Inhibition of Amyloidogenesis by Synthetic Enantiomeric Peptides

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More than 30 human proteins are associated with amyloidoses or amyloid disease, in which these proteins fail to fold into their native state and instead aggregate into insoluble, twisted fibrils. Amyloid fibrils comprise non-covalent fibrillar structures of extended, inter-molecularly hydrogen bonded β-sheets that laterally self-assemble perpendicular to the fibril axis and are characterised by an X-ray diffraction signature known as the cross-β pattern (Nelson *et al*, 2005). Formation of amyloid fibrils presumably involves a transition to β-sheet structure through amyloidogenic intermediates.

There is increasing evidence that soluble intermediate oligomers which arise on the aggregation pathway from monomeric species to polymeric insoluble fibrils are the toxic elements in amyloidogenesis and that the mature fibrils found in amyloid plaques are non-toxic, acting as a reservoir for toxic oligomeric species (Xue *et al*, 2009). An obvious therapeutic strategy is the design of inhibitory peptides which bind to soluble toxic oligomers thus preventing their accumulation and consequent harmful effects.

Toxic soluble oligomeric forms of different amyloidogenic proteins share a common backbone conformation and molecular dynamic (MD) simulations of formation of toxic oligomers by amyloidogenic proteins have revealed the existence of an unusual secondary structure: the α-sheet. This structure was first described as the pleated sheet by Pauling and Corey nearly 70 years ago (Pauling and Corey, 1951). The appearance of the α-sheet structure in low pH MD simulations for a number of non-related amyloidogenic polypeptides, led the group of Valerie Daggett at the University of Washington in Seattle, to put forward the hypothesis that the α-sheet is the defining feature of cytotoxic soluble oligomers (Armen *et al*, 2004; Daggett V, 2006). A recent report of α-sheet structure observed in mutants of the amyloidogenic protein transthyretin (TTR) lends further weight to this hypothesis (Hilaire *et al*, 2018).

The α-sheet is similar to the β-sheet, with one striking difference: in the α-sheet the 'φ' and 'ψ' dihedral angles of consecutive amino acid residues are configured so as to produce alternating L- and D- enantiomeric conformations. The result of this is that α-sheet and β-sheet differ in the orientation of their respective backbone NH and CO groups, the main chain hydrogen-bonding groups. In the β-sheet structure, all amino acid residues are in the L-enantiomeric conformation so that the NH and CO groups from successive residues point in opposite directions and thus alternate on either side of the sheet. Thus, if the NH group of one residue forms a hydrogen bond (H-bond) to the CO of a residue lying in the strand to its 'left', the NH of the next residue will form an H-bond to the CO of a residue lying in the strand to its 'right'. In contrast, in an α-sheet all the NH groups are are aligned on one side of the sheet and all the CO groups on the other. Therefore, the α-sheet has a molecular dipole and a very different H-bonding pattern across the sheet compared to the β-sheet. In an α-sheet each NH or CO group forms two H-bonds with CO or NH groups in the neighbouring strand, whereas in a β-sheet, each NH or CO group forms one hydrogen bond with a CO or an NH group in the neighbouring strand (Figure 1). Once an α-sheet is formed, peptide plane flipping could convert the α-sheet into a β-sheet and ultimately a mature amyloid fibril (Daggett V, 2006; Dobson CM, 1999). Short stretches of α-strand are present in various proteins in the Protein Data Bank (PDB) (Milner-White and Russell, 2008), but extensive α-sheet formation has not been observed in native proteins.

Native proteins are synthesised ribosomally from 22 proteinogenic amino acids, 20 of which, the so-called common amino acids, are encoded by triplet codons in the genetic code; these are glycine which is achiral and 19 L-amino acids (the other two proteinogenic amino acids, selenocysteine and pyrrolysine are termed "non-standard" or "non-canonical" as they are not coded directly by DNA). The presence of D-amino acids in peptides and proteins is usually the result of post-translational modification, although in some prokaryotes they are incorporated through non-ribosomal biosynthesis involving multi-enzyme complexes (Towse *et al*, 2014). D-amino acids are also characteristic of some age-related pathological conditions associated with racemisation. Thus D-amino acids are underrepresented in the Protein Data Bank (PDB) (Towse *et al*, 2014).

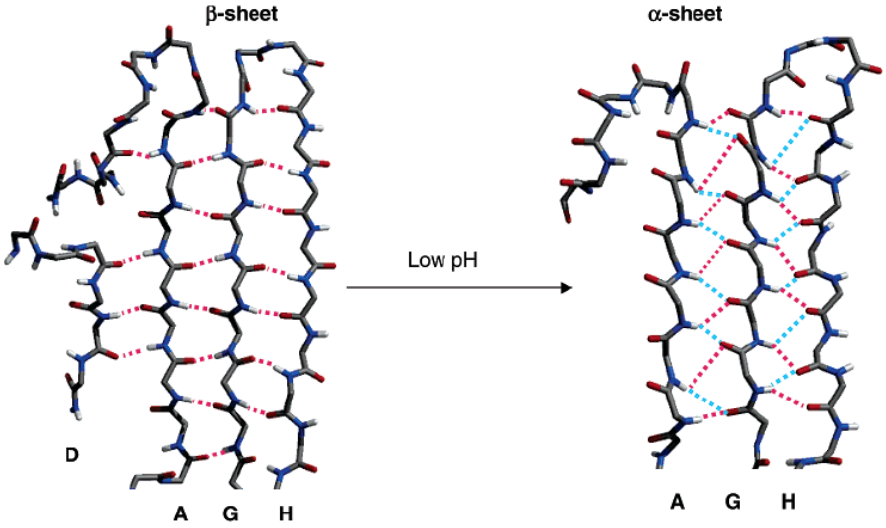

Figure 1. Molecular dynamics (MD) simulations at low pH of crystal structure of the amyloidogenic protein transthyretin with β-sheet secondary structure (TTR) (left) yield intermediate folding conformers with α-sheet secondary structure (right). Adapted with permission from Daggett, V (2006), Copyright 2006 American Chemical Society.

A recent communication reports the successful inhibition of aggregation and reduction of toxic effect in neuroblastoma cells of the β-amyloid peptide, Aβ, implicated in Alzheimer's disease, by a synthetic peptide, AP90, composed of alternating L/D amino acid residues (Maris *et al*, 2018). In contrast, a synthetic peptide with the same sequence, but with all L-amino acid residues, P90, had no inhibitory effect on amyloidogenesis or toxicity of Aβ. The communication by Maris *et al*. reports the latest conclusions of ongoing research which explores the physical and chemical differences between *de novo* designed peptides sharing the same sequence – i.e. the peptides have identical primary structure - yet differ in the enantiomeric state of the residues. Maris and colleagues show that the presence of alternating L/D-amino acid motifs dramatically increases aqueous solubility, enforces α-sheet secondary structure (according to circular dichroism (CD) and Fourier transform infra-red (FTIR) spectroscopy) and inhibits aggregation of β-amyloid implicated in Alzheimer's disease, in addition to neutralizing cytotoxicity. Thus the two peptides have remarkably different chemical and structural behaviour (Maris *et al*, 2018) (Figure 2).

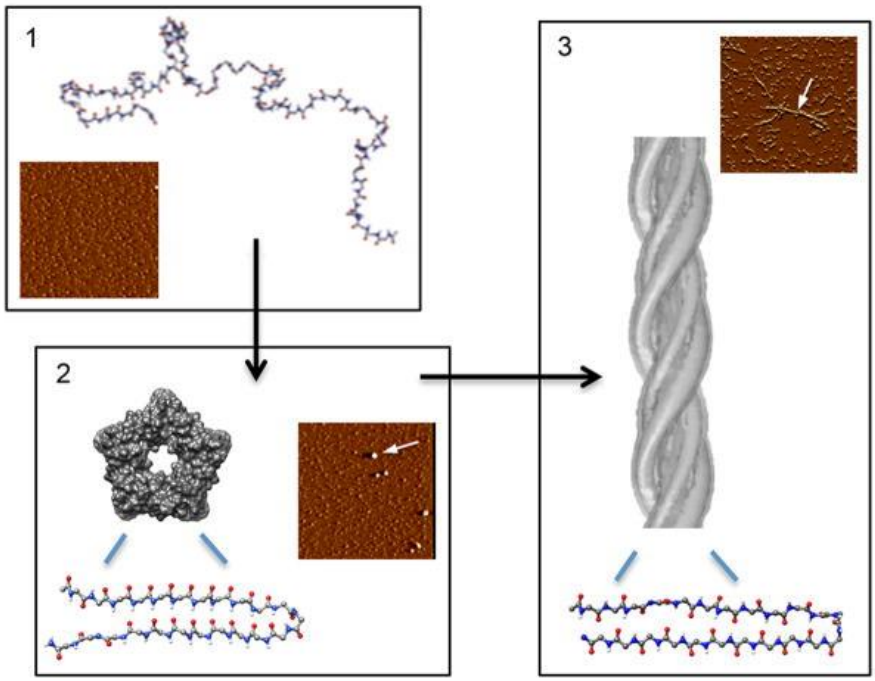

Figure 2. Simplified schematic progression of amyloidogenesis of Aβ. (1) Monomeric aggregation-competent Aβ. Aggregation proceeds, yielding (2) cytotoxic oligomers [with an atomic force microscopy image (AFM)] proposed to contain α-sheet structure, as illustrated in an AP90 model hairpin. (3) Formation of β-sheet fibrils follows, as illustrated by the P90 β-hairpin model and the AFM image of Aβ. AFM images are 2 μm × 2 μm. Reprinted with permission from Maris NL, *et al*. Chemical and Physical Variability in Structural Isomers of an L/D α-Sheet Peptide Designed To Inhibit Amyloidogenesis. Biochemistry. 2018 Feb 6;57(5):507-510. doi: 10.1021/acs.biochem.7b00345. Copyright (2018) American Chemical Society.

The authors conclude that "the striking differences between the two peptides show that even with identical primary structures, altering the chirality of individual amino acid residues has a profound effect on chemical character and functional properties" (Maris *et al*, 2018).

The inhibitory effect of the peptide AP90, is effected by adopting an α-sheet backbone which is complementary to the backbone of the α-sheet structure of toxic oligomers of Aβ. The alternating L- D-enantiomeric forms of amino acid residues enforces the polar α-sheet backbone configuration and encourages complementarity between the designed peptide and its amyloidogenic target (Maris *et al*, 2018).

CONCLUSION

Enantiomeric peptides appear to hold promise as a therapeutic means of combating the deleterious effects of pathogenic amyloidogenesis. The existence of α-sheet as an evolutionary relic raises interesting questions about the functional role of amyloid and its involvement in disease (Milner-White and Russell, 2008). Elucidation of the conformational conversions occurring between intermediate oligomers in amyloidogenesis may shed light on the intriguing question of the origin of chirality of native proteins.

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