Eur Biophys J (2002) 31: 2–8
DOI 10.1007/s002490100172

REVIEW

Danny M. Hatters · Geoffrey J. Howlett

The structural basis for amyloid formation by plasma apolipoproteins: a review

Received: 8 February 2001 / Revised version: 31 May 2001 / Accepted: 31 May 2001 / Published online: 21 July 2001

Abstract The formation of amyloid and other protein deposits in vivo is synonymous with many pathological conditions such as Alzheimer's disease, Creutzfeldt-Jakob disease and Parkinson's disease. Interestingly, many plasma apolipoproteins are also associated with amyloid deposits, including apolipoprotein (apo) A-I, apoA-II and apoE. Apolipoproteins share a number of structural and conformational properties, namely a large proportion of class A amphipathic α-helices and limited conformational stability in the absence of lipid. Other proteins that form amyloid such as α-synuclein and serum amyloid A also contain amphipathic α-helical domains similar to those found in apolipoproteins. In this review we develop a hypothesis to account for the widespread occurrence of apolipoproteins in amyloid deposits. We describe the conformational stability of human apoC-II and the stabilization of α-helical structure in the presence of phospholipid. We propose that lipid-free apoC-II forms partially folded intermediates prone to amyloid formation. Parameters that affect apolipoprotein lipid binding in vivo, such as protein and lipid oxidation or protein truncations and mutations, could promote apolipoprotein-related pathologies including those associated within amyloid deposits of atherosclerotic plaques.

Keywords Amyloid · Apolipoproteins · Amphipathic α-helix · Conformational stability · Atherosclerosis

Abbreviations *apo*: apolipoprotein · *DHPC*: dihexanoyl phosphatidylcholine · *DMPC*: dimyristoylphosphatidyl choline · *GuHCl*: guanidine hydrochloride · *HDL*: high density lipoproteins · *SAA*: serum amyloid A

D.M. Hatters (✉) · G.J. Howlett
Department of Biochemistry and Molecular Biology,
University of Melbourne, Parkville, 3010 Victoria, Australia
E-mail: dannymh@unimelb.edu.au
Tel.: +61-3-83447965
Fax: +61-3-93477730

Introduction

Amyloid is characterized by the self-association of proteins into long semi-regular fibers with a rigid rod or twisted ribbon morphology. Mature fibers are typically composed of several smaller protofilament subunits, approximately 3 nm in width, that pack together in a rope-like configuration. Despite some variation in the superstructural morphologies of amyloids, the basis of assembly is remarkably similar in that the fibers are composed of indefinite β-strands stacked perpendicular to the plane of the fiber axis, forming β-sheets parallel to the fiber axis (Sunde and Blake 1997; Serpell et al. 2000).

In vivo, amyloid formation occurs via alternative folding pathways to those leading to the native structures normally adopted by proteins (Dobson 1999). These alternative folding options generate the predominantly β-sheet forms required for amyloid assembly. Amyloid is associated with several pathological conditions, including systemic amyloidoses with mutants of lysozyme and transthyretin and other diseases such Creutzfeldt-Jakob disease with the prion protein (Kelly 1996). For transthyretin and lysozyme, the formation of amyloid occurs through destabilization of the native state by mutations and the consequent promotion of partially folded intermediates (Booth et al. 1997; Nettleton et al. 1998). Other amyloids form via protein truncation of precursor proteins such as the excision of the amyloid β peptide from the amyloid precursor protein in Alzheimer's disease (Kang et al. 1987).

A number of plasma apolipoproteins are implicated in amyloid formation or influence the amyloid formation of other proteins. These include apolipoprotein (apo) A-I, apoA-II and apoE (Table 1). Our recent work establishes that human apoC-II also readily forms amyloid in the absence of lipids (Hatters et al. 2000). Here we review the occurrence of amyloid formed by apolipoproteins and examine the parameters that characterize the tendency of apolipoproteins to form amyloid in vivo.

The occurrence of apolipoproteins as amyloid

Human apoA-I, apoA-II, apoB100 and apoE co-localize with amyloid in vivo, mostly in atherosclerotic deposits or in the brain (Namba et al. 1991; Wisniewski et al. 1995a, 1995b; O'Brien et al. 1998). More specific cases exist where apoA-I and derivative peptides composed of the N-terminal 80–100 residues are the primary component within the amyloid associated with atherosclerotic plaques (Westermark et al. 1995). Intact apoA-I and N-terminal fragments extracted from atherosclerotic amyloid deposits and shorter synthetic peptides form amyloid in vitro (Westermark et al. 1995; Wisniewski et al. 1995a). As well as the occurrence of native apoA-I with atherosclerosis, several mutant isoforms of apoA-I have been identified in amyloid deposits with both full length and N-terminal fragments of apoA-I (Table 1). Many of these mutations result in a net gain of one charge, leading to the hypothesis that an increase in charge increases the propensity for amyloidosis, either by a change in association of apoA-I with high density lipoproteins (HDL) or a structural change that leads to a changed catabolism (Genschel et al. 1998).

ApoA-II is structurally distinct from apoA-I but has also been reported to form amyloid in vivo in mice (Table 1). One form of heritable senile amyloidosis in mice is linked to mutant forms of apoA-II that contain amino acid differences at four positions compared to non-amyloidogenic isoforms (Higuchi et al. 1991). ApoA-II has also been recently linked to amyloid deposition in humans. A patient with familial systemic amyloidosis in the kidney was found to have a 21 amino acid C-terminal extension of apoA-II as the predominant protein within the amyloid deposits (Benson et al. 2001). Sequencing the apoA-II gene confirmed the presence of a substitution mutation in the stop codon, resulting in the C-terminal extension of the protein sequence (Benson et al. 2001). The most extensive literature on apolipoproteins and amyloid has been that on apoE and the strong genetic linkage of apoE isoforms with Alzheimer's disease (Corder et al. 1993). ApoE exists as three common isoforms, of which the apoE4 isoform confers greater susceptibility to Alzheimer's disease (Corder et al. 1993). The mechanism of this susceptibility is not fully understood. ApoE binds to the amyloid β peptide (Aβ) to form SDS-resistant heterodimers, with residues in the C-terminal domain exerting a direct influence on amyloid formation by Aβ (Strittmatter et al. 1993). Immunohistochemistry of amyloid deposits from Alzheimer's disease plaques shows co-localization of the C-terminal domain of apoE scattered throughout the fibrils (Castano et al. 1995; Naslund et al. 1995). A 10 kDa C-terminal fragment of apoE purified from Alzheimer's disease amyloid deposits forms amyloid in vitro (Wisniewski et al. 1995b). ApoE has been identified immunohistochemically in many types of amyloid, suggesting that the influence of apoE may be more complicated than simply modulating amyloid formation by Aβ. One possibility is that apoE forms mixed amyloid fibrils, as demonstrated recently for two unrelated peptide sequences (MacPhee and Dobson 2000).

Common threads: amphipathic α-helical domains and lipid binding

What, if any, are the common parameters that promote the formation of amyloid by apolipoproteins? Insight may be provided by considering the highly conserved nature of the apolipoprotein superfamily believed to derive from duplications of an ancestral 22 amino acid tandem repeat (Li et al. 1988). This common background generates a number of shared structural features. Plasma apolipoproteins contain a high proportion of

Table 1 Examples of apolipoproteins that form amyloid

Protein	Type/location of deposition	Ref
ApoA-I and N-terminal fragments	Atherosclerotic plaques	Westermark et al. (1995), Wisniewski et al. (1995a)
ApoA-I mutants		
L178H	Cardiac and larynx	de Sousa et al. (2000)
L174S	Cardiac	Obici et al. (1999)
R173P	Cardiac and cutaneous	Hamidi Asl et al. (1999a)
P90L	Cardiac and cutaneous	Hamidi Asl et al. (1999b)
Δ(E70F71W72)	Systemic	Persey et al. (1998)
Δ(60–71)→VT	Hepatic	Booth et al. (1996)
G26R	Systemic	Nichols et al. (1988)
W50R	Systemic	Booth et al. (1995)
L60R	Systemic	Soutar et al (1992)
ApoA-II mutants		
21 amino acid carboxy-terminal extension	Renal	Benson et al. (2001)
Murine	Senile	Higuchi et al. (1991)
ApoE C-terminal fragments	Co-localized with Aβ	Wisniewski et al. (1995b)
ApoC-II (lipid free)	In vitro	Hatters et al. (2000)

class A amphipathic α-helical domains that mediate the binding to lipoprotein surfaces. Class A amphipathic α-helices are characterized by lysine residues on the hydrophilic face near the interface with the hydrophobic face and negatively charged residues at the outermost of the polar face (Segrest et al. 1994). This distribution of charged residues is believed to provide stabilizing interactions with the phosphate and choline moieties of the phospholipid head groups, thus anchoring the protein to the phospholipid surface (Segrest et al. 1994). These amphipathic helical domains bind parallel to the phospholipid surface in rapid equilibrium with unbound forms, in contrast to the firm lipid anchoring characteristic of transmembrane proteins. This mechanism of lipid binding permits switching between lipoprotein classes as part of the remodelling of lipopoproteins that occurs during metabolism (Segrest et al. 1994).

Another significant structural feature of apolipoproteins is their low conformational stability in the absence of lipid. While many apolipoproteins retain α-helical structure in the absence of lipid, including apoA-I, apoA-II, apoA-IV and apoE (Morrisett et al. 1977; Perugini et al. 2000), others have a diminished helical content under lipid-free conditions, including apoC-II (Tajima et al. 1982) and apoC-III (Morrisett et al. 1977). With the exception of the N-terminal domain of apoE, all the exchangeable apolipoproteins have limited conformational stability in the absence of lipid. Denaturation studies on apoA-I (Gursky and Atkinson 1996a), apoA-II (Gursky and Atkinson 1996b), apoA-IV (Weinberg and Spector 1985) and the insect analogue, apolipophorin III (Soulages and Bendavid 1998), indicate low structural stability even though these proteins retain α-helical structure. Specifically, thermal denaturation of apoA-I indicates a molten globular conformation in the absence of lipid (Gursky and Atkinson 1996a), while apoA-IV retains a conformational stability energy near zero in the absence of lipid with a high content (54%) of α-helical structure (Weinberg and Spector 1985). ApoC-I also has limited tertiary interactions in the absence of lipid, perhaps indicative of a conformationally flexible lipid-binding state (Gursky and Atkinson 1998). Even with apoE, the C-terminal domain isolated by thrombin cleavage is α-helical in the absence of lipid and exhibits a low conformational stability similar to other apolipoproteins (Wetterau et al. 1988). The C-terminal domain of apoE is tetrameric and contains the majority of the predicted class A amphipathic α-helical domains in apoE (Aggerbeck et al. 1988). Evidence that loosely folded structures are important for lipid binding is provided by studies on apolipophorin III at variable pH values that show a correlation between lipid binding kinetics and a molten globule conformation (Soulages and Bendavid 1998). Such limited globular stability may reflect the requirement for conformational reordering of amphipathic α-helices upon binding to lipid surfaces, in contrast to typical globular proteins that fold tightly with a well-defined hydrophobic core.

In the case of apoC-II and C-III, lipids induce higher helical content, suggesting that lipid binding is coordinated with protein folding. ApoC-II and C-III have a mostly unordered structure in the absence of lipids (Morrisett et al. 1977; Tajima et al. 1982). Further support for an unordered, monomeric confirmation of apo C-II in the absence of lipid is provided by ultracentrifugation experiments on freshly refolded material that indicate a sedimentation coefficient consistent with an extended axial asymmetry (axial ratio = 4.9; Hatters et al. 2001).

The general lack of conformational stability of apolipoproteins in the absence of lipids suggests a mechanism for the propensity to form amyloid. Several globular proteins show a correlation between a partially folded state and amyloid formation. Mutants that promote molten globular conformations in lysozyme and transthyretin also promote amyloid formation (Booth et al. 1997; Nettleton et al. 1998). Similarly, events that partially denature proteins, such as high concentrations of ethanol (Goda et al. 2000), addition of trifluoroethanol (Chiti et al. 1999) and extreme pH values (McParland et al. 2000), also promote amyloid, leading to the conclusion that amyloid formation is favoured by destablization of the native state (Dobson 1999). In the context of apolipoproteins, lipid binding sequesters hydrophobic groups from the aqueous environment with the lipid providing the core structure. Class A helices are further stabilized by the electrostatic interactions between the lipid headgroups and the amino acid sidechains on the polar face of the helix (Segrest et al. 1994). We propose that events which destabilize the lipid-bound state (the stable native complex) promote a partially folded conformation vulnerable to amyloid formation (Hatters et al. 2000).

The conformational stability of apoC-II

In the absence of lipid, apoC-II readily aggregates into fibrillar ribbons with all the hallmarks of amyloid, including binding to thioflavin T, binding to Congo Red with red/green birefringence under cross-polarized light, and increased β-structure as measured by circular dichroism (CD) spectroscopy at physiological pH, salt concentrations and temperature (Hatters et al. 2000). We examined the conformational stability of apoC-II in the presence and absence of phospholipid using denaturation studies with guanidine hydrochloride (GuHCl). CD spectroscopy was used to monitor the secondary structure of freshly prepared, lipid-free apoC-II. Figure 1A indicates little change in secondary structure over the concentration range 0–5 M GuHCL (Fig. 1A). Parallel experiments were performed with apoC-II bound to dihexanoylphosphatidylcholine (DHPC) micelles (Fig. 1A). The binding of micellar DHPC to apoC-II in the absence of GuHCl induced α-helical structure, as indicated by the large negative ellipticity at 222 nm. The unfolding profile for apoC-II in the pres-

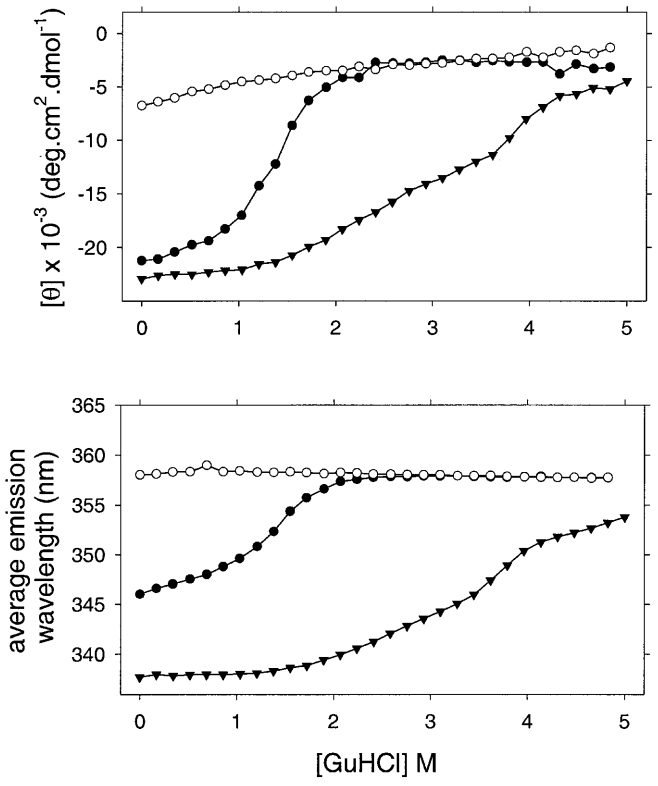

Fig. 1 Equilibrium unfolding curve of 5 μM apoC-II at 20 °C. Unfolding was monitored by circular dichroism spectroscopy at 222 nm (**A**, *top*) and fluorescence emission spectroscopy of the single tryptophan with excitation at 280 nm (**B**, *bottom*). Samples measured include apoC-II alone (*open circles*), apoC-II in the presence of 16 mM DHPC (*solid circles*) and apoC-II in the presence of 500 μM DMPC vesicles (*solid triangles*). ApoC-II is fully bound to the lipid under these conditions in the absence of guanidine hydrochloride. The average emission wavelength is equal to $\Sigma(\lambda i)/\Sigma(i)$, where λ is the emission wavelength and i is the emission intensity. The mean residue ellipticity, $[\theta]$, was calculated from the equation $[\theta] = \theta/(cnl/M)$, where l is the pathlength in mm, θ is the observed ellipticity in millidegrees, c is the concentration of protein (mg/mL), n is the number of amino acid residues, and M is the molecular weight

ence of DHPC shows a cooperative unfolding event with a transition midpoint at approximately 1.5 M GuHCl, comparable to the results observed with the unfolding of globular proteins. Experiments were also performed with apoC-II bound to a longer-chain phospholipid, dimyristoylphosphatidylcholine (DMPC) (Fig. 1A). In this case, GuHCl-induced unfolding also occurs although there appears to be at least two transition phases, indicating stable intermediate species in equilibrium. To further examine the unfolding, the samples were monitored for changes in the fluorescence properties of the single tryptophan residue at position 26. The results in Fig. 1B indicate that the changes in fluorescence correlate to the changes observed in the CD measurements. The shift of the average emission wavelength to a lower energy upon unfolding is consistent with the movement of the tryptophan from a non-polar environment to a more polar environment. The fully bound protein exhibits a substantially lower average emission wavelength when complexed with DMPC rather than DHPC, correlating to a more non-polar environment of the tryptophan when bound to DMPC. This difference may reflect the different dynamics of bilayers compared to micelles and the relative conformational stability of the apoC-II/lipid complexes. The important conclusion is that there is little change in the fluorescence properties of apoC-II with increasing concentrations of GuHCl in the absence of lipid, suggesting that the protein in the lipid-free state is unordered. The results are consistent with a model where the binding of apoC-II to lipid confers conformational stability, with native folding promoted by the hydrophobic core of the phospholipid. The presence of micellar DHPC promotes α-helical structure in apoC-II and dramatically reduces amyloid formation, as indicated by turbidity assays and electron microscopy (Hatters et al. 2001). These studies provide direct evidence that lipid binding provides a competitive folding option to amyloid formation.

Structurally related amyloidogenic proteins

Interestingly, at least two other proteins that form amyloid in vivo have some apolipoprotein-related properties. Serum amyloid A (SAA) is an acute-phase protein associated with amyloid deposition in some patients with chronic inflammatory diseases (Sipe 1992). SAA cleavage produces an amyloidogenic derivative, amyloid A, consisting of the N-terminal two-thirds of the protein (Husebekk et al. 1985). While the physiological functions of SAA are not clear, it is present at high concentrations upon induction (1 mg/mL) and binds to HDL, competing with endogenous apolipoproteins, most notably apoA-I (Uhlar and Whitehead 1999). The N-terminal 11 residues, which are required for amyloid formation, are believed to be the lipid-binding region (Uhlar and Whitehead 1999). Helical wheel analysis of the first 19 N-terminal residues indicates an amphipathic α-helical arrangement similar to that observed with the lipid binding regions of apolipoproteins.

A major amyloid forming protein associated with Lewy bodies in Parkinson's disease pathology, α-synuclein, also has apolipoprotein-like properties. α-Synuclein has limited structure in the absence of lipid and adopts helical structure in the presence of phospholipid (Conway et al. 1998; Davidson et al. 1998). Recent NMR experiments suggest that the 102–103 N-terminal residues are responsible for lipid binding (Eliezer et al. 2001). As with SAA, the N-terminal sequence of α-synuclein contains class A-like amphipathic α-helical domains (Davidson et al. 1998) corresponding to the amyloidogenic region. Two mutants (A53T and A30P) linked to early-onset Parkinson's disease show enhanced oligomerization of amyloid intermediates (Conway et al 2000). These mutants have altered lipid-binding properties which may modulate amyloid deposition in vivo (Jensen et al. 1998; Jo et al. 2000; Perrin et al. 2000). The common structural

characteristics of SAA, α-synuclein and the apolipoproteins are consistent with the hypothesis that amphipathic domains that form α-helical structures in the presence of phospholipid form amyloid-like β-structures in the lipid-free state.

In vivo modulation of amyloid formation by apolipoproteins

Amyloid has been associated with atherosclerotic plaques in 97% of patients over the age of 50 years (Mucchiano et al. 1992). Atherosclerotic plaques consist of foam cells, macrophages and a necrotic core that accumulates oxidatively damaged lipids (Suarna et al. 1995) and proteins (Fu et al. 1998) relative to normal plasma and arteries. Oxidative modification has also been associated with the proteins in amyloid deposits related to Alzheimer's disease, in particular proteins with lipid adducts, side-chain nitration and carbonyl modification (Smith et al. 2000). Studies with peptides containing an N-terminal acyl chain indicate an increased tendency to form β-sheet aggregates from an initial helical conformation relative to the unmodified peptide (Takahashi et al. 1999). Recently, it has been shown that at least some of the α-synuclein deposits in the brain have nitrated tyrosines (Giasson et al. 2000). We propose that these oxidative processes may promote protein truncation or modification of apolipoproteins in a manner that perturbs their lipid binding properties and thus by default promote the amyloidogenic folding options. These modifications may include truncation to a form that is incapable of binding to lipid. Alternatively, changes in the electrostatic interactions between the lysine residues and the zwitterionic choline group near the lipid/water interface may destabilize the anchoring of the class A α-helices to phospholipid surfaces. The reversing of the charge distribution along the amphipathic face of the α-helix in model class A amphipathic α-helical peptides reduces their affinity with neutral phospholipid (Anantharamaiah et al. 1985). With other protein-lipid systems, such as the folding of apocytochrome C in the presence of lipids, electrostatic interactions with negatively charged lipids are critical for the folding onto a lipid surface in a helical conformation (Rankin et al. 1998). Apocytochrome C does not bind to neutral phospholipids and remains in an unordered conformation in solution (Fisher et al. 1973). Given that some amyloid forming mutants of apoA-I involve changes in charge (Table 1), such electrostatic interactions may be critical for the stabilization of the lipid-bound forms. The presence of lipids may also directly affect amyloid formation. For example, the Alzheimer's amyloid peptide Aβ(1–40) binds to ganglioside-containing membranes (Choo-Smith et al. 1997) with a transition from random coil to β-sheet, accompanied by accelerated fibrillogenesis. A similar transformation to β-structure aggregate is seen when Aβ(1-40) binds to negatively charged lipids at low lipid-to-peptide ratios (Terzi et al. 1997).

The presence of oxidatively modified lipids (Suarna et al. 1995) and the products of lipid hydrolysis, such as fatty acids and lysolecithin (Williams and Tabas 1995), may also promote the formation of amyloid. For example, the presence of fatty acids promotes amyloid formation by tau in vitro (Wilson and Binder 1997), while oxidized fatty acids further promote amyloid formation by tau (Gamblin et al. 2000). Recent studies on apoC-II amyloid formation demonstrate that single molecules of submicellar DHPC nucleate amyloid formation (Hatters et al. 2001). This interaction is in contrast to the binding of apoC-II to lipid micelles, which promotes stable α-helical structure.

Conclusions

Current evidence suggests a correlation between apolipoproteins and amyloid formation. We propose that a key to the propensity of apolipoproteins to form amyloid lies in their shared properties and inherent structural instability in the absence of lipids. Further analysis of the physiological conditions that determine the folding options of apolipoproteins will provide insight into mechanisms to combat or reverse these alternate folding pathways and their pathological consequences.

Acknowledgements We thank Cait MacPhee and Chris Dobson for their valuable discussions and access to laboratory space. We thank William Sawyer, Chris MacRaild and Lynne Lawrence for their advice and assistance throughout the preparation of this work.

References

Aggerbeck LP, Wetterau JR, Weisgraber KH, Wu CS, Lindgren FT (1988) Human apolipoprotein E3 in aqueous solution. II. Properties of the amino- and carboxy-terminal domains. J Biol Chem 263:6249–6258

Anantharamaiah GM, Jones JL, Brouillette CG, Schmidt CF, Chung BH, Hughes TA, Bhown AS, Segrest JP (1985) Studies of synthetic peptide analogs of the amphipathic helix. Structure of complexes with dimyristoylphosphatidylcholine. J Biol Chem 260:10248–10255

Benson MD, Liepnieks JJ, Yazaki M, Yamashita T, Hamidi Asl K, Guenther B, Kluve-Beckerman B (2001) A new human hereditary amyloidosis: the result of a stop-codon mutation in the apolipoprotein AII gene. Genomics 72:272–277

Booth DR, Tan SY, Booth SE, Hsuan JJ, Totty NF, Nguyen O, Hutton T, Vigushin DM, Tennent GA, Hutchinson WL, et al. (1995) A new apolipoprotein Al variant, Trp50Arg, causes hereditary amyloidosis. Q J Med 88:695–702

Booth DR, Tan SY, Booth SE, Tennent GA, Hutchinson WL, Hsuan JJ, Totty NF, Truong O, Soutar AK, Hawkins PN, Bruguera M, Caballeria J, Sole M, Campistol JM, Pepys MB (1996) Hereditary hepatic and systemic amyloidosis caused by a new deletion/insertion mutation in the apolipoprotein AI gene. J Clin Invest 97:2714–2721

Booth DR, Sunde M, Bellotti V, Robinson CV, Hutchinson WL, Fraser PE, Hawkins PN, Dobson CM, Radford SE, Blake CC, Pepys MB (1997) Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. Nature 385:787–793

Castano EM, Prelli F, Pras M, Frangione B (1995) Apolipoprotein E carboxy-terminal fragments are complexed to amyloids A

and L. Implications for amyloidogenesis and Alzheimer's disease. J Biol Chem 270:17610–17615

Chiti F, Webster P, Taddei N, Clark A, Stefani M, Ramponi G, Dobson CM (1999) Designing conditions for *in vitro* formation of amyloid protofilaments and fibrils. Proc Natl Acad Sci USA 96:3590–3594

Choo-Smith LP, Garzon-Rodriguez W, Glabe CG, Surewicz WK. (1997) Acceleration of amyloid fibril formation by specific binding of Aβ-(1-40) peptide to ganglioside-containing membrane vesicles. J Biol Chem 272:22987–22990

Conway KA, Harper JD, Lansbury PT (1998) Accelerated *in vitro* fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease. Nat Med 4:1318–1320

Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT Jr (2000) Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA 97:571–586

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923

Davidson WS, Jonas A, Clayton DF, George JM (1998) Stabilization of α-synuclein secondary structure upon binding to synthetic membranes. J Biol Chem 273:9443–9449

Dobson CM (1999) Protein misfolding, evolution and disease. Trends Biochem Sci 24:329–332

Eliezer D, Kutluay E, Bussell R, Browne G (2001) Conformational properties of α-synuclein in its free and lipid-associated states. J Mol Biol 307:1061–1073

Fisher WR, Taniuchi H, Anfinsen CB (1973) On the role of heme in the formation of the structure of cytochrome C. J Biol Chem 248:3188–3195

Fu S, Davies MJ, Stocker R, Dean RT (1998) Evidence for roles of radicals in protein oxidation in advanced human atherosclerotic plaque. Biochem J 333:519–525

Gamblin TC, King ME, Kuret J, Berry RW, Binder LI (2000) Oxidative regulation of fatty acid-induced tau polymerization. Biochemistry 39:14203–14210

Genschel J, Haas R, Propsting MJ, Schmidt HH (1998) Apolipoprotein A-I induced amyloidosis. FEBS Lett 430:145–149

Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM (2000) Oxidative damage linked to neurodegeneration by selective α-synuclein nitration in synucleinopathy lesions. Science 290:985–989

Goda S, Takano K, Yamagata Y, Nagata R, Akutsu H, Maki S, Namba K, Yutani K (2000) Amyloid protofilament formation of hen egg lysozyme in highly concentrated ethanol solution. Protein Sci 9:369–375

Gursky O, Atkinson D (1996a) Thermal unfolding of human high-density apolipoprotein A-1: implications for a lipid-free molten globular state. Proc Natl Acad Sci USA 93:2991–2995

Gursky O, Atkinson D (1996b) High- and low-temperature unfolding of human high-density apolipoprotein A-2. Protein Sci 5:1874–1882

Gursky O, Atkinson D (1998) Thermodynamic analysis of human plasma apolipoprotein C-1: high-temperature unfolding and low-temperature oligomer dissociation. Biochemistry 37:1283–1291

Hamidi Asl K, Liepnieks JJ, Nakamura M, Parker F, Benson MD (1999a) A novel apolipoprotein A-1 variant, Arg173Pro, associated with cardiac and cutaneous amyloidosis. Biochem Biophys Res Commun 257:584–588.

Hamidi Asl L, Liepnieks JJ, Hamidi Asl K, Uemichi T, Moulin G, Desjoyaux E, Loire R, Delpech M, Grateau G, Benson MD (1999b) Hereditary amyloid cardiomyopathy caused by a variant apolipoprotein A1. Am J Pathol 154:221–227

Hatters DM, MacPhee CE, Lawrence LJ, Sawyer WH, Howlett GJ (2000) Human apolipoprotein C-II forms twisted amyloid ribbons and closed loops. Biochemistry 39:8276–8283

Hatters DM, Lawrence LJ, Howlett GJ (2001) Sub-micellar phospholipid accelerates amyloid formation by apolipoprotein C-II. FEBS Lett 494:220–224

Higuchi K, Kitagawa K, Naiki H, Hanada K, Hosokawa M, Takeda T (1991) Polymorphism of apolipoprotein A-II (apoA-II) among inbred strains of mice. Relationship between the molecular type of apoA-II and mouse senile amyloidosis. Biochem J 279:427–433

Husebekk A, Skogen B, Husby G, Marhaug G (1985) Transformation of amyloid precursor SAA to protein AA and incorporation in amyloid fibrils *in vivo*. Scand J Immunol 21:283–287

Jensen PH, Nielsen MS, Jakes R, Dotti CG, Goedert M (1998) Binding of α-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. J Biol Chem 273:26292–26294

Jo E, McLaurin J, Yip CM, St George-Hyslop P, Fraser PE (2000) α-Synuclein membrane interactions and lipid specificity. J Biol Chem 275:34328–34334

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325:733–736

Kelly JW (1996) Alternative conformations of amyloidogenic proteins govern their behavior. Curr Opin Struct Biol 6:11–17

Li WH, Tanimura M, Luo CC, Datta S, Chan L (1988) The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. J Lipid Res 29:245–271

MacPhee CE, Dobson CM (2000) Formation of mixed fibrils demonstrates the generic nature and potential utility of amyloid nanostructures. J Am Chem Soc 122:12707–12713

McParland VJ, Kad NM, Kalverda AP, Brown A, Kirwin-Jones P, Hunter MG, Sunde M, Radford SE. (2000) Partially unfolded states of β_2-microglobulin and amyloid formation *in vitro*. Biochemistry 39:8735–8746

Morrisett JD, Jackson RL, Gotto AM Jr (1977) Lipid-protein interactions in the plasma lipoproteins. Biochim Biophys Acta 472:93–133

Mucchiano G, Cornwell GG III, Westermark P (1992) Senile aortic amyloid. Evidence for two distinct forms of localized deposits. Am J Pathol 140:871–877

Namba Y, Tomonaga M, Kawasaki H, Otomo E, Ikeda K (1991) Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res 541:163–166

Naslund J, Thyberg J, Tjernberg LO, Wernstedt C, Karlstrom AR, Bogdanovic N, Gandy SE, Lannfelt L, Terenius L, Nordstedt C (1995) Characterization of stable complexes involving apolipoprotein E and the amyloid β peptide in Alzheimer's disease brain. Neuron 15:219–228

Nettleton EJ, Sunde M, Lai Z, Kelly JW, Dobson CM, Robinson CV (1998) Protein subunit interactions and structural integrity of amyloidogenic transthyretins: evidence from electrospray mass spectrometry. J Mol Biol 281:553–564

Nichols WC, Dwulet FE, Liepnieks J, Benson MD (1988) Variant apolipoprotein AI as a major constituent of a human hereditary amyloid. Biochem Biophys Res Commun 156:762–768

Obici L, Bellotti V, Mangione P, Stoppini M, Arbustini E, Verga L, Zorzoli I, Anesi E, Zanotti G, Campana C, Vigano M, Merlini G (1999) The new apolipoprotein A-I variant Leu$^{174}\rightarrow$Ser causes hereditary cardiac amyloidosis, and the amyloid fibrils are constituted by the 93-residue N-terminal polypeptide. Am J Pathol 155:695–702

O'Brien KD, Olin KL, Alpers CE, Chiu W, Ferguson M, Hudkins K, Wight TN, Chait A (1998) Comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: colocalization of biglycan with apolipoproteins. Circulation 98:519–527

Perrin RJ, Woods WS, Clayton DF, George JM (2000) Interaction of human α-synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis. J Biol Chem 275:34393–34398

Persey MR, Booth DR, Booth SE, van Zyl-Smit R, Adams BK, Fattaar AB, Tennent GA, Hawkins PN, Pepys MB (1998) Hereditary nephropathic systemic amyloidosis caused by a novel variant apolipoprotein A-I. Kidney Int 53:276–281

Perugini MA, Schuck P, Howlett GJ (2000) Self-association of human apolipoprotein E3 and E4 in the presence and absence of phospholipid. J Biol Chem 275:36758–36765

Rankin SE, Watts A, Pinheiro TJ (1998) Electrostatic and hydrophobic contributions to the folding mechanism of apocytochrome C driven by the interaction with lipid. Biochemistry 37:12588–12595

Segrest JP, Garber DW, Brouillette CG, Harvey SC, Anantharamaiah GM (1994) The amphipathic α-helix: a multifunctional structural motif in plasma apolipoproteins. Adv Protein Chem 45:303–369

Serpell LC, Sunde M, Benson MD, Tennent GA, Pepys MB, Fraser PE (2000) The protofilament substructure of amyloid fibrils. J Mol Biol 300:1033–1039

Sipe JD (1992) Amyloidosis. Annu Rev Biochem 61:947–975

Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G (2000) Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 1502:139–144

Soulages JL, Bendavid OJ (1998) The lipid binding activity of the exchangeable apolipoprotein apolipophorin-III correlates with the formation of a partially folded conformation. Biochemistry 37:10203–10210

Sousa MM de, Vital C, Ostler D, Fernandes R, Pouget-Abadie J, Carles D, Saraiva MJ (2000) Apolipoprotein AI and transthyretin as components of amyloid fibrils in a kindred with apoAI Leu178His amyloidosis. Am J Pathol 156:1911–1917

Soutar AK, Hawkins PN, Vigushin DM, Tennent GA, Booth SE, Hutton T, Nguyen O, Totty NF, Feest TG, Hsuan JJ, et al. (1992) Apolipoprotein AI mutation Arg-60 causes autosomal dominant amyloidosis. Proc Natl Acad Sci USA 89:7389–7393

Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD (1993) Binding of human apolipoprotein E to synthetic amyloid β peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci USA 90:9098–9102

Suarna C, Dean RT, May J, Stocker R (1995) Human atherosclerotic plaque contains both oxidized lipids and relatively large amounts of α-tocopherol and ascorbate. Arterioscler Thromb Vasc Biol 15:1616–1624

Sunde M, Blake C (1997) The structure of amyloid fibrils by electron microscopy and X-ray diffraction. Adv Protein Chem 50:123–159

Tajima S, Yokoyama S, Kawai Y, Yamamoto A (1982) Behavior of apolipoprotein C-II in an aqueous solution. J Biochem (Tokyo) 91:1273–1279

Takahashi Y, Ueno A, Mihara H (1999) Optimization of hydrophobic domains in peptides that undergo transformation from α-helix to β-fibril. Bioorg Med Chem 7:177–185

Terzi E, Holzemann G, Seelig J (1997) Interaction of Alzheimer β-amyloid peptide (1-40) with lipid membranes. Biochemistry 36:14845–14852

Uhlar CM, Whitehead AS (1999) Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 265:501–523

Weinberg RB, Spector MS (1985) Structural properties and lipid binding of human apolipoprotein A-IV. J Biol Chem 260:4914–4921

Westermark P, Mucchiano G, Marthin T, Johnson KH, Sletten K (1995) Apolipoprotein A1-derived amyloid in human aortic atherosclerotic plaques. Am J Pathol 147:1186–1192

Wetterau JR, Aggerbeck LP, Rall SC Jr, Weisgraber KH (1988) Human apolipoprotein E3 in aqueous solution. I. Evidence for two structural domains. J Biol Chem 263:6240–6248

Williams J, Tabas I (1995) The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 15:551–561

Wilson DM, Binder LI (1997) Free fatty acids stimulate the polymerization of tau and amyloid β peptides. *In vitro* evidence for a common effector of pathogenesis in Alzheimer's disease. Am J Pathol 150:2181–2195

Wisniewski T, Golabek AA, Kida E, Wisniewski KE, Frangione B (1995a) Conformational mimicry in Alzheimer's disease. Am J Pathol 147:238–244

Wisniewski T, Lalowski M, Golabek A, Vogel T, Frangione B (1995b) Is Alzheimer's disease an apolipoprotein E amyloidosis? Lancet 345:956–958