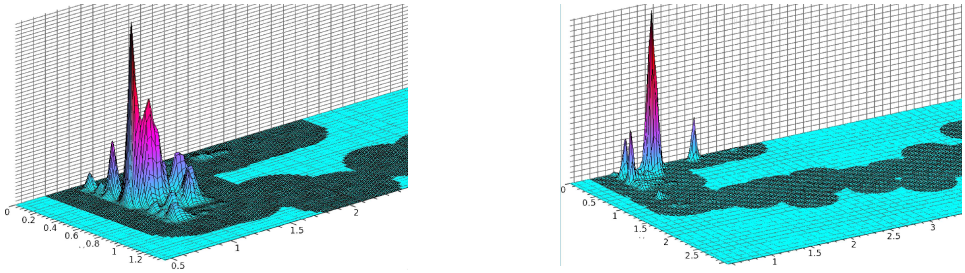

Figure 3. Results from 2-dimensional spectrum analysis of sedimentation velocity data in 3D representation. Left: 21μM Aβ 42/Aβ 42-OG in 10 mM NaP_i, pH 7.4, 4 % DMSO as control. Right: 21μM Aβ 42/Aβ 42-OG in 10 mM NaP_i, pH 7.4, 4 % DMSO with 200μM compound after 5 d incubation at RT (20,000 rpm, 20 ℃).

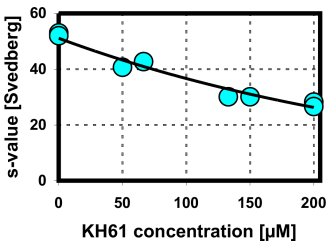

Figure 4. Dose-dependence of the inhibitory effect: Weight averaged s-values as calculated by 2D-SA/MC analyses are plotted against the compound concentration.

2 Methods

The aggregation mixture contained 17.5 μM unlabeled and 3.5 μM Oregon Green-labeled Aβ (1–42) in 10 mM sodium phosphate buffer, pH 7.4 with 4 % final DMSO concentration. DMSO was needed in order to solubilize the inhibitor compound (KH61). Samples (300 μl volume) were incubated slightly agitated at RT for 5 d prior to sedimentation velocity centrifugation. Sedimentation velocity experiments were performed with an XL-A 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 0.002 cm. Scans were recorded at minimal time intervals. To increase the sensitivity and the number of processable samples per run intensity instead of absorption data were recorded in continuous mode. Detection wavelength was 493 nm.

The raw data were transformed to pseudo-absorbance data, processed and evaluated using the UltraScan software package[8] running on a 44 node AMD Opteron cluster under Linux. The $\bar{v}$ value for the Aβ (1–42) as determined from the primary sequence is 0.7377 g/cm^3, the solvent density $\rho = 0.9998$ cm^3/g and viscosity $\eta = 1.0004$ centipoise. The 2-dimensional spectrum analysis (2D-SA) 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 (MC) simulations were used to identify statistically significant solutes.

3 Results

A small organic compounds (<600 Da) designed as β-sheet binder was selected for further studies, which proved to be capable of lowering the amyloid content of Aβ 42 solutions

as measured by a Thioflavin T fluorescence assay (data not shown). The comparative sedimentation velocity analysis (Fig. 1) of Aβ 42 solutions incubated either with or without a 2 to 10fold molar excess (referring to the monomer concentration of Aβ) of the compound revealed a considerable shift of the determined s-values to smaller values as seen in the G(s) distributions determined by van-Holde-Weischet analysis (Fig. 2). This indicated an inhibited growth of aggregates caused by the added compound. An indirect effect of the compound by changing the solvent properties could be ruled out by control experiments with a protein of known s-value in the presence or absence of the compound. The results from 2D-SA/MC analysis (Fig. 4) showed the dependence of the weight averaged s-value of Aβ 42 aggregates on the applied compound concentration. Obviously it is not a single aggregate species which is stabilized by binding of the compound. More probably the measured relationship indicates an end capping mechanism of growing protofilaments or fibrils, leading to a reduced mean length of aggregates. Such a mechanism would also be expected by the design of the compound as a β-sheet binder. As can be seen in the 3D plots (Fig. 3) the number of different species is clearly reduced by compound addition, species with a frictional ratio of about 1.3 at s-values above 40 S are missing. Appropriate models for the aggregates together with further experimental data will be needed in order to interpret the determined shape related frictional ratios.

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