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Biophysical Characterization and Cross-Seeding Properties Of LFAOs, Novel Prion-Like Oligomers of the Amyloid-Beta Peptide Involved In Alzheimer's Disease

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Abstract

The amyloid-beta (Aβ) protein is known to play an important role in the etiology of Alzheimer's disease (AD). Aβ peptide aggregates in the brains of AD patients to form soluble oligomers as well as insoluble fibrils. Oligomers of Aβ are now known to be the primary toxic agents in AD. Evidence is beginning to emerge regarding a conserved prion-like propagation mechanism in AD. One of the principal characteristics of prion diseases is their ability to self-propagate via a template-assisted-corruptive mechanism. Our lab has previously characterized a unique 'Aβ prion' called Large Fatty-Acid-Derived Oligomers (LFAOs). This project examines the ability of LFAOs to self-propagate and cross-seed the formation of aggregates among mutant varieties of the peptide. We observed the self-seeding and cross-seeding reactions of wild-type and Arctic (E22G) mutant varieties of LFAOs. Self-seeding of wild-type Aβ (1-42) (Aβ42) generated an increase in the amount of oligomer of the same size as the formed LFAOs, as did the cross-seeding of wild-type Aβ42 monomer with E22G LFAOs. Self-seeding of E22G monomer with E22G LFAOs, however, resulted in much larger aggregates. Cross-seeding of Aβ (1-40) (Aβ40) monomer with E22G LFAOs resulted in the formation of more oligomer of the same size as the seed. In addition, we have examined the stability of LFAOs in various experimental conditions.

Contents

Introduction

Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder found most often in patients above the age of 60. It is the most common form of dementia, affecting an estimated 5.4 million people nationwide.[1] Although it occurs often in patients with no family history of the disease, familial factors can also play a role in the disease's development.[2] A progressive disorder, AD causes the death of neuronal cells, leading to a gradual decline in cognitive function. AD patients experience memory loss, difficulty completing familiar tasks, and confusion with regards to time and place. AD is at this time considered incurable, and the progression of symptoms eventually results in death.

Amyloid-beta (Aβ) in Alzheimer's disease

Brains of post mortem AD patients show large amounts of insoluble proteinacious 'senile' plaques, as do the brains of patients with similar neurodegenerative disorders. A major component of these plaques is the aggregates of a peptide called Aβ, and evidence suggests that this peptide plays an important role in the development of AD. Amyloid beta (Aβ) is the result of the cleavage of the trans-membrane protein known as the amyloid precursor protein (APP).[3] A pair of aspartyl secretases, β and γ, sequentially cleave APP to generate Aβ peptides with varying lengths (39-43). Among these, Aβ 1-40 (Aβ40) and Aβ1-42(Aβ42) are the most abundantly produced, and form the principal components of the senile plaques. AD is largely a sporadic disease with only ~ 10-15% being familial.[3]. Mutations in the genes encoding APP can cause an individual to

develop AD, as can the over-expression of the normal APP gene such as that present in patients with Down's syndrome. [2, 4]

Aβ mutants and cerebral amyloid angiopathy

In addition to neuronal degeneration, AD patients are also at an increased risk for cerebral amyloid angiopathy (CAA). CAA is a condition in which neuritic plaques build up in the brain vasculature, leading to ischemic stroke. Several mutant Aβ peptides are associated with CAA pathology. These include the Arctic (E22G), Italian (E22K), and Dutch (E22Q) varieties.[5] Patients carrying a point mutation resulting in a change of the 22nd position of the Aβ peptide, particularly a substitution of glutamine for glutamic acid, have been shown to be at increased risk for CAA. [6] In addition, the Arctic mutant of the Aβ peptide, carrying a substitution of glycine for glutamic acid at the 22nd position, has been shown to exhibit enhanced protofibril formation. [7]

Oligomeric species toxicity

For several years after their discovery, amyloid plaques were thought to be the major causative agent in AD. [8] Lue and co workers found that the correlation between the amount of soluble Aβ present in the post-mortem brains of AD patients and the extent of synaptic change was much stronger than that between the accumulation of neuritic plaques and synaptic change. [9] Kuo and co workers found that the amount of water-soluble Aβ, particularly Aβ42, present in the brain of AD individuals was 12 times the amount found in non-AD brains. [10] These results suggest that soluble Aβ is much more likely the primary cause of neurodegeneration in AD than the insoluble fibril form of Aβ. The findings of Lambert and co workers supported this evidence, by demonstrating that

soluble Aβ oligomers are neurotoxic in the absence of any fibril forms of Aβ.[11] Walsh and co workers demonstrated that naturally secreted human Aβ oligomers inhibit long-term potentiation, a process involved with the formation of memories, in mice.[12]

Formation of soluble oligomers

In order to design effective treatments for AD, it is important that we understand the pathways by which neurotoxic soluble oligomers are formed. Barghorn and co workers demonstrated that a globular oligomer (globulomer) of Aβ42 could be formed in a pathway independent of that of fibril formation.[13] They incubated synthetic Aβ42 in 0.2% sodium dodecyl sulfate (SDS), producing and intermediate which was able to self-aggregate to a globulomer form. Rabbits were immunized against these globulomers, and the resulting antibodies were used to demonstrate that this globular form of Aβ exists in the brains of AD patients. They also determined that certain fatty acids could be used to produce the globular form.

Walsh and co workers demonstrated that soluble Aβ oligomers are formed intracellularly in the human brain.[14] After previously demonstrating that Aβ oligomers are present in intracellular spaces, they conducted a series of trials in which monomeric forms of Aβ failed to produce oligomeric forms in CSF incubated at 37°C. They also acquired 56 human CSF samples, which they tested for the presence of oligomeric forms of Aβ using immunoprecipitation. They found soluble oligomeric forms of Aβ in 15 of the 56 samples. This would seem to indicate that formation of Aβ oligomers begins intracellularly, but that oligomers are at some point released to extracellular spaces.

Gellerman and co workers showed that globular formation proceeds independently of the fibril formation pathway.[15] They observed that the presence of Aβ fibrils was able to initiate fibril formation in monomeric Aβ, but did not induce fibril formation of Aβ globulomers. They also investigated the effects of known inhibitors of fibril formation on the formation of globulomeric Aβ, and found that fibril inhibitors did not affect the formation of globulomers. They speculate that because it is possible to produce inhibitors for the two pathways separately, it might eventually be possible to generate a vaccine to inhibit oligomer formation without producing the side-effects associated with targeting fibril plaques.

Chromy and co workers investigated the ability of Aβ to self-aggregate to oligomeric forms as well as the toxicity of the formed species.[16] They found that Aβ was able to assume several oligomeric forms in the absence of fibrils, and demonstrated that some of these oligomers have toxic effects on cells while others do not, suggesting that multiple oligomeric forms are possible. They also examined the possible neuroprotective effects of extracts of *Ginkgo biloba*, and found that these extracts were able to retard the formation of oligomers over short incubation times, but that oligomers formed after longer incubations.

Kumar and co workers investigated a method of producing oligomers using substances that are present under physiological conditions.[17] They used non-esterified fatty-acids, rather than the previously favored SDS, to produce low molecular weight oligomers via two different pathways. They first determined the critical micelle concentration (CMC) of the fatty acids used, and then investigated oligomer formation at fatty acid concentrations below, near, and above the CMC. They found that below the

CMC for a given fatty acid, Aβ42 behaved the same way it did in controls containing no fatty acid, and formed fibrils. Near the CMC, the rate of aggregation of Aβ increased significantly, while above the CMC aggregation was inhibited. In the incubations with fatty acid concentrations below the CMC, they found monomeric and fibril forms of Aβ, just as they did in the control samples containing no fatty acid. Incubations near the CMC produced 12-18mers, while concentrations above the CMC produced 4-5mers. These two forms were found to consist mainly of β-sheets, having subtle structural differences. Both forms were examined using atomic force microscopy. The control samples showed clusters of fibrillar material, while the samples formed near the CMC showed a mixture of fibrillar and small round particles. Samples produced above the CMC showed only round particles. This finding demonstrated that oligomers produced near and above the CMC are structurally different. The oligomers were also evaluated for their ability to seed aggregation of other oligomers, and it was found that oligomers produced near the CMC were able to speed up the aggregation of monomer, acting as a seed, while oligomers produced above the CMC were not able to do so. The 4-5mers were also found to be more thermodynamically stable than 12-18mers. These results suggest that the formation of Aβ is dependent on the nature of the interface in which it is formed. Different aggregate species form under micellar and non-micellar conditions.

Prion proteins and Aβ

Prions are a group of proteins capable of self-propagation through a template-assisted corruptive mechanism. Diseases resulting from prion activity include Creutzfeldt-Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy, better known as mad cow disease.[18] It has been observed that the

activity of Aβ in AD shares several characteristics with the prion diseases. For instance, both diseases result from the buildup of abnormally-folded proteins in the brain, and familial mutations in the proteins involved in each disease can predispose an individual to developing that disease.[18] One important characteristic of prion diseases is that the presence of the toxic, improperly-folded form of the involved protein can induce the improper folding of the healthy form of the protein.

The focus of this project is on the observation and characterization of this same type of self-seeding behavior in the Aβ protein. As already shown by Kumar et al, oligomers formed in fatty acid are capable of seeding the aggregation of more Aβ. It is therefore important that we characterize this self-seeding behavior, to determine whether Aβ corruption occurs in a prion-type mechanism.

Materials and Methods

Materials

Aβ peptides were synthesized at the Mayo Clinic Peptide Synthesis Facility (Rochester, MN) using Fmoc chemistry. The sodium salt of lauric acid was obtained from NuCheck Prep Inc. (Elysian, MN). Sodium dodecyl sulfate (SDS) and all antibodies were obtained from Sigma Aldrich (St. Louis, MO). Biotin was purchased from Millipore (Billerica, MA). Streptavidin-HRP was purchased from Thermo Scientific, and FITC was purchased from Fisher Scientific. All other chemicals were purchased from VWR Inc.

Preparation of Aβ monomers

Aβ peptide stocks were stored at -20°C. Samples of 0.7-1.4 mg of Aβ were dissolved in 35 µL of 0.5 M NaOH, and allowed to stand at room temperature for 7 minutes. Next, 0.5 mL of nanopure water was added, and this stood at room temperature for 10 minutes before size-exclusion chromatography (SEC) to isolate monomeric Aβ. The sample was loaded onto a Superdex 75 10/300 GL column attached to an AKTA FPLC system (GE Healthcare, Buckinghamshire). The column was run with 20 mM TRIS at pH 8.0 at a flow rate of 0.5 mL/min, collecting 1 minute fractions. Fraction concentrations were then determined by UV-Vis spectroscopy using a Cary 50 spectrophotometer (Varian Inc), and a molar extinction coefficient (ε) of 1490 cm^{-1} M^{-1} (www.expasy.org). Monomeric Aβ was stored at 4°C, and fractions were used within 3 days of preparation.

Examination of Stability of LFAOs with respect to temperature and buffer conditions

Wild-type Aβ42 monomer was prepared with various modifications of the procedure described by Kumar et al. [17] Samples of 25 µM Aβ monomer were incubated at room temperature, 4°C, and 37°C with either 5 mM or 20 mM lauric acid in 5 mM TRIS and 50 mM NaCl for 24-48 hours. Alternately, samples containing monomer incubated with 5 mM and 20 mM lauric acid were incubated in 1X phosphate-buffered saline (PBS) at 37°C for 24-48 hours.

Generation of Large Fatty-Acid-Derived oligomers (LFAOs)

Wild-type Aβ42 LFAOs were prepared by incubating 50 µM Aβ42 monomer in 5 mM lauric acid and 50 mM NaCl in 20 mM TRIS (pH 8.0) for 48 hours at 37°C, as

described by Kumar, et al.[17] These were then isolated using SEC as described above, and oligomers were collected from fractions 15-18.

The ideal fatty acid concentration for the generation of Aβ42 E22G oligomers was determined by incubating E22G monomer in the above conditions, with concentrations of 5, 15, and 20 μM lauric acid. For all subsequent preparations, E22G monomer was incubated in 15 mM lauric acid in the conditions described above. E22G oligomers were isolated by SEC and collected from fractions 15-18. All isolated oligomers were stored at 4°C.

Seeding reactions

Monomeric Aβ (20 μM) was incubated with concentrations of LFAOs ranging from 0.4-3 μM in 20 mM TRIS (pH 8.0) for 72 hours at room temperature. Samples were examined using polyacrylamide gel electrophoresis (PAGE) and immunoblotting.

Polyacrylamide gel electrophoresis (PAGE)

Samples were dissolved in 2-(N-morpholino)ethanesulfonic acid (MES) loading buffer containing 1% SDS and loaded onto 4-20% NuPage gels (Novex or BioRad) along with a dye-linked molecular weight marker. The gels were electrophoresed in Laemmli running buffer containing 0.1% SDS. Gels were transferred onto 0.45 μm Immobilon® nitrocellulose membranes (BioTrace™ NT, Life Sciences Inc.) for immunoblotting.

Immunoblotting

After transfer, nitrocellulose membranes were boiled for two minutes, then incubated in blocking buffer containing 1X PBS, 5% nonfat dry milk, and 0.1% tween-20

overnight. Blots were incubated in a solution containing 4 μL Ab9 monoclonal antibody, 1% blocking buffer, and 1X PBS for 1 hour. After rinsing with PBS to remove excess primary antibody, blots were incubated with horseradish peroxidase conjugated anti-mouse IgG and developed with ECL reagent (Thermo Scientific).

Biotin labeling

Samples of Aβ peptide were dissolved in hexafluoro-2-propanol (HFIP) to obtain a 1.3 mM solution, and then shaken at 37°C for 1½ hours before vacuum evaporation until a film was formed. Films were then stored at -20°C until use. The films were re-suspended in 100 μL of PBS. The films were re-suspended in 40 μL of PBS to obtain a 0.3 μM solution. Biotin was added to obtain a 1:10 ratio of Aβ to biotin and mixed by vortexing. This was then allowed to sit at room temperature for 1 ½ hours before purification. The labeled samples were then purified using a Hi Tap 5 mL desalting column attached to an AKTA FPLC purification system in 20 mM TRIS (pH 8.0) at a flow rate of 1 mL/min. Biotin-labeled peptide concentrations were estimated by UV-Vis spectroscopy, and peptides were visualized using horseradish peroxidase (HRP) conjugated streptavidin and ECL reagent, similar to the immunoblotting procedure described above, but without the need for secondary antibody.

Results

LFAO formation is stable in a variety of experimental conditions

Aβ42 wild-type monomer was incubated at room temperature, 37 °C, and 4 °C in Tris (pH 8.0), as well as in PBS (pH 8.0) at 37°C.

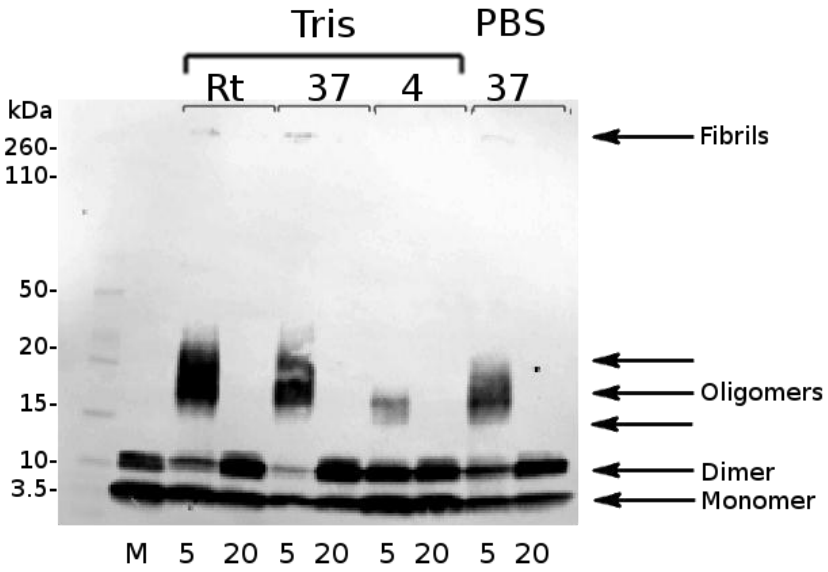

Figure 1: Formation of LFAOs at room temperature, 37 °C, and 4 °C in Tris and PBS. M is monomer incubated with no fatty acid. Fatty acid concentrations (mM) are shown below lanes.

Incubation with 20 mM fatty acid produced distinct bands at 10 kDa, corresponding to dimers, while incubations in 5 mM fatty acid produced bands at both 10 kDa (dimers) and 15-30 kDa (3-7 mers) (with the exception of the incubation at 4°C, which formed a 15-18 kDa band (3-4 mers)) as shown in Figure 1. After 48 hours, the dimeric bands disappeared, and more extensive oligomerization was achieved (data not shown). These findings show us that LFAOs are stable at physiological temperature and pH, as well as that they can be stored in a variety of temperature and buffer conditions.

Aβ42 Arctic (E22G) mutant forms LFAOs

Aβ42 E22G mutant peptide was incubated in 15 mM lauric acid and 50 mM NaCl in 20 mM Tris (pH 8.0) for 48 hours at 37 °C. This produced oligomers of 30-40 kDa corresponding to 6-9 mers as

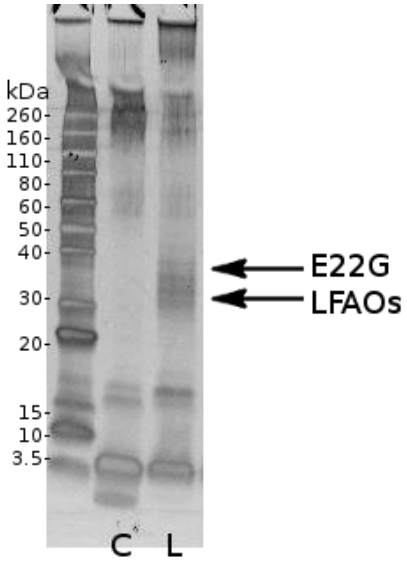

Figure 2: Formation of Aβ42 E22G LFAOs. Lane marked "C" shows E22G monomer with no fatty acid incubated for 48 hours at 37 °C. Lane marked "L" shows E22G LFAOs generated by incubating Aβ42 E22G monomer with 15 mM lauric acid for 48 hours at 37 °C.

shown in Figure 2. These LFAOs were isolated using SEC, and their concentrations determined by UV-Vis spectroscopy before being used for subsequent seeding experiments. The size similarity between wild-type and Arctic LFAOs seems to suggest that Arctic LFAOs may also warrant examination for self-replication properties.

Aβ42 E22G oligomers are capable of self-replication

When 20 μM Aβ42 E22G monomer was incubated with Aβ42 E22G LFAOs, subsequent immunoblotting revealed an 80-200 kDa band (17-45 mers), which was larger than the 30-40 kDa E22G LFAOs (6-9 mers). Fibrils, remaining monomer, and small amounts of dimer were also observed in the samples as shown in Figure 3. The 30-40 kDa band corresponding to the E22G LFAOs originally used as seeds was not observed in self-seeding samples of Aβ42 E22G monomer with Aβ42 E22G LFAOs. The signal strength of the bands increased with an increasing initial seed concentration used, and the total signal strength for each seeded fraction was greater than the signal strength of the LFAOs used as seeds, indicating that seeding took place.

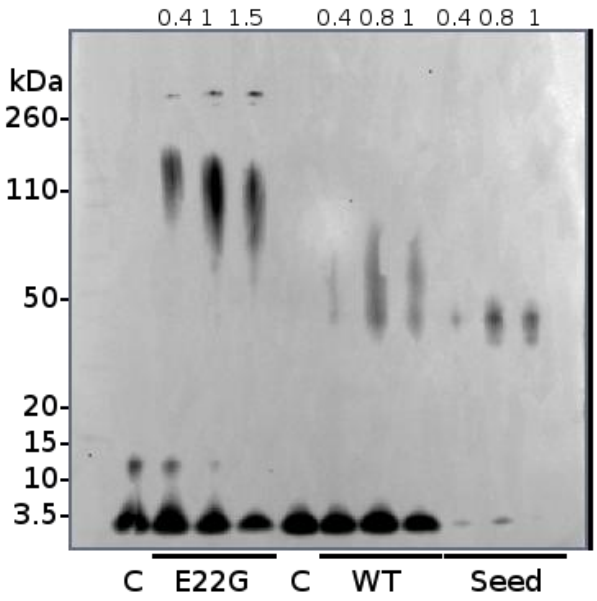

Figure 3: Self-seeding of Aβ42 E22G, and cross-seeding of E22G oligomer with Aβ42 wild-type. C represents control sample with no added seed. Lanes labeled "Seed" contained only oligomers. Numbers above lanes are μM concentrations of oligomer.

Aβ42 E22G oligomers cross-seed Aβ42 wild-type monomer

Cross-seeding of wild-type Aβ42 monomer with Aβ42 E22G LFAOs produced a 40-80 kDa band (8-18 mers), similar to the bands produced by both Aβ42 wild-type and E22G oligomers as shown in Figure 3. These bands overlapped the size range of the E22G LFAOs used for seeding. ECL signal increased as the concentration of seed used increased.

Aβ42 E22G oligomers cross-seed Aβ40 E22G monomer

Cross-seeding of Aβ40 E22G monomer with Aβ42 E22G oligomer resulted in a band of 40-80 kDa (8-18 mers), similar to that of the E22G oligomer seed. The signal strength of the band increased with an increasing seed concentration, and the overall signal strength of each reaction was stronger than its isolated seed alone as shown in Figure 4. This increase in signal strength indicates that the LFAO seeds the formation of more LFAO from the Aβ40 monomer.

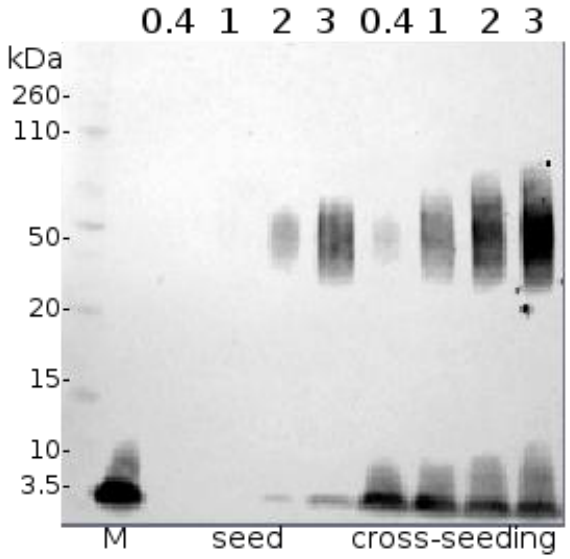

Figure 4: Cross-seeding of Aβ40 E22G monomer with wild-type Aβ42 LFAO. Lane M is Aβ40 monomer alone. Increasing concentrations of E22G LFAO alone are shown above. Increasing concentrations of LFAO incubated with the same concentration of Aβ40 are shown above lanes.

Generation of biotin-labeled Aβ monomers

In order to determine whether the increase in signal strength observed in the seeding samples is due to the conversion of monomer into the oligomeric form, we sought to label the monomer with a detectable tag which could be seen in the oligomeric bands. Biotin, a protein tag that binds strongly to streptavidin, was chosen because HRP conjugated streptavidin could be used in Western blotting, and the results visualized using ECL. Biotin labeling of Aβ monomer was confirmed using a modified immunoblotting technique utilizing streptavidin-HRP. Blots were incubated with streptavidin-HRP for one hour before visualization with ECL reagent. We were able to detect monomeric bands via streptavidin binding, as well as with traditional immunoblotting used to detect Aβ.

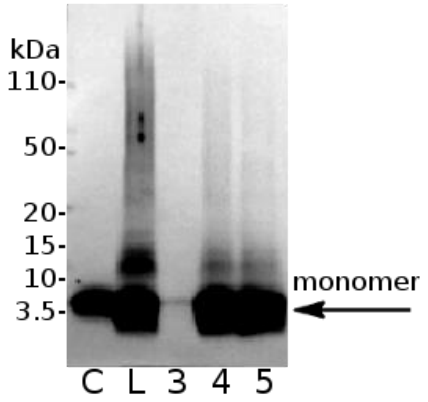

Figure 5: Streptavidin labeling of wild-type Aβ42 monomer. A sample of previously labeled Aβ40 monomer was used for control. Lane L shows the sample before purification by desalting. Numbered lanes correspond to SEC fractions.

Biotin-labeled oligomer formation

To preliminarily determine the detectability of the biotin label within LFAOs, we prepared LFAOs using the above described LFAO generation method, incubating for 72 hours (LFAOs formation is stable over 170 hours).[17] Biotin-labeled oligomers were detected by both streptavidin-HRP, and immunoblotting with Ab9 antibody.

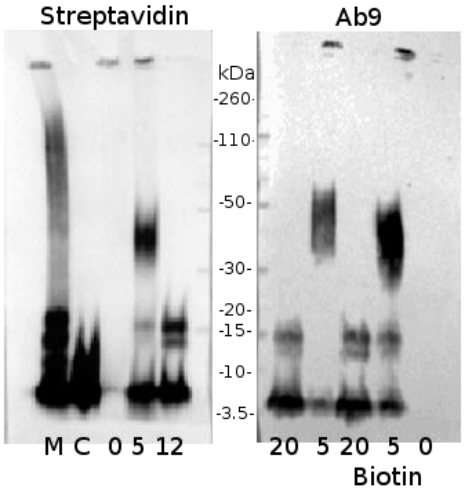

Figure 6: Oligomer formation detected with Ab9 primary antibody, and streptavidin. M is Aβ42 biotin-labeled monomer alone. C is an Aβ40 biotin-labeled control. Numbers represent mM concentrations of lauric acid. For Ab9 blot, lanes not labeled "biotin" are control preparations with 100% unlabeled Aβ42.

At both 20 mM and 5 mM concentrations of fatty acid, 4-5 mers and 12-18 mers, respectively, were detectable using both streptavidin and Ab9 primary antibody.

Self-propagation of biotin-labeled wild-type Aβ42 monomer with wild-type LFAO

A mixture of 10 μM biotin-labeled Aβ42 wild-type monomer and 10μM unlabeled Aβ42 monomer (total concentration of 20 μM) was incubated with unlabeled LFAO seeds in concentrations of 0.4 μM and 0.8 μM seed for 96 hours at room temperature. Detectable LFAO bands were observed upon immunoblotting with Ab9, but only monomeric bands were detected with streptavidin as seen in Figure 7.

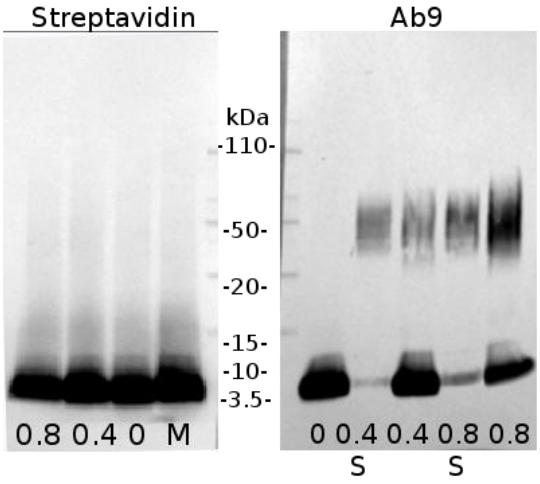

Figure 7: Seeding of biotin-labeled wt Aβ42 with wt LFAO. Left, we see only monomer detected by streptavidin-HRP. Right, we see both monomeric and oligomeric bands detected with Ab9. M is biotin-labeled monomer alone. Numbers are concentrations of LFAO seed in μM. Lanes marked S are LFAO seed alone.

To ensure that the lack of detectable oligomers in the biotin blot was not due to some preferential seeding of unlabeled monomer, we incubated 100% biotin-labeled wild-type Aβ42 monomer with LFAOS, and observed with both Ab9 and streptavidin-HRP. We were again only able to visualize monomer

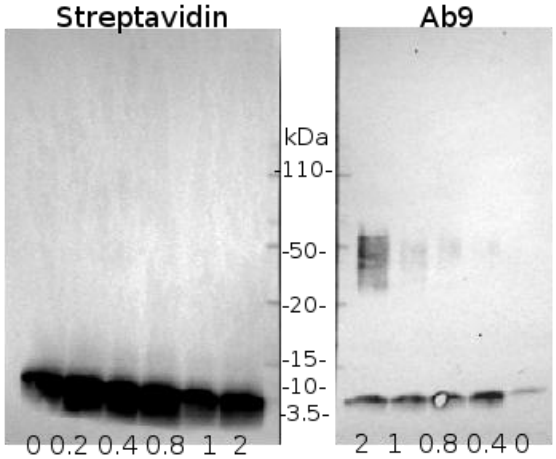

Figure 8: Seeding of biotin-labeled wt Abeta42 with wt LFAO. Numbers correspond to μM concentrations of seed. Streptavidin-HRP detection is shown left. Ab9 detection is shown right. Oligomeric bands are observable in the gel blotted with Ab9.

with streptavidin, while Ab9 detected oligomers as well. We concluded that the seeding interaction must somehow block the binding site for biotin and streptavidin, and so we were not able to use biotin labeling to confirm the incorporation of monomer into the oligomeric form.

Discussion

One of the key distinguishing characteristics of prion diseases is their ability to self-replicate. We have seen that when we seed Aβ monomers with LFAOs, oligomers of the same molecular weight as the LFAOs are formed. In other words, LFAOs self-propagate upon interacting with monomers. Mutant Aβ monomers and LFAOs also tend to cross-seed to form oligomers of similar molecular weight. This suggests that LFAOs could propagate via a prion-like mechanism. In order to further investigate this propagation mechanism, further biophysical characterization of both the LFAOs and the replicated oligomers is necessary. Particularly, it is important to ascertain the degree of structural similarity between the LFAOs and the replicated oligomers. We should also determine whether replicated oligomers, when isolated, are capable of seeding the formation of more replicated oligomers. Continuous self-replication across multiple generations would provide strong evidence that LFAOs replicate via a prion mechanism.

It is important to note that our biotin-labeling experiments revealed a dissimilarity between LFAOs and replicated oligomers. While we were able to detect the biotin label within LFAOs after generation with fatty acid, streptavidin-HRP could not detect the replicated oligomers. This suggests that the binding site for streptavidin to biotin is blocked in the replicated oligomers, but accessible in the LFAOs used for seeds. The

degree of this structural difference is yet to be determined, but serves as evidence against a prion mechanism for LFAO propagation.

Our LFAOs can only be considered to have potential biological relevance if they are able to exist under physiological conditions. Kumar and co workers demonstrated the formation of LFAOs using fatty acids, which are physiological molecules. [17] However, in order to be considered important, LFAOs must not only be formed with the help of physiological molecules, but also be stable under physiological conditions. We determined that LFAOS are stable at physiological pH (37 °C) as well as near-physiological pH (8.0). These findings serve as further evidence that LFAOs might exist within the body, and play a role in AD pathology.

We have demonstrated that mutant E22G LFAOs, which may be involved in CAA, seed the formation of LFAO-sized oligomers when incubated with wild-type Aβ42 monomer. Conversely, Aβ40 monomer oligomerization is less extensive, and E22G monomer forms larger aggregates when self-seeded. This preferential seeding of Aβ42 to form oligomers of the same molecular weight as LFAOs indicates a potential prion-like mechanism for LFAO propagation in AD, which may be influenced by LFAOs of mutant peptides involved in CAA. The formation of larger aggregates upon E22G self-seeding indicates that the Arctic mutant may have a more additive aggregation effect. The disappearance of the E22G LFAO bands from the self-seeding samples suggests that the E22G monomer aggregates with the LFAO seeds to form larger oligomers.

Since we were unable to confirm the incorporation of Aβ monomer into LFAO-sized aggregates using biotin labeling, further experimentation will focus on exploring

other labeling techniques which may be able to show conclusively whether LFAO seeded Aβ monomer is incorporated into the oligomeric form. One promising potential label is FITC, a fluorescent molecule that binds to the amino terminus of the peptide. If FITC is able to label the Aβ monomer without inhibiting aggregation, we will be able to examine seeded oligomers using UV-Vis spectroscopy to determine whether FITC labeled Aβ monomer is incorporated into seeded oligomers.

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