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[2] Preparation of Amyloid β -Protein for Structural and Functional Studies

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Abstract

Amyloid proteins cause a number of progressive, degenerative diseases. Among these is Alzheimer's disease (AD), the etiology of which is linked to the formation of neurotoxic assemblies by the amyloid β -protein ($A\beta$). The clinical importance of AD has stimulated intense interest in the mechanisms of $A\beta$ folding and self-assembly. Studying these phenomena *in vitro* requires the preparation of $A\beta$ peptide stocks that are well defined and display reproducible biophysical and biological behaviors. Unfortunately, the propensity of $A\beta$ to self-assemble has made this goal difficult. I discuss here a biphasic strategy for preparing $A\beta$ for structural and functional studies. The strategy involves sodium hydroxide pretreatment of synthetic $A\beta$, followed by size fractionation procedures. This approach produces $A\beta$ solutions that have been used successfully in a variety of *in vitro* and *in vivo* experimental systems.

Background

$A\beta$ comprises the fibrils found in the senile plaques that are pathognomonic for AD (Selkoe, 1991). Genetic, physiological, and biochemical data support the hypothesis that $A\beta$ is a causative agent of AD (Selkoe, 2001). This may be a direct result of fibril neurotoxicity (Pike *et al.*, 1991, 1993). However, continuing structure-activity studies have revealed that fibril intermediates and many other types of $A\beta$ assemblies also are neurotoxic (Hoshi *et al.*, 2003; Kirkitadze *et al.*, 2002; Klein *et al.*, 2004; Taylor *et al.*, 2003; Walsh and Selkoe, 2004; Walsh *et al.*, 1999), emphasizing the importance of a full elucidation of $A\beta$ assembly. In particular, recognition of the clinical and biological importance of oligomeric assemblies (Klein *et al.*, 2004) has made determination of conformational and assembly states populated in the structural space between monomer and fibril especially significant. As one might predict (Murphy, 1949), it is within this structural space that the intrinsic propensity of $A\beta$ to self-associate creates the largest experimental impediments. $A\beta$ assembly is a complex process (Buxbaum, 2003; Teplow, 1998; Thirumalai *et al.*, 2003) that produces an array of metastable structures (for a recent review, see Lazo *et al.*, 2005). Metastability and polydispersity largely obviate the use of

solution nuclear magnetic resonance (NMR) and X-ray crystallographic techniques that have been applied so effectively to determine the tertiary structure of homogeneous preparations of natively folded proteins. To overcome these problems, site-specific labeling techniques have been employed to minimize problems arising from population heterogeneity. These include site-directed spin-labeling coupled with electron paramagnetic resonance [EPR] (Torok *et al.*, 2002), hydrogen-deuterium exchange (Kheterpal *et al.*, 2000, 2003), intrinsic fluorescence (Maji *et al.*, 2005), and NMR on isotopically labeled samples (Antzutkin *et al.*, 2000; Balbach *et al.*, 2002; Benzinger *et al.*, 1998; Petkova *et al.*, 2002; Tycko, 2004). In each case, preparation of protein samples with maximal primary, secondary, tertiary, and quaternary structural homogeneity was critical. I discuss here approaches for producing such A β preparations.

Theoretical and Practical Aspects of Amyloid Protein Preparation

Pre Facto Considerations for Studies of A β and Other Amyloid Proteins

A β is produced by solid phase peptide synthesis (SPPS) or recombinant DNA techniques. Unfortunately, substantial compositional variation has been reported among A β preparations, resulting in experimental irreproducibility (Howlett *et al.*, 1995; Simmons *et al.*, 1994; Soto *et al.*, 1995). It is important that the experimentalist verifies that the peptide itself is chemically pure and that nonpeptide components are inert or absent. In practice, this is difficult. Most peptide lyophilizates do contain salts and other components. For example, fluoren-9-ylmethoxycarbonyl (Fmoc)-mediated SPPS coupled with reverse phase high-performance liquid chromatography (HPLC) purification typically produces trifluoroacetic acid (TFA) salts of the resulting peptides. These salts, as well as chemical scavengers, often are present in lyophilized peptide preparations and can complicate the initial solvation and preparation of peptide stock solutions. In addition, these nonpeptide components can alter the biophysical and biological behavior of the peptide. Technical errors also can be made in calculation of nominal peptide concentration if the weights of nonpeptide components are not taken into account.

SPPS cannot produce an A β product that is 100% pure. Failure sequences—peptides missing one or more amino acids—are unavoidable, although with proper synthesis chemistry, their relative amounts can be minimized. Oxidation of Met35 to its sulfoxide form is a common side reaction that can occur during peptide workup and purification. Synthesis-related amino acid racemization and side reactions during peptide cleavage

and deprotection also may be observed, but these generally occur infrequently. Most peptide suppliers perform quantitative amino acid analysis and mass spectrometry to characterize their products. However, because amino acid and simple mass analyses cannot determine primary structure, Edman or mass spectrometric sequence analysis can be used to prove formally that the peptide structure is correct.

For recombinantly-derived $A\beta$, primary structure changes are rare because of the high fidelity of the protein expression systems and the physiological conditions under which these systems operate. In systems in which $A\beta$ is produced as a fusion protein, posttranslational processing with specific endoproteases releases the free peptide. It is important to ensure that the $A\beta$ component of the fusion protein is not contaminated by uncleaved fusion protein, the enzyme itself, or peptide fragments produced through adventitious proteolysis. Because fusion protein cleavage is done under conditions necessary for efficient endoprotease action and these conditions are unlikely to be identical to those desired for subsequent experimentation, buffer exchange or removal may be necessary prior to peptide use.

Metastability and Polydispersity

Metastability and polydispersity are two important factors complicating the study of $A\beta$. The methods discussed here have primary application in the minimization of polydispersity. The reader should note, however, that metastability is an equally important issue. Metastability is an intrinsic property that exists in solution studies of $A\beta$ and other amyloid proteins done under quasiphenological conditions (e.g., neutral pH, isotonic biological buffers). As discussed above, $A\beta$ forms a variety of monomeric, oligomeric, and polymeric structures. The precursor-product relations and equilibria among these assemblies are not entirely understood and are areas of active investigation. Some controversy exists as to the oligomerization state of aggregate-free starting preparations of $A\beta$. Here, I refer to $A\beta$ solutions prepared at micromolar peptide concentrations as low molecular weight (LMW) $A\beta$ (Walsh *et al.*, 1999). I do so because techniques, including quasielastic light scattering (Walsh *et al.*, 1997), chemical cross-linking (Bitan *et al.*, 2001, 2003; LeVine, 1995, 2004), fluorescence resonance energy transfer (Garzon-Rodriguez *et al.*, 1997), and ultracentrifugation (Huang *et al.*, 2000; Seilheimer *et al.*, 1997), have shown that $A\beta$ exists in these solutions as a mixture of monomers and low-order oligomers in quasiequilibrium rather than solely as monomers. The reader should note that for many experimental needs, this issue may be academic, because the primary concern may be the ability to prepare peptide stock solutions identical in

their distribution of peptide assembly states, whatever that distribution may be. The importance of *pre facto* consideration of which assembly state(s) is being monitored cannot be overstated.

Irreproducibility

To conduct studies of amyloid protein assembly, one seeks ideally to produce a homogeneous protein solution within which protein folding and self-association can be initiated synchronously and then monitored. Unfortunately, this has not been possible in studies of A β . Instead, irreproducible behavior of the peptide commonly has been observed, especially among different laboratories. Causes of irreproducibility include the initial structure and aggregation state of the peptide, both in the solid state (Fezoui *et al.*, 2000) and immediately after solvation, the presence in the solvent of nuclei for heterogeneous nucleation, and rapid (<1 s) milieu-dependent protein oligomerization (Bitan *et al.*, 2001, 2003; LeVine, 1995, 2004). The structure of an amyloid protein in the solid state is difficult to control because it depends upon the solution conditions prior to dehydration and the dehydration procedure itself. The issue becomes moot, however, if appropriate solvation procedures are used. These procedures should eliminate preexisting aggregates and create monomeric A β stock solutions from peptide lyophilizates.

Heterogeneous nucleation can be minimized by scrupulous preparation procedures that utilize high-purity water filtered to eliminate nanoparticles, minimize the adherence of particulate matter to glassware and plasticware, and use ultrafiltration methods to eliminate particulates in buffers. These procedures have proven to be effective for biological assays and biophysical studies. It should be noted, however, that electrostatic charging of surfaces and the presence of ionic airborne particulates make complete elimination of particulates almost impossible in the absence of “clean room” procedures of the type used in the semiconductor industry. This fact is particularly relevant for studies using techniques, such as quasielastic light scattering, that have sensitivities directly proportional to analyte molecular weight (Lomakin *et al.*, 1999, 2005). Protein solutions of exceptional clarity (particulate-free) are required if such techniques are to be used successfully.

A “magic formula” for manipulating A β does not exist. Chaotropic agents (dimethylsulfoxide [DMSO]) (Lambert *et al.*, 2001; Stine *et al.*, 2003; Wang *et al.*, 2002), organic acids (TFA) (Zagorski *et al.*, 1999), organic cosolvents (trifluoroethanol [TFE] and hexafluoroisopropanol [HFIP]) (Zagorski and Barrow, 1992), and sodium hydroxide (NaOH) (Fezoui *et al.*, 2000) all have been used either singly or in combination

(Hou *et al.*, 2004) to solubilize and disaggregate A β lyophilizates. For example, examination of the secondary structure of A β dissolved in neat DMSO revealed no β -sheet (Shen and Murphy, 1995). A β treated in this way prior to initiation of fibril formation in biological buffers displayed the slowest polymerization rates relative to samples treated with lower concentrations of DMSO, 0.1% (v/v) TFA, or 0.1% TFA in acetonitrile (Shen and Murphy, 1995). Low (0.1%) TFA concentrations were not effective in disaggregating A β or preventing its self-association. In contrast, pretreatment of A β with concentrated TFA, followed by lyophilization, produced preparations that yielded solutions of protein monomers displaying “random coil” secondary structure following solubilization in biological buffers (Jao *et al.*, 1997). HFIP and TFE disrupt hydrophobic interactions in aggregated amyloid preparations and stabilize α -helical structure (Buck, 1998; Wood *et al.*, 1996; Zagorski and Barrow, 1992), leading to disruption of preexistent β -sheet structure. HFIP pretreatment of A β has been shown to yield peptide solutions of uniform globular morphology with predominantly α -helical and random coil secondary structure and less than 1% β -sheet (Stine *et al.*, 2003).

Preparing A β for Biophysical and Biological Study

In this section, I discuss the technique and benefits of pretreating synthetic A β with NaOH. I then present procedures using membrane filtration or gel permeation chromatography that produce LMW A β from the pretreated lyophilized peptide stocks.

Alkaline Pretreatment of A β

Introduction. The majority of biophysical work done on A β involves synthetic peptides. The chemical synthesis procedures involve cleavage and deprotection of peptide-resins with TFA or hydrogen fluoride (HF), depending on whether FMOC or *t*-BOC (tert-butyloxycarbonyl) chemistry, respectively, is employed. Peptide purification then is accomplished by reverse phase HPLC, typically using gradient elution with TFA in acetonitrile. The result of these procedures is a peptide lyophilizate containing residual, avidly bound trifluoroacetate or fluoride ions. The bound ions produce an acidic milieu following solubilization of the peptide in water. This low pH (≈ 3 – 4) can facilitate peptide solubilization and has proven useful in spectroscopic studies of fibril nucleation and elongation (Lomakin *et al.*, 1996, 1999). However, solvation of A β lyophilizates in buffers of neutral pH produces a pH transition from acidic (≈ 2) to neutral as residual TFA or HF is neutralized. During this transition, the solution pH

passes through the isoelectric point of A β (5.5), at which A β aggregation propensity is maximal and solubility is minimal (Barrow *et al.*, 1992; Wood *et al.*, 1996). The result is conversion of A β monomers into a polydisperse population of interacting low-order oligomers and higher order polymers. This population has irreproducible assembly behavior characterized by significant pH-dependent morphologic and kinetic differences in fibril formation (Wood *et al.*, 1996). We reasoned that if initial solvation of A β could be done without causing pH transitions through the peptide pI, the problems described above could be mitigated. This was accomplished through “presolvation” of the A β lyophilizates with NaOH (pH \approx 10.5–11) followed by relyophilization (Fezoui *et al.*, 2000). Solubilization of the pretreated peptide in neutral buffers then produced a pH shift from the alkaline regimen to neutrality, avoiding the A β pI. Comparative analyses of treated and untreated A β preparations from a variety of commercial and noncommercial sources showed that NaOH pretreatment produced peptide solutions with higher yields of LMW A β and with lower levels of preexistent aggregates (Fezoui *et al.*, 2000). The treated A β preparations reproducibly formed fibrils with conformational and tinctorial properties typical of amyloid fibrils, which were toxic to cultured neurons.

Historical precedent exists for the use of alkali in solubilizing A β . The effects of alkalis on amyloids were examined as early as 1898 (Krakow, 1898). In this work as well as in subsequent studies (Dubois *et al.*, 1999; Hass and Schulz, 1940; Perry *et al.*, 1981; Pras *et al.*, 1969; Shirahama and Cohen, 1967), alkalis, including barium hydroxide, calcium hydroxide, ammonium hydroxide, NaOH, and alkaline borate, and phosphate buffers were used to solubilize amyloids and amyloid proteins from *ex vivo* tissue samples. Ammonium hydroxide (Shirahama and Cohen, 1967) and NaOH (Pras *et al.*, 1969) were found to be particularly effective. Strong alkali treatment thus has proven useful for A β disaggregation and solubilization at both extremes of the polymerization state—monomer and fibril aggregate.

The Method. Dissolve lyophilized A β in 2 mM NaOH to produce a peptide concentration of <1 mg/ml. This may be accomplished conveniently in a 1.5-ml conical microcentrifuge tube. Ensure that the pH of the resulting solution is \geq 10.5. It is very important to add the NaOH and gently agitate the tube only enough to wet the peptide lyophilizate entirely. Allow the solvation to proceed without additional agitation until a visually constant appearance is achieved (\approx 1–3 min). The solution may be clear or remain somewhat turbid. Sonicate the peptide solution in a bath-type sonicator (e.g., a Branson model 1200-R; Branson Ultrasonics Corp., Danbury, CT) for 1 min. Lyophilize the solution and store the lyophilizate at -20° . This NaOH-treated peptide lyophilizate will be the starting material for all subsequent experimental uses.

Filtration and Size Exclusion Chromatography

Introduction. Alkali treatment of A β prior to dissolution is effective in increasing peptide yields and decreasing the number and size of preexistent aggregates (Fezoui *et al.*, 2000). However, this approach alone does not yield peptide solutions amenable to detailed mechanistic study, because aggregates can form during peptide solvation. To produce fully disaggregated peptide solutions, a second preparation step is necessary. This step generally involves size fractionation, which eliminates aggregates, leaving a LMW A β preparation. Filtration methods have been used to remove large and small aggregates from starting solutions of A β (Fezoui *et al.*, 2000). Filtration of A β through a 0.2- μ m nylon microspin Whatman filter at 5000g for 10 min will remove fibrils, fibril aggregates, and other structures larger than 200 nm. However, for most experimental needs, this filtration is insufficient, because assemblies of 200-nm size are relatively large and generally are already fibrillar. Filtration through filters of 20-nm porosity (Anotop 10 Plus; Whatman Inc., Clifton, NJ) or with 10-kDa exclusion limits is a superior method. The latter procedure initially yields monomers and dimers. A second procedure for preparing aggregate-free A β is size exclusion chromatography (SEC). An advantage of SEC relative to simple filtration is its fractionation capability, which allows collection of different populations of oligomers, including protofibrils (Walsh *et al.*, 1997) as well as relatively pure populations of monomer, dimers, and trimers (Bitan and Teplow, 2005).

It is important to reiterate that in peptide concentration regimens of micromola and higher, A β monomers exist in rapid equilibrium with higher order oligomers and that this equilibrium is established within seconds. Most aggregate-free A β solutions therefore comprise an oligodisperse population of assemblies. Nevertheless, because this population can be prepared reproducibly and is not polydisperse, consistent peptide assembly behavior can be observed.

Preparation of LMW A β by Filtration. Filtration is a simple, rapid method that requires few instrumental resources. Microcon YM-10 filters are washed with 200 μ l of 10 mM phosphate buffer, pH 7.4, by adding the buffer to the filter and centrifuging at 16,000g for 20 min. The filtrate is discarded, and the washing is repeated once. The washed filter unit then is placed in a new 1.5 ml microcentrifuge tube. Lyophilized, NaOH-treated A β is dissolved in water at a concentration of \approx 4 mg/ml. An equal volume of 20 mM phosphate buffer, pH 7.4, is added to this solution, which then is sonicated for 1 min and transferred to the filter assembly. The filter unit is centrifuged at 16,000g for 30 min. The filtrate containing LMW A β is collected and used immediately. For experiments involving extended

incubation times, 0.01% (w/v) sodium azide is added to the buffers to prevent microbial growth.

Preparation of LMW $A\beta$ by Size Exclusion Chromatography. Prepare 10 mM sodium phosphate, pH 7.4, using high-purity water. We have found that a Milli-Q Synthesis system (Millipore Corp., Billerica, MA) is an excellent source of water suitable for biophysical and biological studies. Filter the buffer solution through a 0.22 μm polyethersulfone (or equivalent) membrane to remove bacteria and any other large particulates. Use this buffer to wash and equilibrate a 10/30 Superdex 75 HR (Amersham Biosciences, Piscataway, NJ) column at a flow rate of 0.5 ml/min until a flat ultraviolet (UV) trace is observed. The chromatographic pumping system, *per se*, is not relevant to the procedure. It need only provide appropriate flow rates and include a detector capable of determining absorbance in the UV range (200–300 nm). Dissolve 350–400 μg of lyophilized $A\beta$ in DMSO at a concentration of 2 mg/ml in a 1.5 ml

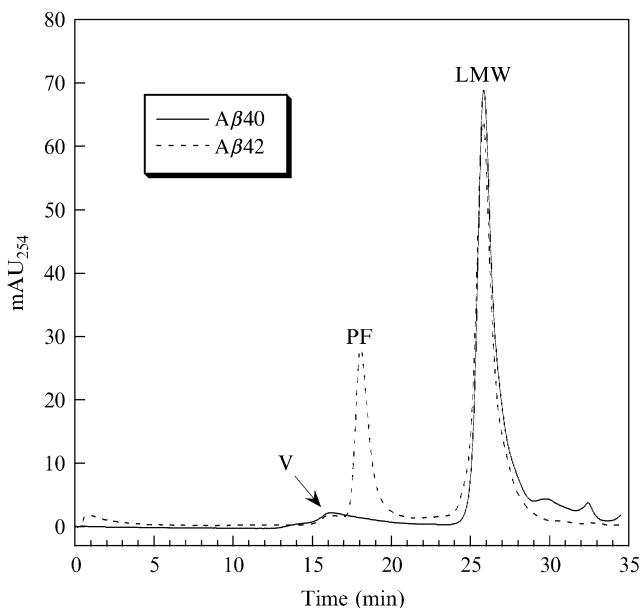


FIG. 1. Preparation of low molecular weight (LMW) amyloid β -protein ($A\beta$) by size exclusion chromatography (SEC). $A\beta_{40}$ (solid line) and $A\beta_{42}$ (dotted line) were fractionated by SEC using a Superdex 75 matrix and 10 mM sodium phosphate, pH 7.4, as the mobile phase. A small void volume peak (V) is observed in both samples, as is a major peak (LMW) in the included volume, which corresponds to LMW $A\beta$. A protofibril peak (PF) is shown in the $A\beta_{42}$ sample. Adapted and reproduced with permission from Bitan *et al.* (2003).

microcentrifuge tube, and then sonicate the tube for 1 min in a bath sonicator. Centrifuge the peptide solution at 16,000g for 10 min to remove any large aggregates. Inject 160–180 μ l of the supernate onto the equilibrated column and monitor the eluate using a UV detector. Protein peaks eluting from the column may be detected at a number of wavelengths, including 215 nm (peptide bonds), 254 nm (mercury line), or 280 nm (tyrosine absorbance). Column calibration with globular and polymeric standards will provide the most accurate indication of apparent molecular weight. Large aggregates elute first in a void volume peak that is followed by a peak comprising protofibrils (Fig. 1). Alkali-treated A β preparations should produce little or none of these components. LMW A β elutes later, with a retention time consistent with globular standards of molecular mass 5–15 kDa. Greatest homogeneity is obtained by collection only of the apex (the middle third, based on collection time) of the LMW peak. The LMW A β fraction should be used immediately after its isolation if structure activity correlations are to be made. Time delays and sample manipulation allow assembly of larger structures, which can complicate interpretation of the experimental data.

LMW A β 40 produced by SEC is qualitatively similar to that produced by filtration (Fig. 2). The oligomerization state of A β 42 differs within LMW fractions prepared by the two methods (Bitan *et al.*, 2003). In

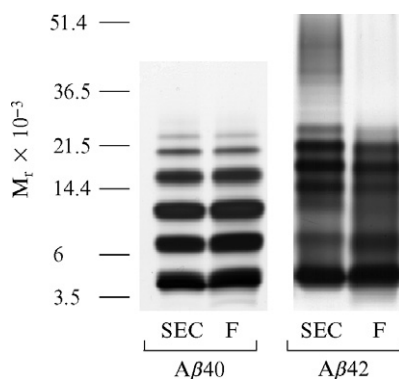


FIG. 2. Amyloid β -protein (A β) oligomer size distributions. Low molecular weight (LMW) A β 40 and A β 42 were isolated either by size exclusion chromatography (SEC) or by filtration through a 10,000 molecular weight cut-off [MWCO] filter. The peptides were photochemically cross-linked to produce a quantitative “snapshot” of the oligomer size distribution (Bitan *et al.*, 2001), and the products were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The mobilities of molecular mass markers are shown on the left. The oligomer size distributions obtained using the two methods are identical for A β 40 but differ for A β 42 (Bitan *et al.*, 2003). Adapted and reproduced with permission from Bitan *et al.* (2001) and Bitan *et al.* (2003).

addition to the relatively narrow (predominantly monomer through heptamer) distribution of oligomers observed with filtered preparations, SEC-isolated LMW A β 42 produces higher order oligomers (Fig. 2).

Preparation of LMW A β by In Situ Filtration. The use of spectroscopic techniques, such as quasielastic light scattering, imposes stringent requirements for sample "cleanliness." Successful measurement of the hydrodynamic radius of LMW molecules depends on the optical purity of the sample. Any high-molecular-weight particulate matter can prevent acquisition of useful spectra. In studies of A β , we have used the intrinsic filtering potential of chromatography column packing materials and a continuous flow procedure for washing the collection vessel (a cuvette) to produce optically pure samples (Fig. 3). Cuvettes first are prepared by heating the top 20 mm of standard 6 \times 50 mm glass test tubes in the flame of a small Bunsen burner or torch. The tops of each tube then are pulled to form

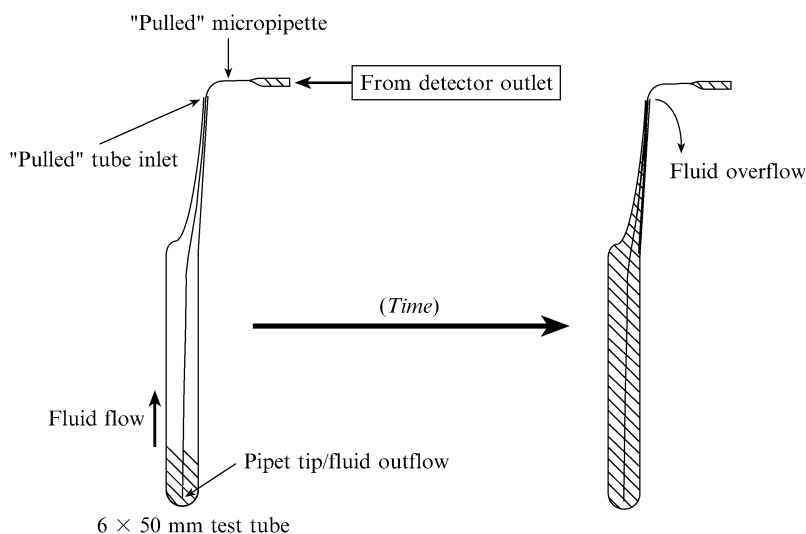


FIG. 3. Direct collection of low molecular weight (LMW) amyloid β -protein (A β) into cuvettes. One end of a 6 \times 50 mm round-bottomed glass test tube is placed in a Bunsen burner, softened, and then pulled to create a narrow inlet. An identical procedure is performed on a glass micropipette. The "pulled" end of the micropipette then is inserted into the bottom of the test tube, and the nonpulled end is connected to the outlet of an ultraviolet detector flow cell. Size exclusion chromatography column eluate then washes the inside of the test tube from the bottom to the top, with the fluid and any dust particles continuously being washed over the lip of the test tube. After a peak of interest is collected, the capillary is removed from the end of the test tube, which is then heat-sealed to create a permanently "clean" environment.

narrow capillaries. A similar procedure is performed on disposable glass micropipettes to form a junction between the HPLC detector and the cuvette. In this case, the untreated end of the micropipette is inserted into the HPLC detector outflow line while the pulled tip of the micropipette is positioned inside the cuvette at its bottom. In this way, "filtered" buffer is constantly washing the interior of the cuvette from the bottom up through the narrow capillary top. To prepare the A β sample, alkali-pretreated peptide is fractionated by SEC. When the peak of interest flows through the detector and fills the cuvette, the micropipette is removed from the cuvette and the end of the cuvette is fire-sealed immediately. This procedure, although somewhat cumbersome, provides excellent dust-free samples. The peak volumes of different A β fractions are large relative to the volume of the cuvette; therefore, the cuvette behaves analogously to the detector flow cell in that cross-contamination among different fractions is not problematic.

Acknowledgments

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References

- Antzutkin, O. N., Balbach, J. J., Leapman, R. D., Rizzo, N. W., Reed, J., and Tycko, R. (2000). Multiple quantum solid-state NMR indicates a parallel, not anti parallel, organization of β -sheets in Alzheimer's β -amyloid fibrils. *Proc. Natl. Acad. Sci. USA* **97**, 13045–13050.
- Balbach, J. J., Petkova, A. T., Oyler, N. A., Antzutkin, O. N., Gordon, D. J., Meredith, S. C., and Tycko, R. (2002). Supramolecular structure in full-length Alzheimer's β -amyloid fibrils: Evidence for a parallel β -sheet organization from solid-state nuclear magnetic resonance. *Biophys. J.* **83**, 1205–1216.
- Barrow, C. J., Yasuda, A., Kenny, P. T. M., and Zagorski, M. (1992). Solution conformations and aggregational properties of synthetic amyloid β peptides of Alzheimer's disease. *J. Mol. Biol.* **225**, 1075–1093.
- Benzinger, T. L. S., Gregory, D. M., Burkoth, T. S., Millerauer, H., Lynn, D. G., Botto, R. E., and Meredith, S. C. (1998). Propagating structure of Alzheimer's β -amyloid(10–35) is parallel β -sheet with residues in exact register. *Proc. Natl. Acad. Sci. USA* **95**, 13407–13412.

- Bitan, G., Kirkitadze, M. D., Lomakin, A., Vollers, S. S., Benedek, G. B., and Teplow, D. B. (2003). Amyloid β -protein ($A\beta$) assembly: $A\beta_{40}$ and $A\beta_{42}$ oligomerize through distinct pathways. *Proc. Natl. Acad. Sci. USA* **100**, 330–335.
- Bitan, G., Lomakin, A., and Teplow, D. B. (2001). Amyloid β -protein oligomerization—Prenucleation interactions revealed by photo-induced crosslinking of unmodified proteins. *J. Biol. Chem.* **276**, 35176–35184.
- Bitan, G., and Teplow, D. (2005). Preparation of aggregate-free, low molecular weight amyloid- β for assembly and toxicity assays. In “Amyloid Proteins—Methods and Protocols” (D. E. M. Sigurdsson, ed.), pp. 3–9. Humana Press, Totowa, NJ.
- Buck, M. (1998). Trifluoroethanol and colleagues: Cosolvents come of age. Recent studies with peptides and proteins. *Q. Rev. Biophys.* **31**, 297–355.
- Buxbaum, J. N. (2003). Diseases of protein conformation: What do *in vitro* experiments tell us about *in vivo* diseases? *Trends Biochem. Sci.* **28**, 585–592.
- Dubois, J., Ismail, A. A., Chan, S. L., and Ali-Khan, Z. (1999). Fourier transform infrared spectroscopic investigation of temperature- and pressure-induced disaggregation of amyloid A. *Scand. J. Immunol.* **49**, 376–380.
- Fezoui, Y., Hartley, D. M., Harper, J. D., Khurana, R., Walsh, D. M., Condron, M. M., Selkoe, P. T., Lansbury, P. T., Fink, A. L., and Teplow, D. B. (2000). An improved method of preparing the amyloid β -protein for fibrillogenesis and neurotoxicity experiments. *Amyloid* **7**, 166–178.
- Garzon-Rodriguez, W., Sepulveda-Becerra, M., Milton, S., and Glabe, C. G. (1997). Soluble amyloid $A\beta$ -(1-40) exists as a stable dimer at low concentrations. *J. Biol. Chem.* **272**, 21037–21044.
- Hass, G., and Schulz, R. Z. (1940). Amyloid. I. Methods of isolating amyloid from other tissue elements. *Arch. Pathol.* **30**, 240–259.
- Hoshi, M., Sato, M., Matsumoto, S., Noguchi, A., Yasutake, K., Yoshida, N., and Sato, K. (2003). Spherical aggregates of β -amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3 β . *Proc. Natl. Acad. Sci. USA* **100**, 6370–6375.
- Hou, L. M., Shao, H. Y., Zhang, Y. B., Li, H., Menon, N. K., Neuhaus, E. B., Brewer, J. M., Byeon, D. G., Ray, D. G., Vitek, M. P., Iwashita, T., Makula, R. A., Przybyla, A. B., and Zagorski, M. G. (2004). Solution NMR studies of the $A\beta$ (1-40) and $A\beta$ (1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. *J. Am. Chem. Soc.* **126**, 1992–2005.
- Howlett, D. R., Jennings, K. H., Lee, D. C., Clark, M. S., Brown, F., Wetzel, R., Wood, S. J., Camilleri, P., and Roberts, G. W. (1995). Aggregation state and neurotoxic properties of Alzheimer β -amyloid peptide. *Neurodegeneration* **4**, 23–32.
- Huang, T. H., Yang, D. S., Plaskos, N. P., Go, S., Yip, C. M., Fraser, P. E., and Chakrabarty, A. (2000). Structural studies of soluble oligomers of the Alzheimer β -amyloid peptide. *J. Mol. Biol.* **297**, 73–87.
- Jao, S. C., Ma, K., Talafous, J., Orlando, R., and Zagorski, M. G. (1997). Trifluoroacetic acid pretreatment reproducibly disaggregates the amyloid β peptide. *Amyloid* **4**, 240–252.
- Kheterpal, I., Lashuel, H. A., Hartley, D. M., Walz, T., Lansbury, P. T., Jr., and Wetzel, R. (2003). $A\beta$ protofibrils possess a stable core structure resistant to hydrogen exchange. *Biochemistry* **42**, 14092–14098.
- Kheterpal, I., Zhou, S., Cook, K. D., and Wetzel, R. (2000). $A\beta$ amyloid fibrils possess a core structure highly resistant to hydrogen exchange. *Proc. Natl. Acad. Sci. USA* **97**, 13597–13601.

- Kirkitadze, M. D., Bitan, G., and Teplow, D. B. (2002). Paradigm shifts in Alzheimer's disease and other neurodegenerative disorders: The emerging role of oligomeric assemblies. *J. Neurosci. Res.* **69**, 567–577.
- Klein, W. L., Stine, W. B., Jr., and Teplow, D. B. (2004). Small assemblies of unmodified amyloid β -protein are the proximate neurotoxin in Alzheimer's disease. *Neurobiol. Aging* **25**, 569–580.
- Krakow, N. P. (1898). Beitdige zur chemie der amyloidenartung. *Arch. Exp. Pathol. Pharmacol.* **40**, 195–220.
- Lambert, M. P., Viola, K. L., Chromy, B. A., Chang, L., Morgan, T. E., Yu, J. X., Venton, G. A., Krafft, G. A., Finch, C. E., and Klein, W. L. (2001). Vaccination with soluble $A\beta$ oligomers generates toxicity-neutralizing antibodies. *J. Neurochem.* **79**, 595–605.
- Lazo, N. D., Maji, S. K., Fradinger, E. A., Bitan, G., and Teplow, D. B. (2005). The amyloid β -protein. In "Amyloid Proteins—The Beta Sheet Conformation and Disease" (J. C. Sipe, ed.), pp. 385–492. Wiley-VCH, Weinheim.
- LeVine, H., III. (1995). Soluble multimeric Alzheimer β (1-40) pre-amyloid complexes in dilute solution. *Neurobiol. Aging* **16**, 755–764.
- LeVine, H., III. (2004). Alzheimer's β -peptide oligomer formation at physiologic concentrations. *Anal. Biochem.* **335**, 81–90.
- Lomakin, A., Benedek, G. B., and Teplow, D. B. (1999). Monitoring protein assembly using quasielastic light scattering spectroscopy. *Methods Enzymol.* **309**, 429–459.
- Lomakin, A., Chung, D. S., Benedek, G. B., Kirschner, D. A., and Teplow, D. B. (1996). On the nucleation and growth of amyloid β -protein fibrils: Detection of nuclei and quantitation of rate constants. *Proc. Natl. Acad. Sci. USA* **93**, 1125–1129.
- Lomakin, A., Teplow, D. B., and Benedek, G. B. (2005). Quasielastic light scattering for protein assembly studies. In "Amyloid Proteins: Methods and Protocols" (E. M. Sigurdsson, ed.), pp. 153–174. Humana Press, Totowa, NJ.
- Maji, S. K., Amsden, J. J., Rothschild, K. J., Condron, M. M., and Teplow, D. B. (2005). Conformational dynamics of amyloid β -protein assembly probed using intrinsic fluorescence. *Biochemistry* **44**, 13365–13376.
- Murphy, E. A. (1949). Murphy's law. Available at: http://www.edwards.af.mil/history/docs_html/tidbits/murphy's_law.html.
- Perry, E. K., Oakley, A. E., Candy, J. M., and Perry, R. H. (1981). Properties and possible significance of substance P and insulin fibrils. *Neurosci. Lett.* **25**, 321–325.
- Petkova, A. T., Ishii, Y., Balbach, J. J., Antzutkin, O. N., Leapman, R. D., Delaglio, F., and Tycko, R. (2002). A structural model for Alzheimer's β -amyloid fibrils based on experimental constraints from solid state NMR. *Proc. Natl. Acad. Sci. USA* **99**, 16742–16747.
- Pike, C. J., Burdick, D., Walencewicz, A. J., Glabe, C. G., and Cotman, C. (1993). Neurodegeneration induced by β -amyloid peptides *in vitro*: The role of peptide assembly state. *J. Neurosci.* **13**, 1676–1687.
- Pike, C. J., Walencewicz, A. J., Glabe, C. G., and Cotman, C. W. (1991). *In vitro* aging of β -amyloid protein causes peptide aggregation and neurotoxicity. *Brain Res.* **563**, 311–314.
- Pras, M., Zucker-Franklin, D., Rimon, A., and Franklin, E. C. (1969). Physical, chemical, and ultrastructural studies of water-soluble human amyloid fibrils. *J. Exp. Med.* **130**, 777–796.
- Seilheimer, B., Bohrmann, B., Bondolfi, L., Muller, F., Stuber, D., and Dobeli, H. (1997). The toxicity of the Alzheimer's β -amyloid peptide correlates with a distinct fiber morphology. *J. Struct. Biol.* **119**, 59–71.
- Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498.
- Selkoe, D. J. (2001). Alzheimer's disease: Genes, proteins, and therapy. *Physiol. Rev.* **81**, 741–766.

- Shen, C. L., and Murphy, R. M. (1995). Solvent effects on self-assembly of β -amyloid peptide. *Biophys. J.* **69**, 640–651.
- Shirahama, T., and Cohen, A. S. (1967). Reconstitution of amyloid fibrils from alkaline extracts. *J. Cell Biol.* **35**, 459–464.
- Simmons, L. K., May, P. C., Tomaselli, K. J., Rydel, R. E., Fuson, K. S., Brigham, E. F., Wright, I., Lieberburg, I., Becker, G. W., Brems, D. N., and Li, W. Y. (1994). Secondary structure of amyloid β peptide correlates with neurotoxic activity *in vitro*. *Mol. Pharmacol.* **45**, 373–379.
- Soto, C., Castano, E. M., Kumar, R. A., Beavis, R. C., and Frangione, B. (1995). Fibrillogenesis of synthetic amyloid- β peptides is dependent on their initial secondary structure. *Neurosci. Lett.* **200**, 105–108.
- Stine, W. B., Dahlgren, K. N., Krafft, G. A., and LaDu, M. J. (2003). *In vitro* characterization of conditions for amyloid- β peptide oligomerization and fibrillogenesis. *J. Biol. Chem.* **278**, 11612–11622.
- Taylor, B. M., Sarver, R. W., Fici, G., Poorman, R. A., Lutzke, B. S., Molinari, A., Kawabe, T., Kappenman, A. E., Buhl, A. E., and Epps, D. E. (2003). Spontaneous aggregation and cytotoxicity of the β -amyloid A β 1–40: A kinetic model. *J. Prot. Chem.* **22**, 31–40.
- Teplow, D. B. (1998). Structural and kinetic features of amyloid β -protein fibrillogenesis. *Amyloid* **5**, 121–142.
- Thirumalai, D., Klimov, D. K., and Dima, R. I. (2003). Emerging ideas on the molecular basis of protein and peptide aggregation. *Curr. Opin. Struct. Biol.* **13**, 146–159.
- Torok, M., Milton, S., Kaye, R., Wu, P., McIntire, T., Glabe, C., and Langen, R. (2002). Structural and dynamic features of Alzheimer's A β peptide in amyloid fibrils studied by site-directed spin labeling. *J. Biol. Chem.* **277**, 40810–40815.
- Tycko, R. (2004). Progress towards a molecular-level structural understanding of amyloid fibrils. *Curr. Opin. Struct. Biol.* **14**, 96–103.
- Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condron, M. M., Lomakin, A., Benedek, D. J., Selkoe, D. J., and Teplow, D. B. (1999). Amyloid β -protein fibrillogenesis—Structure and biological activity of protofibrillar intermediates. *J. Biol. Chem.* **274**, 25945–25952.
- Walsh, D. M., Lomakin, A., Benedek, G. B., Condron, M. M., and Teplow, D. B. (1997). Amyloid β -protein fibrillogenesis—Detection of a protofibrillar intermediate. *J. Biol. Chem.* **272**, 22364–22372.
- Walsh, D. M., and Selkoe, D. J. (2004). Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* **44**, 181–193.
- Wang, H. W., Pasternak, J. F., Kuo, H., Ristic, H., Lambert, M. P., Chromy, B., Viola, K. L., Klein, W. B., Stine, W. B., Krafft, G. A., and Trommer, B. L. (2002). Soluble oligomers of β amyloid (1–42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Res.* **924**, 133–140.
- Wood, S. J., Maleeff, B., Hart, T., and Wetzel, R. (1996). Physical, morphological and functional differences between pH 5.8 and 7.4 aggregates of the Alzheimer's amyloid peptide A β . *J. Mol. Biol.* **256**, 870–877.
- Zagorski, M. G., and Barrow, C. J. (1992). NMR studies of amyloid β -peptides: Proton assignments, secondary structure, and mechanism of an α -helix \rightarrow β -sheet conversion for a homologous, 28-residue, N-terminal fragment. *Biochemistry* **31**, 5621–5631.
- Zagorski, M. G., Yang, J., Shao, H., Ma, K., Zeng, H., and Hong, A. (1999). Methodological and chemical factors affecting amyloid- β peptide amyloidogenicity. *Methods Enzymol.* **309**, 189–204.