

Biophysical characterization of amyloid β -derived diffusible ligands (ADDLs).

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Abstract

Amyloid β is a peptide naturally synthesized in the human brain at low levels. Over-expression, or lack of clearance of this peptide will result in the formation of insoluble protein fibers which bundle together to form amyloid plaques, a hallmark of the neurodegenerative disorder known as Alzheimer's disease.

In the last decade, soluble forms of amyloid β known as amyloid-derived diffusible ligands (ADDLs), have been implicated as the toxic species responsible for the neurodegeneration associated with Alzheimer's disease. Identification and characterization of these soluble species have been hampered by the nature of the amyloid β peptide, which has a strong tendency to self-associate.

We have used analytical ultracentrifugation and size exclusion chromatography coupled with multi-angle laser light scattering to characterize various amyloid β preparations with the ultimate goal of being to correlate those results with the results of in-vitro toxicity assays and cell binding studies.

Our results indicate that ADDLs exist in solution as a binary mixture of primarily monomeric and higher molecular weight (>150kDa) oligomeric species, with minimal amounts of intermediate oligomers present. These results are in contrast to published data, which relies heavily on atomic force microscopy and SDS gel electrophoresis for characterization of amyloid oligomers, and it represents a step forward in the understanding of the solution-state behavior of these important protein oligomers.

Methods

ADDLs are prepared from synthetic amyloid β peptide obtained from one of several commercial sources. The peptide is first treated with hexafluoroisopropanol (HFIP) to remove pre-existing "seeds" which could promote rapid, uncontrolled aggregation. The treated peptide is divided into known amounts, dried under vacuum, and stored at -70°C . An ADDL preparation is made by dissolving HFIP-treated peptide in anhydrous dimethyl sulfoxide (DMSO). The DMSO/peptide solution is then diluted in cold culture media (HAM's F12) and incubated at $2-8^\circ\text{C}$ for 16-20 hours. Following this incubation, the samples are centrifuged at $\sim 10,000 \times g$ for 10 minutes to remove any aggregates, and the supernatant is used as the ADDL preparation in all subsequent studies.

ADDL characterization was accomplished using the orthogonal techniques of analytical ultracentrifugation and size exclusion chromatography - multi-angle laser light scattering (SEC/MALLS).

Analytical ultracentrifugation is a technique in which the forces of radial acceleration push molecules in a fixed direction past a detector. The movement of these molecules is dependent primarily upon their mass. Sedimentation coefficients for these molecules are mathematically converted to very accurate mass measurements.

Multi-angle laser light scattering utilizes a completely different method to determine the size of molecules in solution. When polarized light of a specific wavelength is passed through a solution, the molecules in that solution will scatter the incoming photons in many directions. The intensity of this scattered light is mathematically related to the molecular mass of the solute. The specific angle at which molecules scatter light is a function of their molecular size or radius. When coupled with the separation properties of size exclusion chromatography, you have a very powerful system for the determination of molecular weight and size of molecules in a complex mixture.

Atomic force microscopy and SDS gel electrophoresis have been used extensively in the literature to characterize preparations of amyloid β peptides. Atomic force microscopy and SDS gel electrophoresis have been used to characterize our ADDL preparations to provide a reference for comparing our data to that of other laboratories.

Results

Figure 1. Atomic force microscopy was performed on ADDLs that were adsorbed to freshly cleaved mica. The mica was then washed and dried prior to imaging. ADDL preparations imaged by AFM typically show the compact, spherical globules evident in this figure. The size of these structures are on the order of 4-7 nanometers in diameter.

Figure 2. SDS-PAGE Western blot analysis of ADDLs (lane 1), monomer (lane 2), and fibrils (lane 3). All samples were loaded at equivalent masses. Western blot detection was accomplished using MAB 6E10. The migration position of molecular weight reference standards is shown to the left.

Figure 1.

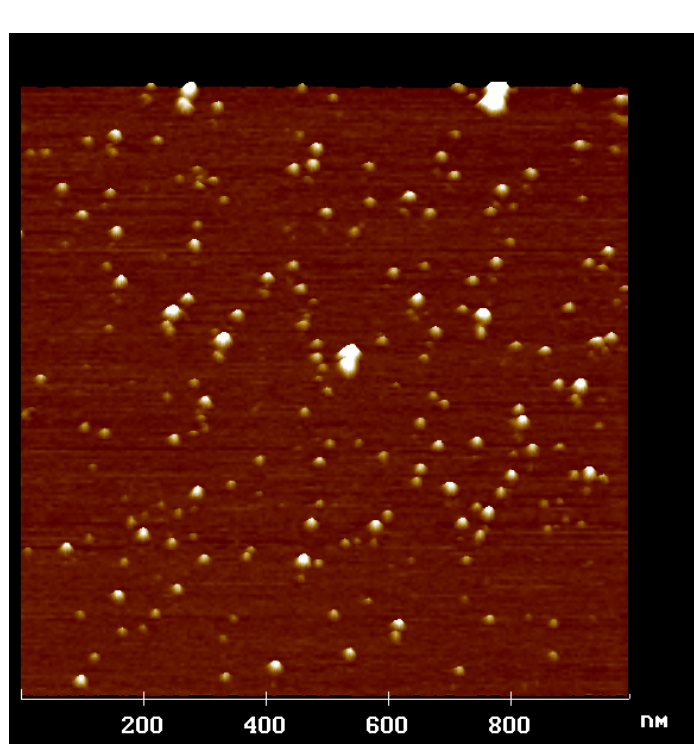


Figure 2.

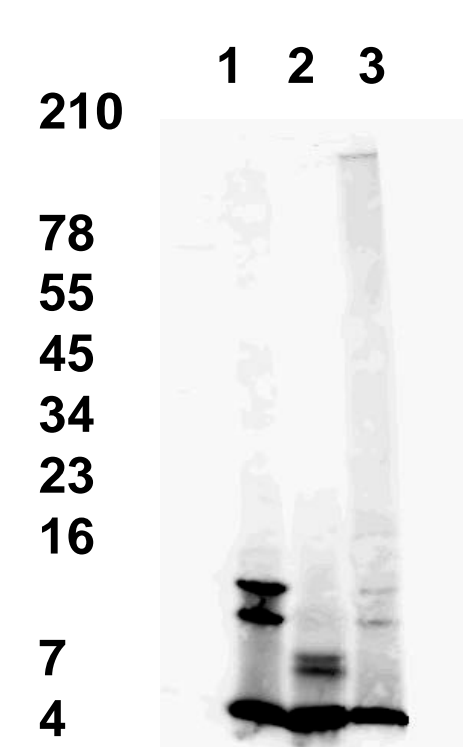
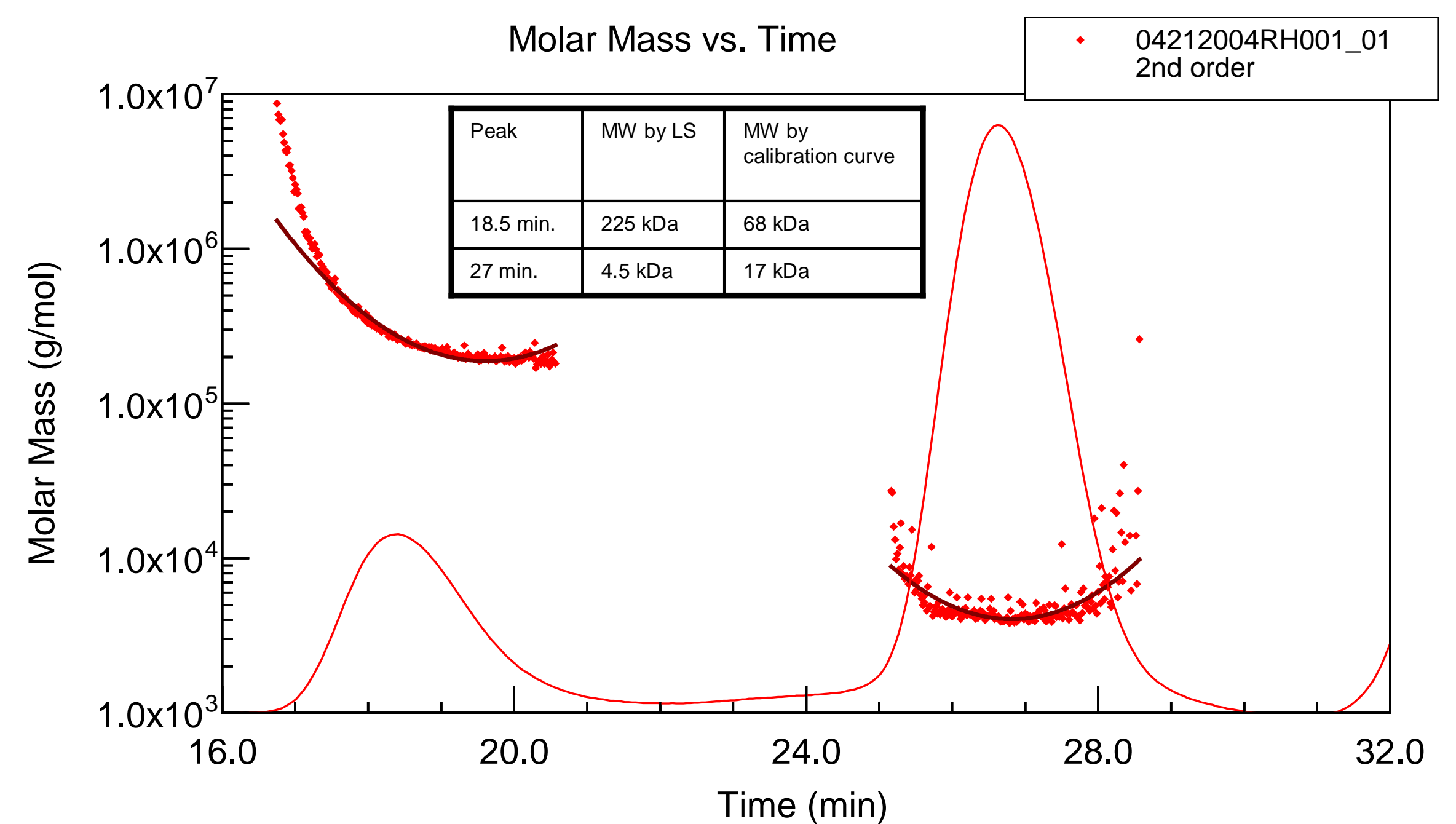
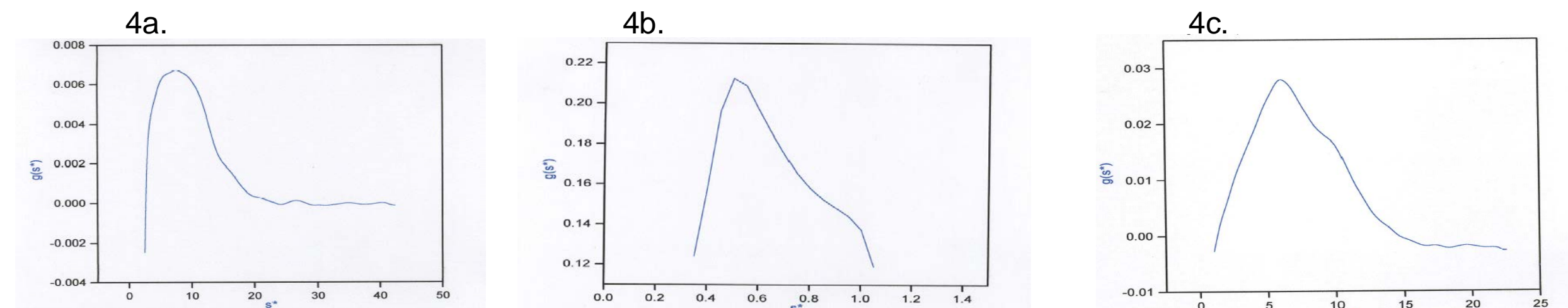


Figure 3. SEC/MALLS analysis of ADDLs. The chromatogram represents the refractive index signal while the overlay represents the calculated molecular weight for each slice of the chromatogram. The lines embedded in the molar mass data represent 2^{nd} order polynomial fits of the molar mass versus time. Calculation of the molecular weights of these two peaks based on calibration vs. globular protein molecular weight markers places the 18.5 and 27 minute peaks at 68 kDa, and 17 kDa, respectively; however, MALLS data indicates that the 18.5 minute peak is composed of oligomers with molecular weights of 225 kDa and higher, while the 27 minute peak is monomeric peptide at 4.5 kDa.



Figures 4a, b, and c. Sedimentation coefficient distribution from sedimentation velocity run of a. a standard ADDL preparation (broad sedimentation coefficient distribution of 4S-12S), b. SEC purified low molecular weight peak (average sedimentation coefficient - 0.55 S), and c. SEC purified high molecular weight peak (average sedimentation coefficient - 6.7 S).



Conclusions

Amyloid-derived diffusible ligand preparations are not a homogeneous solution of a single, or relatively few distinct oligomeric species. They are instead a complex mixture of monomeric peptide and a polydisperse population of higher molecular weight oligomers.

Characterization of these preparations by atomic force microscopy and SDS gel electrophoresis alone present a misleading picture of the macromolecular composition of this material. SEC analyses using external calibration to protein molecular weight markers would also provide misleading information due to the non-ideal chromatographic behavior of this peptide.

Analytical ultracentrifugation and SEC/MALLS analyses of these preparations provide a more accurate analysis of the solution state composition of this mixture.

The data obtained from AU and SEC/MALLS is complementary in nature and presents a similar picture of the size and distribution of the various macromolecular species present in an ADDL preparation.

Further Reading:

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