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# Molecular determinants for amyloid fibril formation: lessons from lung surfactant protein C

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## Summary

Amyloid fibrils are polymers composed of proteins in β-sheet conformation, which are found in at least 20 diseases for which no cure is available. These diseases include Alzheimer's disease and spongiform encephalopathies, in which the amyloid β-peptide (Aβ) and the prion protein (PrP), respectively, form amyloid. All fibrils are morphologically very similar although the native structures of the corresponding proteins are widely different. Proteins that are not known to form fibrils *in vivo* can do so under conditions where unfolding intermediates are well populated. This indicates that fibril formation can arise from most, if not all, polypeptide chains under certain conditions, and that nature has found ways to avoid fibrillogenic protein conformations. In support of this, it was recently found that unpaired β-strands present in native proteins are prevented from forming intermolecular β-sheets, by strategic placement of prolines and charged residues for example. Structural studies of the lung surfactant-associated protein C (SP-C) have revealed determinants for amyloid fibril formation. The poly-valine α-helix of SP-C spontaneously converts to β-sheet aggregates *in vitro* and SP-C amyloid fibrils are found in pulmonary alveolar proteinosis. Aβ, PrP, and SP-C harbour an α-helix which is strongly predicted to form a β-strand, and in all cases investigated so far such α-helix/β-sheet discordance correlates with the ability to form β-sheet aggregates and fibrils.

*Key words: amyloid β-peptide; protein misfolding; Alzheimer's disease; prion protein; prion disease; α-helix to β-strand transition; discordant helix; SP-C*

## Amyloid diseases and amyloid fibril formation

The term amyloid emanates from early histological examinations where tissue deposits associated with certain diseases were thought to be composed of carbohydrates, but amyloid is now known to be made up of proteins which have lost their native, soluble, structure and turned into insoluble β-sheet polymers [1]. Amyloid fibrils are defined by their ability to stain with the dye Congo red and then show birefringence under polarised light, by the appearance of 5–10 nm unbranched fibrillar structures detectable by electron microscopy, and by typical X-ray diffraction pattern. Amyloid fibrils are likely to be built from a polypeptide chain that has adopted a cross-β-sheet structure, ie, the individual β-strands are oriented perpendicular to the fibril direction [2, 3]. The approximately 20 known amyloid diseases represent a perplexing medical problem, including such diverse entities as Alzheimer's disease (fibrils from the amyloid β-peptide, Aβ), the spongiform encephalopathies or prion diseases, including Creutzfeldt-Jakob disease in humans, scrapie in sheep and bovine spongiform encephalopathy (mad-cow disease) (fibrils from the prion protein, PrP), familial polyneuropathy (fibrils from transthyretin), type 2 diabetes mellitus (fibrils from islet amyloid polypeptide or amylin), systemic amyloidosis (fibrils from lysozyme or transthyretin), Parkinson's disease (fibrils from α-synuclein) and Huntington's disease (fibrils from huntingtin) [4–6]. Amyloid diseases occur in sporadic and hereditary forms, whilst the prion diseases are also infectious. Notably, the formation of amyloid fibrils is not necessarily the result of abnormal processes, but can be used for specific functions. They form a protective shell in the silk moth [7], and spiders' web fibres have been proposed to have amyloid-like properties [8]. The proteins implicated in disease are invariably present in the corresponding amyloid deposits, but also other proteins and additional constituents can be

Abbreviations:
Aβ amyloid β-peptide
PrP prion protein
SP-C surfactant protein C

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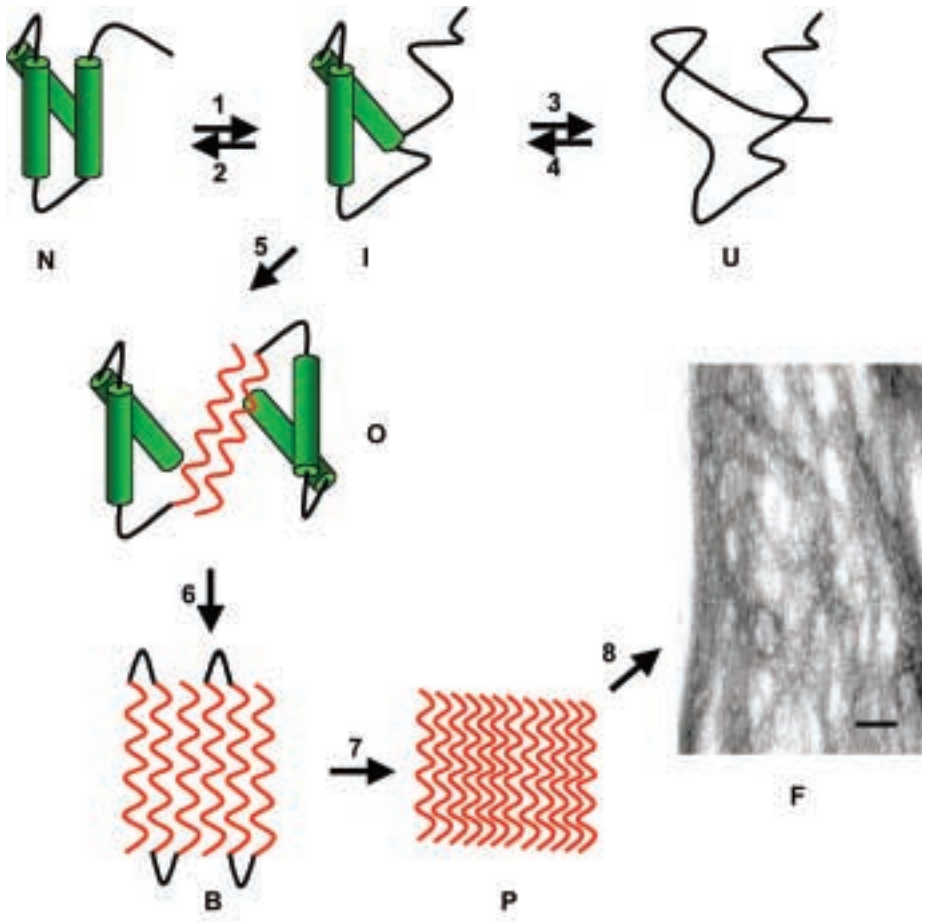



**Figure 1**

Pathway for formation of amyloid fibrils. Schematic representation of the current understanding of molecular mechanisms involved in fibril formation. N represents the native structure, U a completely unfolded state, and I represents an intermediate in which parts of the polypeptide chain are in non-native conformation. Formation of intermolecular β-sheet mediated by unfolded regions in I leads to protein oligomerisation (O), which in turn leads to further formation of β-strand structure (B), formation of prefibrillar oligomers/protofibrils (P) and eventually to fibril formation (F). The scale bar in the electron micrograph represents 50 nm.

found. For example, the amyloid plaques found in Alzheimer's disease have been found to contain, in addition to Aβ, proteoglycans, $\alpha_1$-antichymotrypsin and apolipoprotein E [9]. Moreover, the protein serum amyloid P component (SAP) binds to amyloid deposits irrespective of what protein is the primary constituent. SAP-amyloid binding can be reduced by low molecular weight SAP ligands, a property which has been explored as a means to facilitate amyloid clearance [10]. For some fibril-forming proteins, eg, Aβ and PrP, both wild-type and mutant proteins form amyloid, while for others, e.g., lysozyme, destabilising point mutations are required for fibril formation [11]. Point mutations related to inherited forms of human prion diseases do not cause formation of the amyloid form of PrP *in vitro* and are not as a rule destabilising [12].

All fibrils appear to be made from very similar cross-β-sheet structure [2], but the native states of fibril-forming proteins vary considerably. For example, PrP contains a structurally disordered part, three α-helices and a short β-sheet [13]. Aβ is mainly flexible, but under membrane-mimicking conditions its central (residues 15–25) and C-terminal parts (residues 30–40) form helices, see [2]. The conversion of the soluble form of PrP to its fibrillar counterpart is accompanied by reduction in α-helix content and increase in β-sheet structure, and Aβ fibril formation also involves α-helix to β-strand conversion [14, 15]. This indicates that proteins with α-helical structure in their native states must undergo α-helix→β-sheet conversion before or during fibrillation. Fibril formation, however, does not necessarily involve α-helix→β-sheet conversion; transthyretin is a tetramer where each subunit is composed of all-β-sheet structure, and α-synuclein is structurally disordered in its native state. Amyloid fibril formation is promoted under conditions where conformations present during unfolding are well populated [16–18]. A current understanding of the events leading to transformation of a native protein into a fibril with β-sheet structure is presented in figure 1. Salient features of this process are (i) local unfolding of the native protein, or dissociation of a multimeric protein, leading to exposure of regions which are prone to self-associate, (ii) intermolecular association, (iii) further structural rearrangement leading to increased β-sheet content, and (iv) acquisition of a cross-β-sheet structure and polymerisation into fibrils.

The ability to form amyloid fibrils is not limited to the proteins that form amyloid in human and mammalian disease. Fibrils similar to those found in disease can be formed *in vitro* from virtually any protein under partly denaturing conditions [19, 20]. Although any peptide sequence may be able to form a cross-β-sheet structure under some conditions, the ability to do so under physiological conditions is apparently limited to a few proteins. This raises the question: what distinguishes the small number of proteins that form amyloid under physiological conditions? *In vitro* fibril formation of a globular protein and of short

peptides can be modulated by changes in the amino acid sequence [21–23]. For natural amyloidogenic proteins, there is data indicating that specific regions of their amino acid sequences determine fibrillation. Aβ is highly fibrillogenic, while peptides lacking residues 14–23 are not [24]. In $\beta_2$-microglobulin, which forms fibrils in haemodialysis-related amyloidosis, five of seven β-strands are preserved in a partly unfolded fibrillogenic intermediate, and largely the same region forms a rigid β-sheet in $\beta_2$-microglobulin fibrils [25, 26]. Interestingly, when represented as synthetic peptides in isolation, only one of these strands forms fibrils *in vitro* [27]. Unexpectedly, studies of lung surfactant protein C (SP-C) have lead to the identification of structural features, which appear to play a role for the fibrillogenic behaviour of fibril-forming proteins.

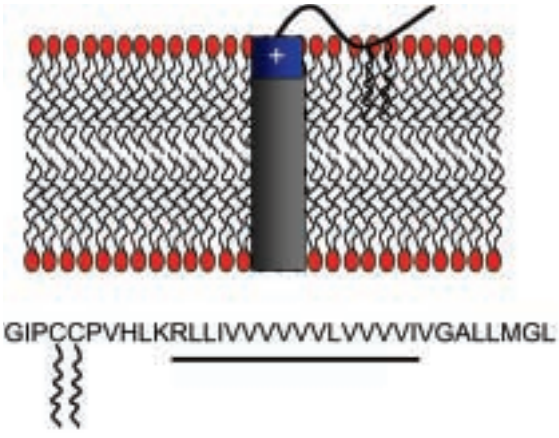
## Lung surfactant protein C

SP-C is one of four surfactant-associated proteins which are involved in preserving alveolar patency and in lung host-defence [28, 29]. SP-C is evolutionarily highly conserved and the peptide is found only in lung surfactant [30]. SP-C is mainly implicated in lung surface properties [31], and the characterisation of SP-C has been fuelled by the fact that it is found in preparations used for therapy of respiratory failure [32]. However, SP-C binds lipopolysacharides, suggesting that it may also be involved in lung host-defence [33–35]. SP-C is extremely hydrophobic owing to the presence of two fatty acyl chains and almost 30 residues (out of 35) with aliphatic side-chains (figure 2). The three-dimensional structure of SP-C encompasses an α-helix covering positions 9–34, which is perfectly adopted to span a lipid bilayer [36, 37] (figure 2).

The helical structure of SP-C exhibits long-term stability when inserted into lipid micelles or membranes, but is metastable in aqueous organic solvents, i.e., once it opens up into a non-helical structure, it does not refold again but forms β-sheet aggregates [38]. The short-term stability of the SP-C poly-valine α-helix stems from tight interactions between the valine side-chains, giving high activation energy for SP-C helix unfolding [39, 40]. The structural conversion of SP-C from α-helix to β-sheet aggregates results in formation of amyloid fibrils, and occurs in association with pulmonary alveolar proteinosis [41]. Whether these fibrils are formed as a result of altered intraalveolar metabolism in pulmonary alveolar proteinosis, or are involved in the pathogenesis of the disease remains to be investigated. The metastability of helical SP-C and the problems in folding SP-C into a helix may be involved in other human diseases. Familial forms of human interstitial lung disease are associated with point mutations in the proSP-C gene [42, 43], and formation of aggresomes occurs in cells transfected with mutated proSP-C [44]. The near-complete lack of mature SP-C observed in the disease cases is likely a dominant negative effect caused by interactions between mutant and wild-type proSP-C [45, 46]. Transgenic expression of the mature SP-C peptide leads to marked lung underdevelopment [47], while genetic ablation of SP-C gives variable effects on respiratory function [29, 48]. It is thus possible that interstitial lung disease is caused to a large extent by cellular injury due to SP-C aggregation. It will be interesting to find out whether β-sheet formation and fibril formation of SP-C are associated with the familial forms of interstitial lung disease.

**Figure 2**

Structure of SP-C. The cylinder represents the α-helix of SP-C, covering residues 9–34 in the structure determined by NMR, and which corresponds to the thickness of a fluid dipalmitoyl-phosphatidylcholinebilayer. The plus sign in the N-terminal part of the helix corresponds to the evolutionarily conserved Lys11–Arg12 dibasic pair. The two palmitoyl groups linked to Cys5 and Cys6 are depicted by wavy lines. The amino acid sequence of human SP-C is given using one-letter abbreviations for the amino acid residues. The discordant region is underlined.



## α-Helix/β-strand discordant helices and fibril formation

The experimentally determined SP-C α-helix, composed essentially of a long stretch of valine residues, is unusual since valines are well-known to be over-represented in β-strands and under-represented in helices [36, 49]. Intriguingly, synthetic peptides with the SP-C amino acid sequence are inefficient in helix formation, and form insoluble aggregates [49]. In contrast, peptides in which the poly-valine sequence of SP-C is replaced with that of a helical part of bacteriorhodopsin or a poly-leucine stretch, readily form helices and do not aggregate [49, 50]. These sequences have high helical propensities. When three-dimensional structures in the protein data bank were compared with their predicted secondary structures, about three percent of the proteins turned out to contain a ≥7-residue α-helix strongly predicted to form a β-strand. These α/β discordances include PrP (helix 2, positions 179–191), Aβ (positions 16–23), and SP-C (positions 12–27, figure 2). Coagulation factor XIII, a triacylglycerol lipase, and a transpeptidase likewise contain a long discordant helix. None of these proteins were known to be fibrillogenic, but were found to form fibrils in physiological-like solutions, suggesting a correlation between α-helix/β-strand discordant stretches and amyloid fibril formation [51].

Regarding Aβ, two lines of evidence indicate that the discordant region is crucial for fibril formation; positions 16–20 are essential for formation of Aβ intermolecular contacts and fibril formation [52], and destabilisation of the helix covering residues 17–24 is critical for α-helix→β-sheet conversion and fibril formation [53]. Experimental support for the possibility that discordant helices represent underlying structural ambivalence can be found also in a study of isolated synthetic peptides corresponding to transmembrane helices of bacteriorhodopsin [54]. This showed that of the seven transmembrane regions, only the discordant one (the most C-terminal of the helices) forms a hyperstable β-sheet aggregate, while five of the other regions form stable transmembrane helices and one region does not form any stable secondary structure. The authors pointed out that C-terminally located helices may have a lower intrinsic stability, and their efficient folding requires rapid folding of the segments which are synthesised first, so that they can serve as folding templates for the distal segments [54]. It is possible that discordant helices require the presence of scaffolding, e.g., a folded protein nucleus or a lipid membrane, for proper folding in order to compensate for their low intrinsic potential for helix formation.

In PrP helix 2 is discordant, while helices 1 and 3 are correctly predicted to be helical [51]. In line with this, an early prediction of PrP secondary structure gave contradictory results for the helix 2 region, suggesting that alternative conformations of PrP can coexist [55]. It was recently found that PrP helix 2, in particular its C-terminal part, is "frustrated" in its helical state and this region may play a role in α→β transition [56]. Moreover, in a crystal structure of dimeric PrP, the last turn of the discordant helix 2 is transformed to a β-strand, while helices 1 and 3 remain intact. It was suggested that formation of the dimeric form could represent a first step towards fibril formation of PrP [57]. This shows experimentally that parts of the PrP discordant helix can change the structure into β-strand, as suggested from the secondary structure predictions, and lends support to the concept that discordant helices may represent starting sites for α-helix→β-sheet conversions. The Cys residue in PrP helix 2 makes a disulphide bond with helix 3. In the domain-swapped dimer, this disulphide bond has spontaneously rearranged from an intramolecular to an intermolecular interaction [57]. This suggests that the disulphide connecting helices 2 and 3 can be opened *in vivo*. Interestingly, a reduced form of a recombinant PrP fragment (positions 91–231), but not the oxidised form, can form a soluble monomer composed almost entirely of β-sheet [58]. This β-sheet-rich monomer was prone to aggregate into fibrils.

Modulation of the sequences of α/β discordant segments, so that their helical propensities are increased and β-strand propensities are decreased reduces fibril-formation. The mutations K16A/L17A/F20A and V18A abrogate Aβ fibril formation *in vitro* [52, 59]. These changes reverse the discordant nature of the 16–23 region of Aβ. Likewise, by replacing valines in SP-C with leucines (SP-C(Leu)), an α-helix is found experimentally and by predictions [50, 51]. SP-C(Leu) does not form β-sheet aggregates or fibrils under conditions where SP-C forms abundant fibrils. Studies of SP-C and SP-C(Leu) using hydrogen/deuterium exchange and mass spectrometry indicate that unfolded SP-C rapidly converts into aggregates, while for unfolded SP-C(Leu) the lowest energy barrier is the one going back to helical conformation [60] (figure 3). Hydrogen/deuterium exchange in combination with NMR indicates that the activation energy for unfolding α-helical SP-C is ~100 kJ/mol [38]. The α-helical conformation of PrP is likewise kinetically stabilised relative to a β-rich isoform, with an energetic barrier of ~80 kJ/mol [61]. SP-C and PrP thus retains an α-helical fold not because of thermodynamic stability but because of a high energetic barrier to unfolding.

Studies of the two-helix protein acylphosphatase show that regions which determine folding and aggregation can be localised to different regions of the polypeptide chain [62, 63]. Mutations in mainly one of the α-helices influence the aggregation properties, while mutations in the other helix affect the folding properties [63]. Analysis of SP-C and SP-C(Leu) gives some clues

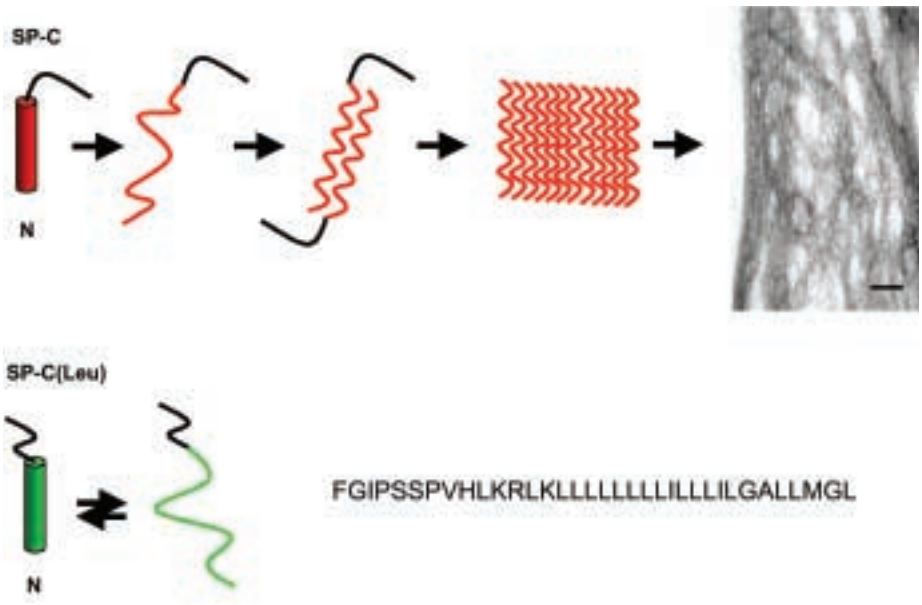



**Figure 3**

Folding and aggregation of SP-C and SP-C(Leu). Schematic representation of the different behaviour of SP-C compared to SP-C(Leu) regarding folding and aggregation, as derived from hydrogen/deuterium exchange in combination with NMR and mass spectrometry. Both peptides are helical in their native states (N). SP-C(Leu) (green) refolds into a helix after unfolding, while SP-C (red) forms β-sheet aggregates and amyloid fibrils after unfolding, with no detectable refolding of helical structure. The amino acid sequence of SP-C(Leu) is shown, see figure 2 for the amino acid sequence of SP-C.

as to what may identify an aggregation-determining region and a folding-determining region, respectively. There is no major difference between SP-C and SP-C(Leu) in size, polarity or structure of the native state (Figs. 2 and 3), but their secondary structure preferences differ significantly. The α-helix/β-strand propensity ratio for Val is 0.46, and for Leu 1.2 [60]. Thus there is an excellent agreement between the secondary structure propensities and the fates of the unfolded peptides; SP-C forms β-sheet aggregates and SP-C(Leu) reforms an α-helix (figure 3). This supports the observations that partitioning between aggregation and folding can be attributed to conformational preferences of the polypeptide chain [62, 64]. However, studies of peptides derived from β-strands of $\beta_2$-microglobulin gave no correlation between secondary structure propensities and fibril formation [27]. One caveat is that the secondary structure propensities used in the predictions described herein are mainly derived from water-soluble proteins. Using secondary structure propensities determined for peptides in non-polar environments [65] a helical structure of SP-C is predicted, suggesting that its discordant nature could reflect that the proper non-polar membrane environment is not taken into account. However, also in non-polar environments SP-C forms β-sheet aggregates [49, 60, 66], while SP-C(Leu) forms a helix in all solvents used [50]. This shows that folding of the SP-C poly-valine segment into a helical conformation is not accomplished by placing the mature peptide in a hydrophobic environment; at least proSP-C is probably necessary for correct folding (see above).

Fibrillogenic proteins may under physiological conditions form partially unfolded intermediates that are prone to oligomerise (figure 1). It appears reasonable to assume that if a discordant helix unfolds, the high β-strand propensity will make it less able to refold into a helical conformation compared to a region for which a helix is predicted, analogous to the situation observed for SP-C *vs* SP-C(Leu) [60] (figure 3). Unfolded discordant helices have high intrinsic preferences to adopt β-strand conformation and could then form β-sheets *via* protein oligomerisation. In the pathway of figure 1, unfolding of a discordant helix, relative to unfolding of a "normal" helix, would favour reaction 5 over reaction 2, thereby diverting a partly unfolded intermediate to fibril formation. This suggestion is in agreement with recent data. Analysis of β-sheet edges in a large sample of β-sheet proteins showed that all free edge strands were protected from making intermolecular β-sheet interactions [67]. The mechanisms involved include strategically placed prolines and charged residues, very short edge strands and loop coverage. In contrast, edge strands that natively form β-sheet dimers or rings lack such protections. Naturally occurring monomeric β-sheet proteins have thus evolved to avoid edge-to-edge aggregation [67]. Introduction of charged residues in *do novo* designed polypeptides was found to indeed prevent formation of amyloid-like fibrils and instead promote formation of monomeric β-sheets [68]. Prevention of amyloid fibril formation thus includes avoidance of that unpaired β-strands that can form intermolecular contacts. Protection against intermolecular β-sheet formation has likely not evolved for regions that are natively helical, and transformation of a discordant helix into an unpaired β-strand may thus promote fibril formation.

## Modulation of fibril formation

The pathophysiological mechanisms in amyloid diseases may, in principle, include loss of functional protein due to deposition as insoluble amyloid, but most evidence indicates that the major underlying mechanism is rather destructive effects elicited by the deposits [69, 70]. Regarding Aβ, the levels of both Aβ (1–40) and Aβ (1–42/43) in the cerebral cortex are significantly higher in Alzheimer's disease than in normal ageing [71]. Notably, in Alzheimer's disease brains a marked shift in Aβ from the predominantly soluble form in normal ageing to very large insoluble pools was seen. Näslund and co-workers found that the cortex levels of Aβ ending at positions 40 and 42 were elevated early in dementia, and levels of both peptides strongly correlated with cognitive decline [72]. These studies indicate an important role for Aβ in mediating initial pathogenic events in Alzheimer's disease. However, no correlation is found between the amounts of Aβ amyloid deposits and the degree of dementia, see eg, [73]. The reason for this is not known but may be related to the question whether intermediates in the pathway leading to fibril formation are the dominant toxic species [74]? Evidence is accumulating that prefibrillar soluble aggregates, including species referred to as protofibrils, may be more toxic than the mature fibrils [75–80]. Oligomeric, prefibrillar forms may form membrane-penetrating pores, similar in structure to pores made from bacterial toxins, and thereby cause cell lysis [81]. It has been proposed that incorporation of the prefibrillar species into mature fibrils is a way of protecting against their toxic effects, although it remains possible that both oligomers and mature fibrils are destructive.

Obstruction of amyloid and/or oligomer/protofibril formation may prevent the occurrence or progression of amyloidoses, and several ways have been explored to accomplish this [82]. Compounds which can abrogate fibril formation by interfering with peptide-peptide contacts in fibrils, which are expected to interfere with reactions 5 through 8 in figure 1, have been identified [52, 83, 84]. A potential drawback with such compounds is that they not only reduce fibril formation, but may also increase the amounts of oligomers/protofibrils, see figure 1. Another way to prevent fibril formation is to stabilise the native structure. This approach is effective in preventing fibril formation of acylphosphatase under partly denaturing conditions [85]. The possibility that α/β discordant helices are involved in the fibrillation process for some proteins suggest that stabilisation of these segments in helical conformation could prevent amyloid formation. Such an approach is analogous to the stabilisation of the native, non-amyloidogenic transthyretin tetramer, which prevents the dissociation into subunits which can form fibrils [86]. Stability of the SP-C helix is inversely correlated with its potency for forming fibrils; removal of one or both of the palmitoyl groups of SP-C *in vitro* results in faster unfolding of the α-helical conformation, as well as faster and more prominent formation of insoluble aggregates [41, 66]. Moreover, fibrillar SP-C associated with pulmonary alveolar proteinosis is partly non-palmitoylated [41], while in healthy individuals SP-C is almost exclusively di-palmitoylated [87].

For Aβ and SP-C, removal of the discordant nature by residue replacements abrogates fibril formation *in vitro*, see above and figure 3, further supporting that increasing the helical occupancy can reduce the fibril formation. This hypothesis is supported by the finding that optimal stabilisation of helical Aβ by addition of trifluoroethanol completely prevents fibril formation [88]. However, partial stabilisation of helical structure of Aβ instead accelerates formation of β-sheet aggregates and fibrils [88]. Furthermore, transient formation of α-helix structure prior to formation of β-sheet structure and fibrils has been observed, suggesting that partially helical forms of Aβ may be on-pathway to fibril formation [89]. Further studies are needed in order to understand the steps leading to fibril formation, so that this most devastating result of protein misfolding can be controlled.

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## References

1 Sipe JD, Cohen AS. History of the amyloid fibril. J Struct Biol 2000;130:88–98.
2 Serpell LC. Alzheimer's amyloid fibrils: structure and assembly. Biochim Biophys Acta 2000;1502:16–30.
3. Sunde M, Blake CCF. From the globular to the fibrous state: protein structure and structural conversion in amyloid formation. Quart Rev Biophys 1998;31:1–39.
4 Glenner GG. Amyloid deposits and amyloidosis. The β-fibrilloses (first of two parts). N Engl J Med 1980;302:1283–92.
5 Kelly JW. Alternative conformations of amyloidogenic proteins govern their behavior. Curr Opin Struct Biol 1996;6:11–7.
6 Rochet JC, Lansbury PT Jr. Amyloid fibrillogenesis: themes and variations. Curr Opin Struct Biol 2000;10:60–8.

7 Iconomidou VA, Vriend G, Hamodrakas SJ. Amyloid protect the silkmoth oocyte and embryo. FEBS Lett 2000;479:141–5.
8 Kenney JM, Knight D, Wise MJ, Vollrath F. Amyloidogenic nature of spider silk. Eur Z Biochem 2002;269:4159–63.
9 Atwood CS, Martins RN, Smith MA, Perry G. Senile plaque composition and posttranslational modification of amyloid-β peptide and associated proteins. Peptides 2002;23:1343–50.
10 Pepys MB, Herbert J, Hutchinson WL, Tennent GA, Lachmann HJ, Gallimore JR, et al. Targeted pharmacological depletion of serum amyloid P component for treatment of human amylodidosis. Nature 2002;417:254–9.
11 Booth DR, Sunde M, Bellotti V, Robinson CV, Hutchinson WL, Fraser PE, et al. Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. Nature. 1997;385:787–93.
12 Liemann S, Glockshuber R. Influence of amino acid substitutions related to inherited human prion diseases on the thermodynamic stability of the cellular prion protein. Biochemistry 1999;38:3528–67.
13 Zahn R, Liu A, Luhrs T, Riek R, von Schroetter C, Lopez Garcia F, et al. NMR solution structure of the human prion protein. Proc. Natl Acad Sci USA 2000;97:145–50.
14 Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, et al. Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci USA 1993;90:10962–6.
15 Barrow CJ, Yasuda A, Kenny PT, Zagorski MG. Solution conformations and aggregational properties of synthetic amyloid β-peptides of Alzheimer's disease. Analysis of circular dichroism spectra. J Mol Biol 1992;225:1075–93.
16 Kelly JW. The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. Curr Opin Struct Biol 1998;8:101–6.
17 Ramirez-Alvarado M, Merkel JS, Regan L. A sytematic exploration of the influence of the protein stability on amyloid fibril formation in vitro. Proc Natl Acad Sci USA 2000;97:8979–84.
18 Canet D, Last AM, Tito P, Sunde M, Spencer A, Archer DB, et al. Local cooperativity in the unfolding of an amyloidogenic variant of human lysozyme. Nat Struct Biol 2002;9:308–15.
19 Chiti F, Webster P, Taddei N, Clark A, Stefani M, Ramponi G, et al. Designing conditions for in vitro formation of amyloid protofilaments and fibrils. Proc Natl Acad Sci USA 1999; 96:3590–4.
20 Fändrich M, Fletcher MA, Dobson CM. Amyloid fibrils from muscle myoglobin. Even an ordinary globular protein can assume a rogue guise if conditions are right. Nature 2001; 410:165–6.
21 Dalal S, Regan L. Understanding the sequence determinants of conformational switching using protein design. Protein Sci 2000;9:1651–9.
22 Tjernberg L, Hosia W, Bark N, Thyberg J, Johansson J. Charge attraction and β propensity are necessary for amyloid fibril formation from tetrapeptides. J Biol Chem 2002;277:43243–6.
23 Reches M, Porat Y, Gazit E. Amyloid fibrils formation by penta- and tetrapeptide fragments of human calcitonin. J Biol Chem 2002;277:35475–80.
24 Tjernberg LO, Callaway DJE, Tjernberg A, Hahne S, Lilliehöök C, Terenius L, et al. A molecular model of Alzheimer amyloid β-peptide fibril formation. J Biol Chem 1999;274: 12619–25.
25 McParland VJ, Kalverda AP, Homans SW, Radford SE. Structural properties of an amyloid precursor of $\beta_2$-microglobulin. Nat Struct Biol 2002;9:326–31.
26 Hoshino M, Katou H, Hagihara Y, Hasegawa K, Naiki H, Goto Y. Mapping the core of the $\beta_2$-microglobulin amyloid fibril by H/D exchange. Nat Struct Biol 2002;9:332–6.
27 Jones S, Manning J, Kad NM, Radford SE. Amyloid-forming peptides from $\beta_2$-microglobulin-insights into the mechanism of fibril formation in vitro. J Mol Biol 2003;325:249–57.
28 Creuwels LA, van Golde LM, Haagsman HP. The pulmonary surfactant system: biochemical and clinical aspects. Lung 1997;175:139.
29 Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. N Engl J Med 2002;347:2141–8.
30 Johansson J. Structure and properties of surfactant protein C. Biochim Biophys Acta 1998;1408:161–72.
31 Pérez-Gil J, Keough KM. Interfacial properties of surfactant proteins. Biochim Biophys Acta 1998;1408:203–17.
32 Bradbury J. Could treatment of neonatal RDS improve further? Lancet 2002;360:394–5.
33 Augusto L, Le Blay K, Auger G, Blanot D, Chaby R. Interaction of bacterial lipopolysaccharide with mouse surfactant protein C inserted into lipid vesicles. Am J Physiol Lung Cell Mol Physiol 2001;281:L776–L785.
34 Augusto AL, Li J, Synguelakis M, Johansson J, Chaby R. Structural basis for interactions between lung surfactant protein C and bacterial lipopolysaccharide. J Biol Chem 2002;277: 23484–92.
35 Augusto AL, Synguelakis M, Johansson J, Pedron T, Girard R, Chaby R. Interaction of pulmonary surfactant protein C with CD14 and lipopolysaccharide. Infect Immun 2003;71:61–7.
36 Johansson J, Szyperski T, Curstedt T, Wüthrich K. The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a valyl-rich α-helix. Biochemistry 1994;33:6015–23.
37 Johansson J, Szyperski T, Wüthrich K. Pulmonary surfactant-associated polypeptide SP-C in lipid micelles: CD studies of intact SP-C and NMR secondary structure determination of depalmitoyl-SP-C(1–17). FEBS Lett 1995;362:261–5.
38 Szyperski T, Vandenbussche G, Curstedt T, Ruysschaert JM, Wüthrich K, Johansson J. Pulmonary surfactant-associated polypeptide C in a mixed organic solvent transforms from a monomeric α-helical state into insoluble β-sheet aggregates. Protein Sci 1998;7:2533–40.
39 Kovacs H, Mark AE, Johansson J, van Gunsteren WF. The effect of environment on the stability of an integral membrane helix: molecular dynamics simulations of surfactant protein C in chloroform, methanol and water. J Mol Biol 1995;247: 808–22.
40 Zangi R, Kovacs H, van Gunsteren WF, Johansson J, Mark AE. Free energy barrier estimation of unfolding the α-helical surfactant-associated polypeptide C. Proteins 2001;43:395–402.
41 Gustafsson M, Thyberg J, Näslund J, Eliasson E, Johansson J. Amyloid fibril formation by pulmonary surfactant protein C. FEBS Lett 1999;464:138–42.
42 Nogee LM, Dunbar AE, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N Engl J Med 2001; 344:573–9.
43 Thomas AQ, Lane K, Phillips III J, Prince M, Markin C, Speer M, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med 2002;165:1322–8.
44 Kabore AF, Wang WJ, Russo SJ, Beers MF. Biosynthesis of surfactant protein C: characterization of aggresome formation by EGFP chimeras containing propeptide mutants lacking conserved cysteine residues. J Cell Sci 2001;114:293–302.
45 Nogee LM. Abnormal expression of surfactant protein C and lung disease. Am J Respir Cell Mol Biol 2002;26:641–4.
46 Wang W-J, Russo SJ, Mulugeta S, Beers MF. Biosynthesis of surfactant protein C (SP-C). Sorting of SP-C proprotein involves homomeric association via a signal anchor domain. J Biol Chem 2002;277:19929–37.
47 Conkright JJ, Na C-L, Weaver TE. Overexpression of surfactant protein-C mature peptide causes neonatal lethality in transgenic mice. Am J Respir Cell Mol Biol 2002;26:85–90.
48 Glasser SW, Burhans MS, Korfhagen TR, Na CL, Sly PD, Ross GF, et al. Altered stability of pulmonary surfactant in SP-C-deficient mice. Proc Natl Acad Sci USA 2001;98:6366–71.
49 Johansson J, Nilsson G, Strömberg R, Robertson B, Jörnvall H, Curstedt T. Secondary structure and biophysical activity of synthetic analogues of the pulmonary surfactant polypeptide SP-C. Biochem J 1995;307:535–41.
50 Nilsson G, Gustafsson M, Vandenbussche G, Veldhuizen E, Griffiths WJ, Sjövall J, et al. Synthetic peptide-containing surfactants – Evaluation of transmembrane versus amphipathic helices and surfactant protein C poly-valyl to poly-leucyl substitution. Eur J Biochem 1998;255:116–24.
51 Kallberg Y, Gustafsson M, Persson B, Thyberg J, Johansson J. Prediction of amyloid fibril-forming proteins. J Biol Chem 2001;276:12945–50.
52 Tjernberg LO, Näslund J, Lindqvist F, Johansson J, Karlström AR, Thyberg J, et al. Arrest of β-amyloid fibril formation by a pentapeptide ligand. J Biol Chem 1996;271:8545–8.
53 Janek K, Rothemund S, Gast K, Beyermann M, Zipper J, Fabian H, Bienert M, Krause E. Study of the conformational transition of Ab(1–42) using D-amino acid replacement analogues. Biochemistry 2001;40:5457–63.
54 Hunt JF, Earnest TN, Bousché O, Kalghati K, Reilly K, Horváth C, et al. A biophysical study of integral membrane protein folding. Biochemistry 1997;36:15156–76.

55 Huang Z, Gabriel J-M, Baldwin MA, Fletterick RJ, Prusiner SB, Cohen F.E. Proposed three-dimensional structure for the cellular prion protein. Proc Natl Acad Sci USA 1994;91:7139–43.
56 Dima RI, Thirumalai D. Exploring the propensities of helices in PrPc to form β sheet using NMR structures and sequence alignments. Biophys J 2002;83:1268–80.
57 Knaus KJ, Morillas M, Swietnicki W, Malone M, Surewicz WK, Yee VE. Crystal structure of the human prion protein reveals a mechanism for oligomerization. Nat Struct Biol 2001;8:770–4.
58 Jackson GS, Hosszu LLP, Power A, Hill AF, Kenney J, Saibil H, et al. Reversible conversion of monomeric human prion protein between native and fibrilogenic conformations. Science 1999;283:1935–7.
59 Soto C, Castano EM, Kumar A, Beavis RC, Frangione B. Fibrillogenesis of synthetic amyloid-β peptides is dependent on their initial secondary structure. Neurosci Lett 1995;200:105–8.
60 Hosia W, Johansson J, Griffiths WJ. Hydrogen/deuterium exchange and aggregation of a poly-valine and a poly-leucine α-helix investigated by matrix-assisted laser desorption ionization mass spectrometry. Mol Cell Proteomics 2002;1:592–7.
61 Baskakov IV, Legname G, Prusiner SB, Cohen FE. Folding of prion protein to its native α-helical conformation is under kinetic control. J Biol Chem 2001;276:19687–90.
62 Chiti F, Taddei N, Baroni F, Capanni C, Stefani M, Ramponi G, Dobson CM. Kinetic partitioning of protein folding and aggregation. Nat Struct Biol 2002;9:137–43.
63 Taddei N, Capanni C, Chiti F, Stefani M, Dobson CM, Ramponi G. Folding and aggregation are selectively influenced by the conformational preferences of the α-helices of muscle acylphosphatase. J Biol Chem 2001;276:37149–54.
64 Ciani B, Hutchinson EG, Sessions RB, Woolfson DN. A designed system for assessing how sequence affects α to β conformational transitions in proteins. J Biol Chem 2002;277: 10150–55.
65 Liu LP, Deber CM. Uncoupling of hydrophobicity and helicity in transmembrane segments. α-Helical propensities of the amino acids in non-polar environments. J Biol Chem 1998;273: 23645–8.
66 Gustafsson M, Griffiths WJ, Furusjö E, Johansson J. The palmitoyl groups of surfactant protein C reduce unfolding into a fibrillogenic intermediate. J Mol Biol 2001;310:937–50.
67 Richardson JS, Richardson DC. Natural β-sheet proteins use negative design to avoid edge-to-edge aggregation. Proc Natl Acad Sci SA 2002;99,:2754–9.
68 Wang W, Hecht MH. Rationally designed mutations convert de novo amyloid-like fibrils into monomeric β-sheet proteins. Proc Natl Acad Sci USA 2002;99:2760–5.
69 Weissmann C. Molecular genetics of transmissible spongiform encephalopathies: an introduction. J Toxicol Sci 2002;27:69–77.
70 Price DL, Tanzi RE, Borchelt DR, Sisodia SS. Alzheimer's disease: genetic studies and transgenic models. Annu Rev Genet 1998;32:461–93.
71 Wang J, Dickson DW, Trojanowski JQ, Lee VM-Y. The levels of soluble versus insoluble brain Aβ distinguish Alzheimer's disease from normal and pathologic aging. Exp Neurol 1999;158: 328–37.
72 Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD. Correlation between elevated levels of amyloid β-peptide in the brain and cognitive decline. J Am Med Assoc 2000;283:1571–7.
73 Davis II JN, Chisholm JC. The "amyloid cascade hypothesis" of AD: decoy or real McCoy? Trends Neurol Sci 1997;20:558–9.
74 Dahlgren KN, Manelli AM, Stine Jr WB, Baker LK, Krafft GA, LaDu M.J. Oligomeric and fibrillar species of amyloid-β peptides differentially affect neuronal viability. J Biol Chem 2002;277:32046–53.
75 Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, et al. Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 1998;95:6448–53.
76 Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, et al. Amyloid β-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. J Biol Chem 1999;274:25945–52.
77 Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, et al. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. Nature 2002;416:535–9.
78 Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, et al. The "Arctic" APP mutation (E693G) causes Alzheimer's disease by enhanced Aβ protofibril formation. Nat Neurosci 2001;4:887–93.
79 El-Agnef OMA, Nagala S, Patel BP, Austen BM. Non-fibrillar oligomeric species of the amyloid ABri peptide, implicated in familial british dementia, are more potent at inducing apoptotic cell death than protofibrils or mature fibrils. J Mol Biol 2001;310:157–68.
80 Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, et al. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 2002;416: 507–11.
81 Lashuel HA, Hartley DM, Petre BM, Walz T, Lansbury PT Jr. Amyloid pores from pathogenic mutations. Nature 2002;418: 291.
82 Sacchettini JC, Kelly JW. Therapeutic strategies for human amyloid diseases. Nat Rev Drug Discov 2002;4:267–75.
83 Soto C, Sigurdsson EM, Morelli L, Kumar RA, Castano E, Frangione B. β-Sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy. Nat Med 1998;4:822–6.
84 Soto C, Kindy SM, Baumann M, Frangione B. Inhibition of Alzheimer's disease amyloidosis by peptides that prevent β-sheet conformation. Biochem Biophys Res Commun 1996;226: 672–80.
85 Chiti F, Taddei N, Stefani M, Dobson CM, Ramponi G. Reduction of the amyloidogenicity of a protein by specific binding of ligands to the native conformation. Protein Sci 2001;10: 879–86.
86 Peterson SA, Klabunde T, Lashuel HA, Purkey H, Sacchettini JC, Kelly JW. Inhibiting transthyretin conformational changes that lead to amyloid fibril formation. Proc Natl Acad Sci USA 1998;95:12956–60.
87 Voss T, Schäfer KP, Nielsen PF, Schäfer A, Maier C, Hannappel E, et al. Primary structure differences of human surfactant-associated proteins isolated from normal and proteinosis lung. Biochim Biophys Acta 1992;1138:261–7.
88 Fezoui Y, Teplow DB. Kinetic studies of amyloid β-protein fibril assembly: Differential effects of α-helix stabilization. J Biol Chem 2002;277:36948–54.
89 Kirkitadze MD, Condron MM. Teplow DB. Identification and characterization of key kinetic intermediates in amyloid β-protein fibrillogenesis. J Mol Biol 2001;312:1103–19.

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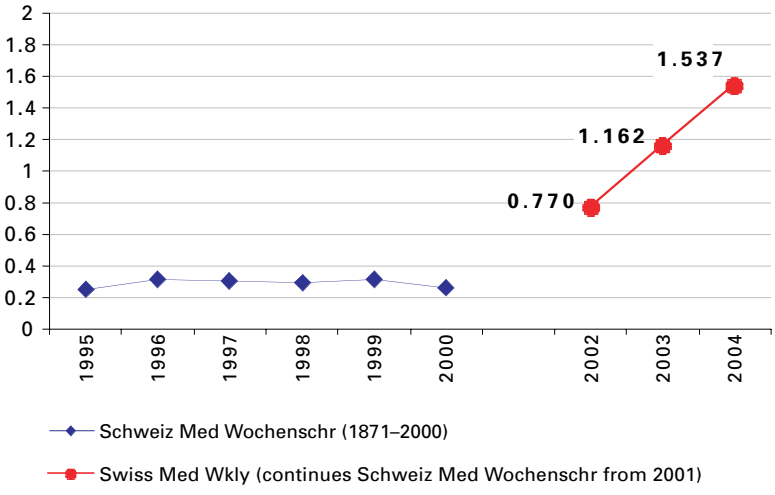


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