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Biochemistry of Amyloid β -Protein and Amyloid Deposits in Alzheimer Disease

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Progressive cerebral deposition of the amyloid β -protein ($A\beta$) in brain regions serving memory and cognition is an invariant and defining feature of Alzheimer disease. A highly similar but less robust process accompanies brain aging in many nondemented humans, lower primates, and some other mammals. The discovery of $A\beta$ as the subunit of the amyloid fibrils in meningeocerebral blood vessels and parenchymal plaques has led to innumerable studies of its biochemistry and potential cytotoxic properties. Here we will review the discovery of $A\beta$, numerous aspects of its complex biochemistry, and current attempts to understand how a range of $A\beta$ assemblies, including soluble oligomers and insoluble fibrils, may precipitate and promote neuronal and glial alterations that underlie the development of dementia. Although the role of $A\beta$ as a key molecular factor in the etiology of Alzheimer disease remains controversial, clinical trials of amyloid-lowering agents, reviewed elsewhere in this book, are poised to resolve the question of its pathogenic primacy.

THE LASTING IMPACT OF THE DISCOVERY OF AMYLOID β -PROTEIN ON THE ELUCIDATION OF ALZHEIMER DISEASE

With the benefit of hindsight, it is now clear that the isolation and partial sequencing of the meningeovascular amyloid β -protein ($A\beta$) by George Glenner and Caine Wong in 1984 provided a turning point for modern research on the fundamental mechanism of Alzheimer disease (AD). Ever since Alzheimer peered through the microscope at the brain of his first patient and wrote prophetically “scattered through the entire cortex . . . one found miliary foci that were caused by the deposition

of a peculiar substance . . .,” neuropathologists had sought the nature of the amyloid material found in the senile plaque. By the early 1980s, as compositional analyses of the neurofibrillary tangle were beginning (see Mandelkow and Mandelkow 2011), a few investigators turned their attention to the identity of the amyloid protein in vascular and plaque deposits. In this chapter, we will review how our biochemical understanding of the amyloid deposits emerged and has advanced, and we will describe many features of the peptides that comprise this hallmark lesion of AD and certain molecules associated with them. The trafficking and proteolytic processing of amyloid precursor protein (APP), including the generation of

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A β , and the proteolytic degradation of the peptide are covered in other chapters (see Haass et al. 2011; Saido and Leissring 2011, respectively) and will not be discussed here.

BIOCHEMISTRY OF A β IN MENINGOVASCULAR AMYLOID DEPOSITS AND AMYLOID PLAQUE CORES

Because George Glenner's earlier research on the circulating precursors of nonneural amyloid deposits (e.g., AL amyloid) convinced him that the amyloid in AD might well be derived from a serum precursor, he focused his attention on the amyloid in meningeal vessel walls. By stripping the meninges from postmortem AD brains, Glenner and Wong enriched for amyloid-bearing microvessels and discarded the cerebral tissue with its potentially "contaminating" amyloid plaques and neurofibrillary tangles (Glenner and Wong 1984b). They used the chaotropic salt guanidine hydrochloride (at 6M) to solubilize and then chromatographically enrich the amyloid subunit, which ran as a 4.2 kDa band on SDS-PAGE. HPLC purification of the protein and amino-terminal sequencing to residue 24 revealed a unique sequence (their report of a glutamine rather than glutamate at position 11 was corrected in their subsequent sequencing of Down's syndrome meningeovascular A β). In this initial report, Glenner and Wong suggested that this novel peptide might turn out to be derived from a serum precursor and that it could "provide a diagnostic test for Alzheimer's disease and a means to understand its pathogenesis." Whereas the first of these three predictions turned out not to be true, the second and third clearly did.

Shortly after this paper appeared, Glenner and Wong published a highly similar study (Glenner and Wong 1984a) which showed that the meningeovascular amyloid subunit in Down's syndrome brains was the same " β -protein," as they had dubbed it. Glenner called attention to this evidence of a key biochemical relationship between Down's syndrome and AD, a concept he had touted as early as 1979 in a prescient article in *Medical Hypotheses* (Glenner 1979). He stressed that Down's

syndrome may be a "predictable model" for AD and further suggested that "the genetic defect in Alzheimer's disease is localized on chromosome 21." Glenner reasoned that, because trisomy 21 led to Alzheimer-type A β accumulation in vessels and plaques, familial AD itself might well involve a defect in the precursor of the β -protein on this chromosome. This prediction turned out to be true in part; the first gene implicated in a familial form of AD was indeed the β -amyloid precursor protein. What Glenner apparently did not recognize—or at least state at the time—was the heterogeneity of familial forms of AD as well as the notion that many cases may not be genetically determined. Nevertheless, these two brief papers in *Biochemical and Biophysical Research Communications*, although not viewed as potentially seminal in the months after their publication, turned out to provide both the factual and conceptual underpinnings for all subsequent research on β -amyloidosis in AD.

During the years 1983–1985, efforts independent of those of Glenner were made in several laboratories to isolate and sequence the amyloid in senile plaque cores (Fig. 1) from AD brains. These efforts began before the identification of the vascular A β peptide by Glenner and Wong, but they were greatly facilitated by it. In 1983, Allsop, Landon, and Kidd reported a method for isolating intact neuritic plaque cores from postmortem AD brain and found them to be insoluble in various denaturants (Allsop et al. 1983). They published an amino acid composition which did not resemble any previously described amyloid protein. The authors described a variety of contaminants in their final preparations, including bacteria, leading to concerns about the accuracy of this composition, although subsequent methods produced core preparations of greater purity but generally similar composition, signifying the relative insensitivity of the amino acid composition of partially purified proteins as a biochemical comparator.

In the laboratories of Masters and Beyreuther, Roher, Selkoe, and Frangione, distinct but partially related methods for purifying

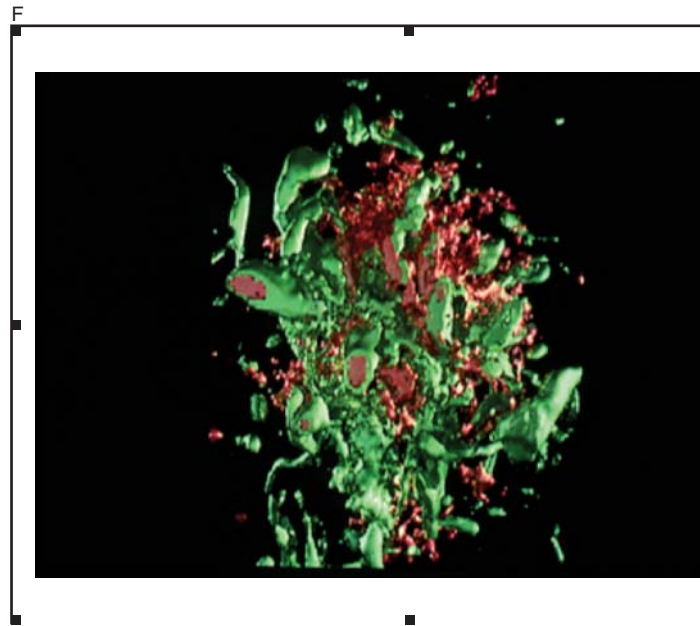


Figure 1. Three-dimensional reconstructed image by confocal microscopy of a neuritic (senile) plaque in the cortex of a patient dying with Alzheimer disease. Red labeling is by an antibody to amyloid β -protein which reveals the extracellular amyloid; green labeling is with an antibody against p-Tau which reveals intimately associated dystrophic neurites. Note that this plaque core is not a solid mass of amyloid but is fragmented and porous and contains abnormal cell processes intercalated within it. (Image courtesy of Dr. Eliezer Masliah, University of California, San Diego, CA.)

and solubilizing amyloid plaque cores from postmortem AD brain were developed. Masters and colleagues first reported the protein subunit of amyloid plaque cores, using a method which used nonionic detergent extraction of brain, pepsin digestion, and sucrose density gradient fractionation (Masters et al. 1985). The resultant cores were found to be approximately 90% pure by microscopy and were partially soluble in high concentrations (10%) of SDS and BME and fully soluble in approximately 70% formic acid. By both HPLC and SDS/urea PAGE, the formic acid-solubilized core protein ran not only at ~ 4.3 kDa but also at ~ 8 , 12, and 16 kDa, demonstrating the ready association of the monomer into SDS-stable oligomers. Masters and Beyreuther pointed out that the molecular mass, amino acid composition and amino-terminal sequence of the protein they isolated from cores were essentially identical to those described for vascular A β

by Glenner, although their analyses showed considerable amino-terminal “raggedness” in the plaque-derived protein. They concluded that the shared 4 kDa subunit indicated a common origin for the plaque and vascular amyloids in AD. Again, A β peptides isolated from AD and Down’s syndrome plaques were indistinguishable. The amino-terminal heterogeneity reported by Masters and Beyreuther was striking, in that only 12% of the sequenced protein began at Asp1, with 64% starting at Phe4 and the remainder at downstream residues, perhaps deriving in part from their use of pepsin digestion during plaque purification. In Masters’ report, Glenner’s 24-residue sequence was extended to residue 28, although the identities of two of those additional four residues were later revised.

Plaque core purifications and analyses performed at that time in three other laboratories provided largely consistent findings.

The various methods employed took advantage of the insolubility of the amyloid cores in detergents such as SDS and their relative resistance to quantitative digestion by proteases. In the studies of Roher et al. (1986) and Gorevic et al. (1986), as in that of Masters et al. (1985), peptidases were used to diminish contaminants, but this approach raised the possibility of partial digestion of A β itself and the creation of some of the observed amino-terminal heterogeneity. In the study of Selkoe et al. (1986) the use of extensive SDS extraction of the cores, then sucrose gradient centrifugation, and then a two-step fluorescence-activated particle sorting (FACS) led to SDS-insoluble plaque cores that were >90–95% pure by electron microscopy, enabling an estimate (via amino acid analysis) of the protein content of a single plaque core: 60–130 pg. However, the attempts of Selkoe and colleagues to sequence this purified plaque amyloid after its solubilization in formic acid or saturated guanidine thiocyanate showed a blocked amino terminus. In subsequent years, biochemical and immunocytochemical studies from several laboratories made clear that the amino termini of plaque A β peptides are heterogeneous and include derivatized and amino-terminally blocked species, e.g., pyroglutamate at residue 3. It is likely that the degree of amino-terminal heterogeneity and the precise termini obtained in various biochemical analyses of plaque cores depends in considerable part on the biochemical nature and harshness of the extraction protocol. It has been shown that particular purification reagents can chemically alter A β structure, for example, the oxidation of Met35 in the presence of formic acid. Other types of amino acid modifications of plaque A β , such as racemization and isomerization of its aspartates (e.g., D-aspartate and L- and D-is-aspartates) or formylation of serines during formic acid solubilization, have been reported. The former changes may occur during the prolonged aging of the deposited amyloid proteins in vivo, whereas the latter is an artifact of an in vitro method of solubilization.

One amino-terminal modification that has received particular attention is the proteolytic removal of residues 1 and 2 (Asp and Ala) and

the subsequent cyclizing of residue 3 (Glu) to a pyroglutamate (designated N-3pE). First described in biochemical extracts of AD cortex (Mori et al. 1992), this truncated species was found to be detectable immunohistochemically in many diffuse (i.e., mostly nonfibrillar) plaques in AD and DS cortex (Saido et al. 1995). This truncation increases the aggregation kinetics of A β (D'Arrigo et al. 2009; Sanders et al. 2009; Wirths et al. 2010) and also obviates the amino-terminal binding of those therapeutic antibodies which target Asp1 of A β (Gardberg et al. 2009). Recent work has shown that glutaminyl cyclase, an enzyme in brain and other tissues which cyclizes exposed glutamates, can do so with high efficiency at Glu3 (Seifert et al. 2009) after removal of the first two residues by aminopeptidases (Schlenzig et al. 2009; Sevalle et al. 2009). The amount of A β pE3 in the brains of APP transgenic mice can increase with time, suggesting that the deposits begin with full-length A β _{1-x}, some of which is first truncated by local aminopeptidase activity and then modified by glutaminyl cyclase (Wirths et al. 2010). Other changes in the amino terminus, including pathogenic mutations at residues 6 and 7 (Ono et al. 2010), may have major effects on oligomerization.

Taken together, the early biochemical analyses of the amyloid fibrils in meningeal vessels and cerebral plaque cores established that the subunit in both cases was a highly hydrophobic ~4 kDa protein with a unique sequence that had a strong tendency to self-aggregate into stable dimers, trimers, and tetramers, higher oligomers and, ultimately, typical 8 nm amyloid fibrils. One interesting sidelight of these studies was the observation that the A β derived from plaque cores was generally more insoluble than that from vascular deposits. For example, 6 M guanidine hydrochloride could effectively solubilize the latter but not the former, whereas the stronger chaotropic salt, guanidine thiocyanate, at saturated (6.8 M) concentrations, could solubilize the cores (Selkoe et al. 1986). The use of concentrated formic acid by Masters and coworkers provided a reagent that appeared to bring even the most insoluble amyloid fibers in AD brains into

solution, and it has subsequently been widely used for this purpose. That it can do so indicates that, in general, cerebral A β proteins which assemble into amyloid fibrils undergo little or no covalent cross-linking.

Although the identity of the protein subunit of Alzheimer amyloid was thus well established by 1986, the carboxy-terminal sequence beyond residue 28 and the molecular origin of this small peptide remained unclear. As detailed in Haass et al. (2011), it was the power of molecular biological approaches that enabled the elucidation of its full length and how it actually arose from proteolysis of a large precursor polypeptide (Kang et al. 1987).

In the almost 30 years since the biochemical characterization of AD amyloid deposits commenced, we have come to realize that the complexity of this relatively short peptide is determined in part by the microenvironment in which it is generated and resides. Although small amounts can be produced in the endoplasmic reticulum and other vesicular organelles in the secretory pathway, much of the peptide appears to arise from APP that has trafficked to the cell surface and is then sequentially processed by β - and γ -secretase (both are aspartyl proteases) in the mildly acidic environment of recycling endosomes (Selkoe 1994; Kaether et al. 2006; Cirrito et al. 2008; and see Haass et al. 2011). As mentioned above, the peptide isolated from fibrillar amyloid plaques shows substantial heterogeneity at both its amino and carboxyl termini. Its biochemical properties vary significantly depending on its terminal residues, particularly at the hydrophobic carboxyl terminus. Although the field has focused until recently on two peptide lengths, the most abundantly produced species (A β _{1–40}) and the far less abundant but more aggregation prone A β _{1–42}, this is a simplification, as the variability of carboxy-terminal lengths created by γ -secretase extends at least from A β ₃₆ to A β ₄₃ (Kang et al. 1987). This heterogeneity arises secondary to the initial ϵ -cleavage of APP by the presenilin/ γ -secretase complex at Leu49–Val50 (Weidemann et al. 2002), followed by processive intramembrane processing

by this protease in an amino-terminal direction (i.e., first ϵ , then ζ and then γ cleavages) (QiTakahara et al. 2005; Takami et al. 2009; and see Haass et al. 2011).

AMYLOID FIBRILS OF A β : STUDIES OF THEIR STRUCTURE AND PROPERTIES

The pathognomonic lesions of AD are the fibrillar extracellular deposits of A β in parenchymal plaques and vascular amyloid and the intraneuronal neurofibrillary tangles, which also have the tinctorial properties of amyloid (Serrano-Pozo et al. 2011). How A β , including its buffer-soluble oligomeric forms, may induce the formation of intracellular tangles of the tau protein is discussed elsewhere (Mandelkow and Mandelkow 2011; Mucke and Selkoe 2011). Here we will review the pathway which converts the A β region from its largely α -helical conformation when APP is embedded in the lipid membrane to its gradual aggregation into large polymers (filaments) rich in cross- β sheet structure in the extracellular space of the brain. The conversion of α -helix or random coil stretches within normally soluble proteins into principally β -sheet rich assemblies is a common theme in several neurodegenerative diseases. Drawing on the analogous prion theory, it is also possible for a β -sheet conformer to induce or “seed” an α -helical conformer (or some other metastable intermediate) to adopt β -sheet structure (Eisele et al. 2009, 2010). What structural relationship such intermediates in fibrillogenesis have to the soluble, diffusible oligomers of A β detected in AD brain remains uncertain. Despite more than 50 years of structural analysis, the atomic resolution of classical amyloid fibrils remains incomplete. Although many techniques have been applied (including solid-state nuclear magnetic resonance and cryo-electron microscopy), the basic noncrystalline subunit in the A β fibril has prevented progress (Caspar 2009; Kajava et al. 2010). Moreover, most of the data available on the structure of A β and its fibrils come from studies of synthetic A β peptides, and it remains unclear whether these accurately model the natural A β assemblies found in AD brain.

Theoretical Computational and Molecular Dynamic Models of the A β Amyloid Fibril

Although the monomeric subunit of fibrils is thought to consist of two β -strands connected by a turn, the ambiguous nature of the amino and carboxyl termini have precluded development of a detailed model (Olofsson et al. 2009b; Paparcone and Buehler 2009; Ramos et al. 2009). The convoluted carboxy-terminal folding seen in a constrained oligomeric structure (Streltsov et al. 2011) provides a caveat that the simple, U-shape β -turn may be an oversimplification. Some variability in this turn region has now been identified using molecular dynamic (MD) simulations of dimers compared to trimers/pentamers (Horn and Sticht 2010) and a triangular subunit forming a three-fold hexamer (Zheng et al. 2010). Constraining the β -turn by linking Asp23 to Lys28 also results in a system with increased fibrillogenic propensity (Reddy et al. 2009a). Placing the β -turn on a constraining physical interface also affects assembly (Fu et al. 2009), and this could have implications for A β assembly when some of the peptide is bound to cell membranes, as is likely in the brain. Assembly at low or neutral pH may have an effect on the registration of the subunits within the fibril (Negureanu and Baumketner 2009). Interpeptide hydrogen bonds may play a major role in fibril growth, based on MD modeling (Reddy et al. 2009b; Takeda and Klimov 2009b, 2010). Oxidation of Met35 and assembly in quiescent versus agitated conditions have also been modeled and found to have effects on the hydrophobic surfaces exposed on the fibrils (Wu et al. 2010a).

Structural Studies of Synthetic A β Fibrils

Low-resolution (8 Å) cryo-electron microscopy of synthetic A β_{40} and A β_{42} reveals similar protofilament structures, with approximately 2.5 peptides per cross- β repeat per protofilament (Schmidt et al. 2009). The lack of an integral number per repeat suggests that the assembly may have undefinable amino termini within a tetrameric structure (Caspar 2009). Other low-resolution (10 Å) cryo-EM reconstructions

have suggested that the carboxyl terminus forms the inside wall of a hollow core (Zhang et al. 2009d). Two-dimensional infrared spectroscopy discloses intramolecular water molecules around residues 17/34 and 18/36 in a conformation with a presumptive β -turn at 23/28. Substitutions around Glu22 and Asp23, either artificial or mimicking the pathogenic “Arctic” or “Iowa” FAD mutations, produce major effects on rates of aggregation (Perálvarez-Marín et al. 2009; Tycko et al. 2009), perhaps through a mechanism that involves off-registry side chain interactions (Takeda and Klimov 2009c).

DIFFUSIBLE OLIGOMERS OF A β

If one considers the trajectory of biochemical studies of A β , it is clear that the field has moved over the last dozen years from an initial emphasis on the fibrillar state found in amyloid plaques and meningeocerebral vessels to a range of smaller, oligomeric A β assemblies that are relatively soluble and diffusible and thus more able to exert a toxic effect on the neuronal plasma membrane, including synapses. A rich and confusing vocabulary has developed to describe the oligomers of A β as they assemble along pathways which may or may not lead to the classical 8 nm amyloid fibrils found in plaques and blood vessels (Table 1). The methods of analysis often determine nomenclature: biochemical characterization of synthetic or natural (cellular and brain) A β peptides using SDS-PAGE or size exclusion chromatography has led to descriptions of assemblies containing a few (e.g., 2–20) monomers, usually designated soluble oligomers; other methods, particularly those using morphological or biophysical approaches on synthetic A β such as electron microscopy or atomic force microscopy, may describe linear protofilaments (often \sim 4 nm in diameter) or spherical/globular particles, each of which has been interpreted as a precursor of amyloid fibrils. It is important to emphasize here that many of the synthetic A β assembly forms reported in the literature have been made in vitro using supraphysiological concentrations of a single-length peptide (e.g., A β_{1-40}), and

Table 1. A β assemblies described in the literature

A rich vocabulary that depends on the source of the peptide, the method of analysis, and the laboratory involved
A β oligomers of natural or synthetic origin, as visualized chromatographically and/or on denaturing protein gels: monomers (A_4), dimers (A_8), trimers, tetramers (A_{16}), pentamers, hexamers, dodecamer/12-mer ($A\beta^*56$), lower/higher order oligomers [$(A\beta)_n$]
Other synthetic oligomeric A β assemblies: amyloid- β -derived diffusible ligands (ADDLs); A β micelles, annular (pore-like) structures, (pre-)globulomers (globular oligomers), growth-arrested colloid particles, metastable aggregates, nanopore-like structures, nanoparticles, paranuclei/nucleating seeds, on/off pathway intermediate states, spherical aggregates, etc.
Synthetic A β fibrillar assemblies: protofibrils, prefibrils, fibrillar oligomers, nanofibrils

the occurrence of closely similar or identical species in AD brain tissue may not have been explicitly confirmed structurally (immunochemical cross-reaction would not be sufficient confirmation). One caveat in this regard is that natural A β oligomers isolated from AD brain tissue or APP-expressing cell cultures are far more potent in electrophysiological or cytotoxicity assays than are synthetic assembly mixtures such as ADDLs (“A β -derived diffusible ligands”) (Lambert et al. 1998) or protofibrils (Harper et al. 1997; Walsh et al. 1997), which require high nanomolar concentrations to induce biological effects, suggesting that they contain many “off-pathway” (unnatural) assembly forms that do not interact with neuronal membranes the way natural oligomers do. Indeed, some such synthetic “oligomers” have not been proven to be truly soluble in aqueous buffers (i.e., not pelletable at 100,000 g in an ultracentrifuge), which is the case for natural oligomers (see, for further reviews of the complexity of A β assembly forms, Haass and Selkoe 2007; Walsh and Selkoe 2007; Di Carlo 2010; Sakono and Zako 2010).

Synthetic A β as a Substrate for Oligomer Formation

Ever since the sequence of A β became known, the easiest approach to study assembly has been to aggregate synthetic peptides at supra-physiological concentrations in vitro (Castano et al. 1986; Gorevic et al. 1986; Kirschner et al. 1987). Classical biochemical analyses of the synthetic aggregates have been supplemented with

newer biophysical methods such as scanning tunneling microscopy (Liu et al. 2009a; Ma et al. 2009), atomic force microscopy (Wu et al. 2010b), quartz crystal microbalance (Ogi et al. 2009), hydrogen exchange mass spectrometry (Zhang et al. 2009a), electron capture dissociation Fourier-transform ion cyclotron resonance mass spectroscopy (Sargaeva et al. 2009), single-molecule spectroscopy (Ding et al. 2009), fluorescence photobleaching and quenching (Reinke et al. 2009; Edwin et al. 2010), click peptide technique (Taniguchi et al. 2009), and ion mobility coupled with mass spectrometry (Bernstein et al. 2009). The experimental conditions for assembling synthetic A β monomers into oligomers vary enormously with regard to the roles of temperature, salts, detergents, lipids, metal ions, fatty acids, and other molecules (Sahoo et al. 2009; Yu et al. 2009; Ahmed et al. 2010; Ladiwala et al. 2010; Ryan et al. 2010), and each such condition provides constraints on the techniques that can be used to study the synthetic peptide. Stabilization of synthetic dimers by cross-linking oxidized tyrosine-10 residues (Ono et al. 2009) or introduced cysteine residues (O’Nuallain et al. 2010) also provides a way to study this smallest oligomer and its role in the dynamic equilibrium of A β assembly.

Amino acid substitutions and modifications can readily be introduced into synthetic peptides. However, to what degree these synthetic changes adequately model the in vivo situation is often disregarded by investigators. For example, the familial intra-A β mutations that occur in dominant fashion at position 22 or

23 (E22G, E22Q, E22K, and D23N) result in the accrual in vivo of a mixture of mutant and wild-type peptides of heterogeneous lengths, but this is often not modeled in vitro (Masuda et al. 2009; Murray et al. 2009b; Brorsson et al. 2010). Nevertheless, synthetic A β studies of this critical region do provide some information on the effects of these mutations on the β -turn at 25/26 versus 22/23 and consequent effects on oligomerization and toxicity in vitro.

Molecular Dynamic Approaches to Understanding Synthetic A β Oligomers

Although some progress in obtaining atomic resolution of the amino terminus and carboxyl terminus of synthetic A β has been made, the overall structure(s) of the oligomer(s) at different assembly points remains elusive. A plethora of MD simulations and theoretical modeling has emerged. Starting at the amino terminus, Takeda and Klimov (2009a) find that amino-terminal truncation has an effect on oligomer (dimer) formation. The metal-binding region of A β around residues 6–14 has not yet been adequately addressed by MD studies (see below), and there are conflicting results on models obtained for the loop and β -strands associated with the residue 16–35 region (Chebaro et al. 2009; Miller et al. 2009; Murray et al. 2009a; Hamley et al. 2010; Wei et al. 2010). The oxidation state of Met35 has long been of interest (Haeffner et al. 2010); this residue in the hydrophobic carboxyl terminus may play a role in oligomerization driven by hydrophobic interactions (Zhao et al. 2009). Although it has long been suspected that the highly hydrophobic carboxyl terminus of A β ₄₂ is a principal determinant of aggregation, its biophysical role in oligomerization has only recently begun to be addressed using synthetic peptides (Murray et al. 2009a; Li et al. 2010). The structure of the carboxyl terminus may involve novel metastable conformations (β -hairpin at 35–37), but these remain to be confirmed by crystallographic methods (Wu et al. 2009). Higher order oligomers (pentameric/hexameric assemblies) have been studied by MD and show different effects between the central hydrophobic and

carboxy-terminal regions when A β ₄₀ and A β ₄₂ are compared (Urbanc et al. 2010). Synthetic oligomers are also being used in attempts to discover small molecules which are able to target these specific assemblies (Davis and Berkowitz 2009a; Davis et al. 2009; Feng et al. 2009; Liu et al. 2009a; Nerelius et al. 2009; Pitt et al. 2009; Riviere et al. 2009; Smith et al. 2009; Sun et al. 2009; Yamin et al. 2009; Hawkes et al. 2010; Ladiwala et al. 2010).

Recombinant A β Oligomers

A β oligomers obtained using recombinant techniques appear to be as challenging to work with as their synthetic counterparts (Picone et al. 2009; Walsh et al. 2009; Streltsov et al. 2011). Nevertheless, recombinant constructs have provided a way to conformationally constrain oligomerized A β assemblies and are beginning to provide novel insights into lower-order oligomers (e.g., dimers and tetramers) at atomic resolution (see Fig. 2; Streltsov et al. 2011). Recombinant A β ₄₂, with its strong fibrillogenic propensity, is coming under study (Zhang et al. 2009c; Finder et al. 2010). As a bacterially derived product, it is difficult to fully exclude impurities and adventitious factors that might interact with and co-purify with the recombinant peptide. Although purified synthetic A β peptides can also contain impurities (e.g., racemized or truncated peptides), the faster aggregating and more potent toxic properties of the recombinant material raise the question of the presence of pro-aggregating seeds and are worthy of further investigation (Finder et al. 2010).

Tissue- and Cell-Derived Natural Oligomers

The ultimate goal of studying the biochemistry of A β is to understand its nature and biological properties as it accumulates in the human brain. Early studies detected SDS-stable low-n oligomers on western blots of AD brain extracts (e.g., Masters et al. 1985; Roher et al. 1993), although their biological activities were not studied at that juncture. The major advance of generating mouse lines transgenic for human

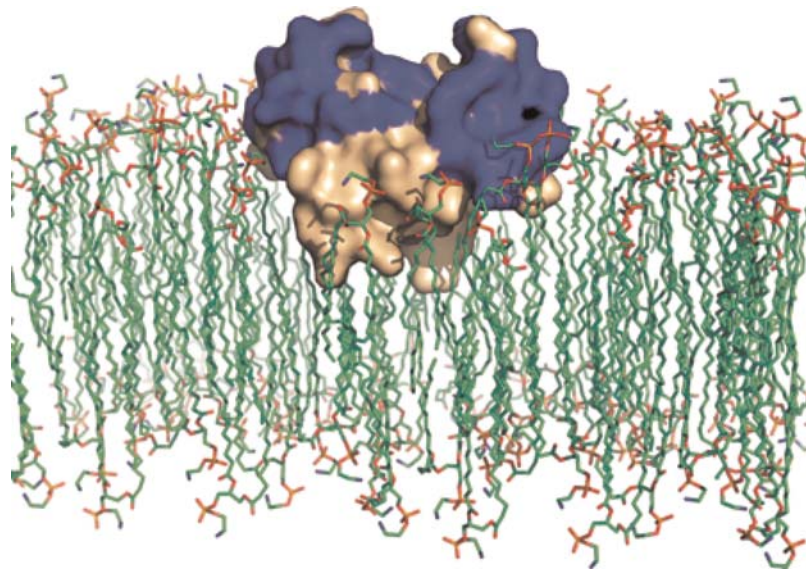


Figure 2. Model of potential interactions of $A\beta_{18-41}$ dimer with membrane lipid bilayers. The hydrophobic dimer–dimer interface of the $A\beta_{18-41}$ tetramer is intercalated into the membrane surface through non-electrostatic interactions, whereas hydrophilic aspects (blue) with metal-binding sites (black) are on the membrane surface. (From Streltsov et al. 2011; reprinted, with permission, from the author.)

APP (Games et al. 1995; Hsiao et al. 1996) has provided dynamic information about which species accrue most quickly (principally $A\beta$ ending at residue 42) and how they aggregate and deposit over time (e.g., Hamaguchi et al. 2009; Philipson et al. 2009; Tomiyama et al. 2010; and see LaFerla and Duff 2011). The in situ association of $A\beta$ oligomers with lipid membranes (Liu et al. 2010b), including post-synaptic densities (Koffie et al. 2009), in APP transgenic mouse brains helps us understand how these potentially toxic $A\beta$ species are compartmentalized. However, only a small number of studies characterizing soluble oligomers per se in transgenic mouse brains has been published (Kawarabayashi et al. 2001; Lesne et al. 2006; Shankar et al. 2009; Pham et al. 2010).

Turning now to studies of human brain tissue, soluble (aqueously extractable and non-pelletable) forms of $A\beta$ in postmortem AD cortex, which include monomers and various oligomers, have become recognized as stronger quantitative correlates of degree of cognitive impairment shortly before death than are

amyloid plaques (see Fig. 3; McLean et al. 1999; Tomic et al. 2009; Woltjer et al. 2009; McDonald et al. 2010). Soluble $A\beta$ oligomers extracted from the cortex of typical AD subjects have been shown to potently inhibit long-term potentiation (LTP), enhance long-term depression (LTD), and reduce dendritic spine density in slices of normal rodent hippocampus (Shankar et al. 2008). The extracts of soluble oligomers also disrupted the memory of a learned behavior after intracerebroventricular injection in normal rats. These effects could be principally attributed to dimers, the major SDS-stable oligomer detected on western blots of AD cortex. Importantly, insoluble amyloid plaque cores from the same brains did not impair LTP unless they were first solubilized to release $A\beta$ dimers and other oligomers, suggesting that plaque cores per se have low bioactivity but sequester $A\beta$ dimers that can be synaptotoxic if released (Shankar et al. 2008). There is also evidence that soluble $A\beta$ oligomers isolated from AD cortex can induce hyperphosphorylation of tau protein at AD-relevant epitopes, followed by progressive collapse of the

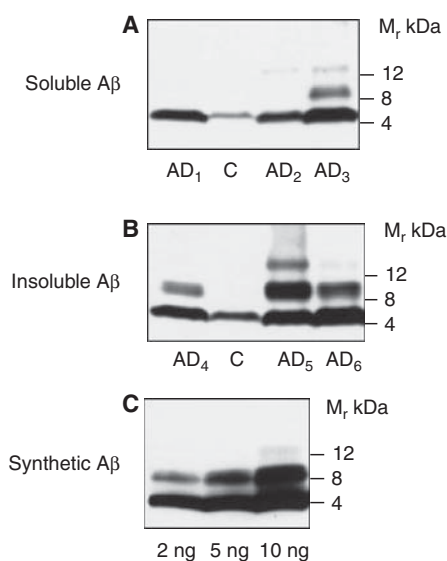


Figure 3. Representative western blots showing Aβ in frontal cortex of selected Alzheimer disease (AD) and control subjects. (A) Soluble Aβ in 175,000 g supernatants after a single extraction in phosphate-buffered saline. (B) Insoluble Aβ extracted from the 175,000 g pellets. (C) To enable quantification and between-gel comparisons, synthetic Aβ₄₀ standard curves were run on each gel. The markers designate monomeric (4 kDa), dimeric (8 kDa), and trimeric (12 kDa) forms of Aβ. (From McLean et al. 1999; reprinted, with permission, from the author.)

microtubule cytoskeleton and neuritic dystrophy (Jin et al. 2011).

Although these results suggest a synaptotoxic role for dimers, there are other soluble oligomers detectable in AD brain, including a ~56 kDa putative dodecamer (Lesne et al. 2009). A similar species has been detected in the brains of some APP transgenic mice and its level is shown to correlate with the occurrence of behavioral deficits; isolation of this species from mouse brain and subsequent icv injection into wild-type rats induced decreased spatial memory performance (Lesne et al. 2006; LaFerla and Duff 2011). In vivo, it is likely that there exists an array of low- and medium-sized oligomers, at least some of which appear to be in equilibrium with fibrils in plaques. The latter notion is supported by the occurrence of a halo

of dystrophic neurites immediately around fibrillar plaques, with the neuritic dystrophy diminishing as one moves farther from the plaque; this halo zone is also immunoreactive with certain antibodies that selectively detect small oligomers of Aβ (Meyer-Luehmann et al. 2008; Koffie et al. 2009).

Using immunoaffinity techniques, Noguchi et al. (2009) have isolated 10–15 nm spherical Aβ assemblies (mass >100 kDa) from AD cortex. How these relate to the lower order SDS-stable oligomers discussed above remains to be determined. Other post-translationally modified Aβ species, such as partial aspartate isomerization (Tomidokoro et al. 2010) and carboxy-terminal heterogeneity that includes longer Aβ₄₃ peptides (Welander et al. 2009), are being uncovered by isolating Aβ directly from postmortem human brain. In addition, direct analysis of human brain Aβ fibrils may disclose structural differences not predictable from similar analyses of synthetic fibrils (Paravastu et al. 2009).

Neuronally generated Aβ monomers and perhaps various oligomers are presumed to equilibrate within the interstitial fluid of the brain and to turn over in relation to the rates of Aβ production, clearance, and aggregation into amyloid fibrils. From the interstitial compartment or brain parenchyma, soluble Aβ monomers and oligomers may enter into the CSF compartment (Englund et al. 2009; Fukumoto et al. 2010) and even the peripheral blood circulation (Roher et al. 2009; Xia et al. 2009). Although much more work is required to establish the existence of blood-borne Aβ oligomers and confirm their cerebral origin, there is preliminary evidence that blood dimer levels may correlate with clinical features of AD (Villemagne et al. 2010; see also Blennow et al. 2011). Interestingly, such blood-borne dimers are associated with blood cellular membranes (mainly white cells and platelets) and may increase as the natural history of AD advances (Villemagne et al. 2010). In contrast, levels of Aβ₄₂ monomers in both the CSF and plasma are generally considered to fall as AD progresses (Lui et al. 2010a; Blennow et al. 2011).



THE INTERACTIONS OF $A\beta$ WITH OTHER MOLECULES: SERENDIPITOUS BYSTANDERS AND/OR INTIMATE PARTNERS IN PATHOGENESIS?

Early compositional analyses performed on isolated amyloid plaque cores suggested that $A\beta$, although clearly the major component, was not the sole protein constituent. Moreover, nonproteinaceous components were also identified to varying degrees in enriched—albeit not fully purified—plaque core preparations. It has been difficult to determine on a biochemical basis alone which of these additional constituents are important and integral components of the amyloid plaques and which might become adventitiously associated with $A\beta$ during plaque purification from homogenized brain tissue. When a non- $A\beta$ component of plaques is identified and antibodies are raised to it, these can be used to attempt to label amyloid plaques in situ at both the light and electron microscopic levels. Positive results suggest that a particular protein is indeed associated with the amyloid deposits, although not an integral component of the amyloid fibrils because, like other tissue amyloids, the fibrils should be composed solely of the specific subunit protein. Indeed, the ability to reconstitute amyloid fibrils with an ultrastructure closely resembling the fibrils seen in situ from synthetic $A\beta$ peptides alone has strongly suggested that the sole component of the amyloid filaments in vivo is $A\beta$. A careful proteomic analysis of amyloid plaque cores isolated from postmortem AD cortex by laser capture microdissection concluded that the only protein constituent detectable by mass spectrometry in the isolated cores was $A\beta$ (Soderberg et al. 2006), supporting the conclusion that the plaque amyloid fibrils are composed of just this protein type. It should be noted, however, that the fibrils may consist in part of heteropolymers of slightly different $A\beta$ peptides, rather than just homopolymers of a single peptide length (e.g., $A\beta_{1-42}$).

Nevertheless, a variety of other molecules has been found to be loosely or more tightly associated with amyloid deposits during their

isolation and can sometimes be shown immunocytochemically in diffuse and/or compacted plaques in situ. Because the morphology of senile plaques indicates the presence of several distinct cellular elements that are intimately apposed, including dystrophic axons and dendrites (Fig. 1) and the processes of activated microglia and reactive astrocytes, any of these as well as local microvessels could potentially be sources of various non- $A\beta$ constituents of the plaques. In short, mature amyloid (neuritic) plaques are heterogeneous mixtures of proteinaceous and nonproteinaceous constituents, and the temporal sequence of accrual of these elements onto the presumed initial $A\beta$ polymer has been difficult to determine.

A recently completed interactome of APP disclosed more than 200 different entities which interact with different domains of APP (128 validated, 74 putative), including a significant proportion interacting with the $A\beta$ region (Perreau et al. 2010). One of the earliest to be identified was the enzyme acetylcholinesterase (AChE; Friede 1965), perhaps paradoxical because of its subsequent role as a therapeutic target for AD and surprising in that the mechanistic basis for the co-location of AChE with the amyloid plaques remains uncertain (De Ferrari et al. 2001). Most of the identified molecular interactors of $A\beta$ in the brain remain equally mysterious and often raise the question of bystander versus functionally significant partner.

Metal Ions

Because of their ubiquitous presence in human tissues, the bioavailable metal ions, Cu, Zn, and Fe, have been obvious choices for investigation of amyloid association. For decades, uncertainty has reigned over the quantitative elemental analysis of whole brain homogenates—and of isolated plaques or tangles—in AD compared to normal aged controls and other neurodegenerative diseases. At the level of grey matter homogenates, there is no agreement that any particular metal ion is specifically elevated or lowered in AD brain. Most techniques have detected elevations in Cu, Zn, or Fe in AD amyloid plaques, either in situ or after their

purification (see, for example, Rajendran et al. 2009), but such analytical approaches have never been entirely convincing. The observations that both APP and A β have sequences consistent with metal-binding motifs and metallo-complexing activities add a new dimension to this line of enquiry (Faller 2009; Duce et al. 2010). Measuring the affinities of metal-protein interactions is challenging (Xiao and Wedd 2010) but, as technologies have improved, the general rule has emerged that the metal affinities increase as the proteins move toward their sites of final subcellular compartmentalization and utilization. Thus, certain other proteins act as chaperones to take the metal ions into compartments where their higher affinity end-user proteins reside. The synapse has proven to be a subcellular site where ions such as Zn²⁺ and Cu²⁺ are used to modulate the activities of key excitatory NMDA/AMPA receptors. It is in the vicinity of this cellular compartment that A β may interact with these divalent cations in a fashion that can alter the peptide's conformation. This metal-based mechanism, as well as the overall level of local excitatory neurotransmission (Cirrito et al. 2005), could help provide an explanation for the topographic selectivity of A β aggregation and extracellular deposition in the AD brain, as there is an intriguing overlap between those areas of the brain rich in glutamatergic terminals, free vesicular zinc, and A β amyloid plaques in certain APP transgenic mice (Stoltenberg et al. 2007).

With an emerging understanding of the pathways leading to soluble oligomer or insoluble fibril production, therapeutic strategies loosely termed as "anti-A β aggregation" need to be refocused on the specific steps being targeted (Rodriguez-Rodriguez et al. 2009; Yadav and Sonker 2009; Dickens and Franz 2010), particularly with regard to the concept of "therapeutic chelation" of metal ions. The concept of therapeutic chelation needs to be qualified by the relative affinities each metal ion has for its target protein. Thus, metal "chaperone" is a preferred concept when discussing the reversible interactions divalent cations can have with A β , regardless of which oligomeric or fibrillar assembly is being considered.

A β and Copper

The average K_D of Cu²⁺ for A β is about 10⁻¹⁰ M (i.e., low nanomolar) for both soluble and fibrillar forms of the synthetic peptide in vitro (Rózga et al. 2010; Xiao and Wedd 2010). This means that other metallo-chaperone proteins with higher (i.e., high picomolar or greater) affinities will prevent Cu²⁺ binding to A β . This criterion would include human serum albumin (Perrone et al. 2009), suggesting that A β in locations (e.g., CSF or blood) remote from parenchymal brain compartments such as neurites and synapses should be unmetallated. Furthermore, therapeutic compounds designed to act as metal-ion chaperones with low picomolar affinities would be expected to compete with A β for Cu²⁺ binding only within the brain parenchyma.

A β may have more than one Cu²⁺-binding site (Behbehani and Mirzaie 2009; Jun et al. 2009; Sarell et al. 2009). Depending on the stoichiometry, Cu²⁺-A β interactions can cause synthetic A β to aggregate in vitro principally via an oligomer-forming pathway or a fibrillogenic pathway (Brzyska et al. 2009; Moore et al. 2009; Olofsson et al. 2009a; Tōugu et al. 2009; Haeflner et al. 2010). That is, at sub-equimolar Cu²⁺:A β ratios, amyloid fibrils form; at supra-equimolar ratios, stable oligomers form first, then dityrosine cross-linkages occur (Smith et al. 2007). The principal Cu²⁺-binding site is coordinated within the first 16 residues and involves His6, His13, and His14, together with the first two residues (Asp1, Ala2) (Dorlet et al. 2009; Drew et al. 2009a,b; Hureau et al. 2009a,b). This coordination environment is pleiotropic (Drew et al. 2009b), adding to the complexity of the analysis (Drochioiu et al. 2009; Hureau et al. 2009a). As was predicted when the A β sequence became known, the protonation of the histidine residues, dependent on pH, should have a major effect in the A β folding pathway: metallation and folding of A β in the endosome/lysosome pathway will probably be quite different from that in the extracellular or peri-synaptic compartments.

Cu²⁺ as a redox-active entity can also induce oxidative modification to A β , particularly



at Tyr10 with consequent dityrosine covalent cross-links (Drew et al. 2009a,b; Moore et al. 2009; Jiang et al. 2010). Other residues, such as Met35, may participate (Barman et al. 2009; Butterfield et al. 2010), but this is not proven (da Silva et al. 2009). Whether the metal-modified A β is capable of pro- or anti-oxidant activity is also uncertain (Baruch-Suchodolsky and Fischer 2009), but it is an important question that needs to be resolved in terms of understanding the toxicity of A β oligomers. Reducing intracellular Cu²⁺ bioavailability has an inhibitory effect on A β oligomer formation (Crouch et al. 2009a).

A β and Zinc

In contrast to Cu²⁺, Zn²⁺ is redox inactive and therefore cannot be directly involved in any oxidative processes involving A β . In common with Cu²⁺, Zn²⁺ has pleotropic binding sites on A β (Damante et al. 2009; Talmard et al. 2009; Miller et al. 2010) which can serve to drive synthetic A β aggregation in vitro. The Zn²⁺-induced formation of cytotoxic A β oligomers in proximity to excitatory glutamatergic synapses is believed to be a mechanism contributing to synaptic degeneration in AD (Deshpande et al. 2009). Zn–A β complexes also become more resistant to proteolytic degradation in in vitro experiments (Crouch et al. 2009b), potentially allowing metal-bound A β fibrils to accumulate in the extracellular space.

A β and Iron

Studies of Fe³⁺/Fe²⁺ complexes with A β indicate a potential pro-aggregating role for this abundant metal (Jiang et al. 2009a; Uraga et al. 2010), especially if evidence that A β has significantly higher affinity for Fe²⁺ than does transferrin (Jiang et al. 2009a) is confirmed.

A β INTERACTIONS WITH CELL MEMBRANES, LIPOPROTEINS, AND MEMBRANE-ASSOCIATED PROTEINS

The proteolytic release of A β from APP is believed to occur principally in an endosomal/lysosomal compartment or from the

surface of the plasma membrane (see Haass et al. 2011). Given the amphiphilic nature of A β , it is not surprising that many potential interactions can occur once it is a free peptide. These interactions can be driven by phase/interface effects, electrostatic (charge) interactions dependent on the pH of the microenvironment, and hydrophobic interactions if the hydrophobic carboxy-terminal region is able to re-associate with the lipid bilayer. These types of bonding apply also to A β interactions with the lipoprotein particles formed with ApoE, ApoA, and ApoJ, as well as with other membrane-associated macromolecular complexes in the vicinity of synapses such as NMDA, AMPA, insulin, and nicotinic ACh receptors. All of these complex protein interactions are dependent in part on the conformation and state of assembly of A β itself. When sequential fractions of postmortem AD brain homogenates are analyzed, the major pool of A β lies in the detergent-insoluble (e.g., formic acid- or guanidine-extractable) fraction, presumably representing rather insoluble amyloid plaques, but a considerable amount is apparently loosely associated with cellular membranes (e.g., the sodium carbonate-extractable fraction). The diffusible, aqueously extractable fraction (e.g., buffered saline extract) is generally less than 1–2% of the total recoverable brain A β . It is the membrane-associated pool of brain A β which can be recovered in sodium carbonate or Triton that we will focus on now (see Relini et al. 2009 for a recent review).

Phase/Interface Effects

Most in vitro studies of the α -helix to β -sheet conversion and aggregation of A β peptides are conducted at concentrations 3–4 orders of magnitude greater than those found in vivo. Moreover, the special microenvironment in which A β aggregation is believed to occur in vivo is not always taken into account, e.g., the relatively high concentrations of metal ions in and around synapses. The interface between the interstitial fluid phase and the surface of the plasma membrane is likely to be a critical factor in influencing the aggregation pathway

of A β . A number of in vitro studies find this, showing interface clustering of A β (Chi et al. 2010) that slows the lag phase of fibril formation (Hellstrand et al. 2010) by providing an environment for a hydrophobic layer adjacent to the membrane interface (Jiang et al. 2009b). Physical movement/agitation at the water-membrane interface may also promote fibrillogenesis (Morinaga et al. 2010; Wu et al. 2010a). The nature of this interface may therefore strongly affect the A β folding pathway (Kayed et al. 2009). In contrast to the hydrophilic ectodomains of various proteins proposed to function as A β receptors, membrane lipid surfaces seem a more biophysically plausible receptor for the highly hydrophobic A β oligomers.

Electrostatic/Charge Effects

The role of negatively charged phospholipid head groups, sphingolipids, sialic acid, etc. in affecting the binding and oligomerization of A β is being increasingly examined (Kayed et al. 2009; Salay et al. 2009; Kotarek and Moss 2010; Sureshbabu et al. 2010). Smaller (1–2 nm diameter) synthetic A β oligomers have a greater tendency to bind such charged species than do those of larger (4–5 nm) size (Cizas et al. 2010). Local membrane charge may also alter the β -turn of synthetic A β peptides (Grimaldi et al. 2010). Exposed phosphatidylserine has been proposed as a mediator of A β -membrane surface interactions (Simakova and Arispe 2007). MD modeling (Davis and Berkowitz 2009a,b) suggests the induction of subtle changes in conformation around the β -turn of A β fold on its membrane binding, and in vitro studies show the effect of pH and the protonation of His13 and/or His14 when the amphiphilic domain A β _{11–22} is used for membrane binding studies (Ravault et al. 2009). Using L- and D-handed enantiomers of A β ₄₂, Ciccotosto et al. (2011) have reported that synthetic A β binds directly to cell membranes in vivo through phosphatidylserine and that this interaction is stereospecific. The toxicity of A β oligomers may therefore be related in part to some aspect of its specific electrostatic interactions with phosphatidylserine. Gangliosides

provide another charged interactor for A β on the cell surface (Nakazawa et al. 2009; Peters et al. 2009; Utsumi et al. 2009; Yagi-Utsumi et al. 2010), with potential effects on the folding pattern of the peptide (Mao et al. 2010; Ogawa et al. 2011).

Hydrophobic Interactions of A β

After the release of the A β monomer from its partially transmembrane location, a portion of resultant A β assemblies may bind and re-insert into the hydrophobic lipid bilayer. There has been a longstanding controversy in the field as to whether this re-insertion event leads to the formation of a complete transmembrane pore or whether membrane association and partial insertion can disrupt the bilayer to such an extent that its structural integrity is compromised. MD simulations and in vitro artificial lipid membrane models of this insertional event are plentiful (Friedman et al. 2009; Lemkul and Bevan 2009; Miyashita et al. 2009; Qiu et al. 2009; Song et al. 2009; Yang et al. 2009a,b; Morita et al. 2010; Schauerte et al. 2010; Wang et al. 2010), but rigorous evidence for a hydrophobic membrane-traversing interaction in vivo is lacking. Using photobleaching Förster resonance energy transfer, there was a loss of signal from the hydrophobic carboxyl terminus of A β as it interacts with the plasma membrane of PC12 cells, which may indicate its sequestration within the lipid bilayer (Bateman and Chakrabarty 2009). Addition of synthetic A β ₄₂ oligomers to N2a and HT22 neuronal cell lines led to significant cellular stiffening/rigidity (Lulevich et al. 2010). Peripheral membrane association of A β ₄₂ (but not A β ₄₀) oligomers with lysosomes has also been suggested as evidence of in vivo membrane insertion (Liu et al. 2010b). Clearly, more evidence is required to prove actual transmembrane insertion of the peptide in vivo.

A β Interaction with Lipoproteins

Electrostatic or hydrophobic interactions of A β with the various lipoprotein particles (ApoE, ApoA, ApoJ) are discussed elsewhere in this

volume (Holtzman et al. 2011). We note in vivo evidence of direct ApoE–A β and ApoA1–A β interactions (Bales et al. 2009; Paula-Lima et al. 2009), and that ApoE found in the brain is more heavily sialylated than that in the peripheral circulation (Kawasaki et al. 2009), potentially facilitating electrostatic interactions with A β (see above).

A β Interactions with Selected Membrane Polypeptides (e.g., NMDA and ACh Receptors)

Growing evidence suggests the occurrence of at least functional—if not physical—interactions of A β oligomers with NMDA or α 7-nicotinic ACh receptors or the cellular prion protein (Hu et al. 2009; Lauren et al. 2009; Li et al. 2009; Liu et al. 2009b; Zhang et al. 2009b). However, much of this evidence comes from studies showing that antagonists or downstream regulators of these and other cell-surface receptors (e.g., AMPA and insulin receptors) can mitigate or fully prevent the effects of soluble A β oligomers on synaptic form and function (see, e.g., Shankar et al. 2007; Li et al. 2009). Such studies only indicate that the expression and normal function of the receptor in question is necessary for some of the downstream effects of A β on neurons to occur, not that these cell-surface polypeptides are the direct receptors for A β in vivo. Instead, the binding of extracellular A β oligomers—via their exposed hydrophobic residues—to certain lipids in the plasma membrane could alter the biophysical properties of the bilayer and secondarily and somewhat nonspecifically perturb the structures (and thus the functions) of a variety of membrane-anchored neuronal receptor proteins. Moreover, as mentioned above, the high concentrations of Zn²⁺ and Cu²⁺ found in and around NMDAR-containing post-synaptic elements may be involved in the actions of A β oligomers at the membrane.

Other A β Interactions

Over the past 30 years, many other proteins have been described as being associated with A β extracellular deposits, using a variety of

immunohistochemical or biochemical approaches. Among these, two broad categories of proteins stand out: extracellular matrix factors and inflammatory/stress response factors. The latter include members of the complement cascade, cytokines, immunoglobulins, acute phase proteins, components of the inflammasome, etc. The serine protease inhibitor, α 1-antichymotrypsin, is an acute phase protein that may be tightly associated with amyloid plaque cores (Abraham et al. 1988). Biochemical isolation approaches have also yielded co-purifying proteins of unknown pathogenic significance, e.g., a fragment (residues 60–95) of the neuronal protein, α -synuclein, namely, its NAC peptide (i.e., “non-amyloid component” of plaques). The fact that some cortical neurons in AD accumulate aggregates of α -synuclein (Lewy bodies and neurites) provides a possible explanation for the co-purification of this protein fragment from homogenized AD cortex. Many other polypeptides of unknown mechanistic importance in the disease pathogenesis could be cited here. The fact that some amyloid-associated proteins differ in their primary sequences and amounts between human and mouse brain could help explain why APP transgenic mice deposit plaques of human A β but not always with the same local associations and consequences (e.g., without significant neuronal loss).

CONCLUSIONS

Even the wealth of details and accompanying references that we have discussed above cannot do the subject of A β biochemistry justice. Since Glenner and Wong’s seminal paper in 1984, innumerable studies of this small, hydrophobic, and potentially lethal protein have been published. Indeed, several important aspects of its biology, including its mechanisms of formation (Haass et al. 2011) and clearance (Saido and Leissring 2011) and its measurement by brain imaging (Johnson et al. 2011) and in biological fluids (Blennow et al. 2011), are covered extensively in other parts of this volume. The genetics of dominantly inherited AD and the pathobiology of the apolipoprotein ϵ 4 allele in AD have



combined to give A β an apparent initiating role in at least some forms of the AD syndrome. Because these familial forms are largely indistinguishable from “sporadic” late-onset AD, parsimony suggests that an imbalance between A β production and clearance—an A β dyshomeostasis—is a driving force for many or all cases of AD as we define this eponymic syndrome. And yet, precisely why A β accumulates and what upstream events can lead to this accumulation remains unknown for the majority of cases of the disease. Perhaps only through the results of clinical trials of agents that must be working solely on A β (e.g., highly specific anti-A β antibodies) can we adequately test the theory that A β accumulation is a central pathogenic event in AD. For the sake of our patients and their families, one can only hope that the answer to this provocative question lies not too far in the future.

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