Synthesis and characterization of the Alzheimer’s β-amyloid protein

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Overview

Protein self-assembly, or aggregation, is a common biological process associated with everyday bodily function and, in most cases, is considered harmless if not vital. However, aggregation of the β-amyloid protein (Aβ) has been associated with various diseases such as Huntington’s disease, Parkinson’s disease, and most notably Alzheimer’s disease (AD). In specific, the hallmark symptoms of AD are neurofibrillary tangles (composed mostly of the tau protein) and dense, insinuate senile plaques (composed mostly of Aβ), discovered by Aïsle Alzheimer over a century ago. The amyloid plaques have since been implicated in the progressive degradation of neuronal cells that is characteristic of AD, manifesting lead to noticeable and continual loss of memory and cognitive function.

The challenge of studying Aβ resides in the variety of structures it is capable of forming. The most common biological form is a 40 amino acid residue peptide (Aβ40), that is twice cleaved from a larger precursor protein (APP) by the ectodomain; 3-30 and 37-40-residue peptides. As such, Aβ is ultimately responsible for the final length of the peptide, usually forming the aforementioned Aβ40, or a 42 amino acid variant, Aβ42. Each of these peptides is capable of forming amyloid fibrils, stranded aggregate complexes that compose the bulk of senile plaques. However, Aβ has also shown to readily form into a soluble oligomeric species that many now believe to be more indicative of AD pathology.

Recent research has begun to suggest the existence of multiple energetically similar pathways that govern amyloid self-assembly.1 Given this information, we hypothesize that there must exist a disease-specific microenvironment that promotes some type of toxic Aβ aggregation. In testing this hypothesis, we aim to structurally characterize both traditional amyloid species and Aβ oligomers.

Recent years have seen improved techniques for polymerization via pulsed laser deposition (PLD) and magnetic field-induced condensation (MFIC). A common, injury, oxidation of melatonin, was found in our sample and also reported in the literature.6 We subsequently reduced the products form Aβ40 sample below 95 % purity. The 37-40-residue mass spectrometry. Negative- staining transmission electron microscopy (TEM) of the product indicated a mostly homogenous fibrillar mixture with little characterizable both traditional amyloid species and Aβ oligomers. We subsequently reduced the products from Aβ40 sample below 95 % purity. The 37-40-residue mass spectrometry. Negative- staining transmission electron microscopy (TEM) of the product indicated a mostly homogenous fibrillar mixture with little characterizable both traditional amyloid species and Aβ oligomers.

Solid state nuclear magnetic resonance (SSNMR) has served as our main tool for probing protein structure. We performed various one-pulse experiments on Aβ40 in order to deduce structural stability. In specific, we monomeric and oligomeric sample and also examined the effect of hydration. We found that synthesized Aβ40 oligomers can be stable and exhibit structural similarities to fibrils in the water that tends to render more stability.

Amyloid formation and Alzheimer’s disease

A simplified depiction of Alzheimer’s pathology.1 The cleavage of Aβ from APP by γ-secretase is shown at the membrane surface of a neuron. Aggregation into amyloid plaques is associated with neuronal dysfunction and synaptic disruption.

Amyloid self-assembly

Aβ40 synthesis

2 kHz

20 kHz

MAS

Example 1H NMR spectrum of Aβ fibrils. These measurements can aid in determining immobilization dynamics and may offer unexploited information crucial for structural characterization.

NMR experiments

Future work

New breakthroughs in Alzheimer’s treatment has led to the development of γ-secretase modulators (GSMs), drugs that selectively bind to the Aβ region of APP.11 These drugs may prove useful in the effective control of toxic Aβ formation, as they may modify the γ-secretase cleavage procedure. We wish to characterize this relationship by performing 2D Total Correlation Spectroscopy (TOCSY) liquid state NMR and 2D Solid-state NMR experiments with and without the addition of GSMs. In addition, we aim to further pursue Aβ structural characterization with ssNMR measurements on isotopically labeled oligomers.

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References