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Review

Proteolytic generation and aggregation of peptides from transmembrane regions: lung surfactant protein C and amyloid β-peptide

J. Johansson[a,*], T. E. Weaver[b] and L. O. Tjernberg[c]

[a] Department of Molecular Biosciences, Swedish University of Agricultural Sciences, The Biomedical Centre, Box 575, 751 23 Uppsala (Sweden), Fax: +46 18 550762, e-mail jan.johansson@vmk.slu.se
[b] Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229-3039 (USA)
[c] Karolinska Institutet and Sumitomo Pharmaceuticals Alzheimer Center (KASPAC), Neurotec, Novum, 141 57 Huddinge (Sweden)

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Abstract. The formation of amyloid fibrils is associated with several devastating diseases in humans and animals, including e.g. Alzheimer's disease (AD) and the spongiform encephalopathies. Here, we review and discuss the current knowledge on two amyloid peptides: lung surfactant protein C (SP-C) and the amyloid β-peptide (Aβ), implicated in human lung disease and in AD, respectively. Both these hydrophobic peptides are derived from the transmembrane region of their precursor protein, and can transit from a monomeric α-helical state to a β-sheet fibril. The α helices of SP-C and Aβ are composed of amino acid residues with inherently higher propensities for β strand than helix conformation. Their helical states are stabilized by a membrane environment, and loss of membrane association thus promotes structural conversion and fibril formation. We speculate that the loss of structural context for sequences with a high propensity for formation of β sheets may be a common feature of amyloid formation in general.

Key words. Aβ; SP-C; amyloid disease; protein conformation; aggregation.

Synthesis, processing and secretion of SP-C

Surfactant protein C (SP-C) is expressed by only one cell type in the body, the alveolar type II epithelial cell of the lung [1, 2]. Human SP-C is synthesized in the form of a 197-amino acid integral membrane proprotein (proSP-C) that is processed to the 35-amino acid mature peptide within the secretory pathway of the type II cell [3, 4] (fig. 1). The mature peptide (residues 24–58 of proSP-C) encodes a signal sequence that anchors the newly synthesized proprotein in the membrane of the endoplasmic reticulum (ER) orienting the C-terminal peptide (residues 59–197) in the ER lumen [5, 6]. Two-thirds of the 35-amino acid mature peptide resides in the transmembrane domain, while the remaining N-terminal portion is located in the cytosol. The latter domain is palmitoylated on adjacent cysteine residues and is flanked by an N-terminal propeptide (residues 1–23). Trafficking of proSP-C from the ER to the distal secretory pathway is dependent on the cytosolic N-terminal propeptide [6, 7]. In contrast, the lumenal C-terminal domain of proSP-C is dispensable for intracellular trafficking and secretion of SP-C.
Trafficking of proSP-C to the multivesicular body in the distal secretory pathway is necessary for processing to the mature peptide [3, 6]. Proteases in the lumen of the multivesicular body remove the C-terminal peptide from

* Corresponding author.

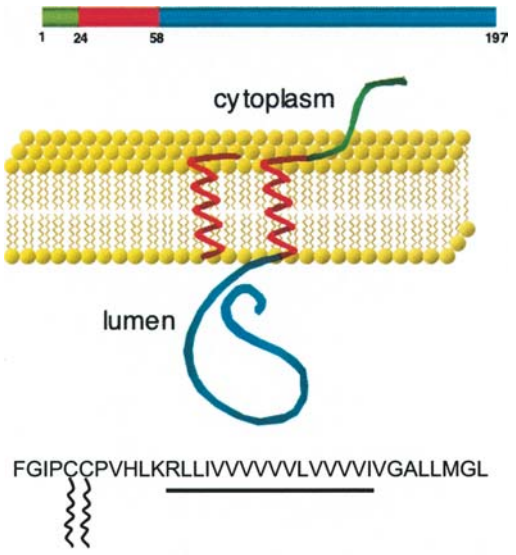

Figure 1. Proteolytic generation of SP-C. A linear presentation of proSP-C is shown at the top, with the part corresponding to the mature peptide (residues 24–58) marked in red, the N-terminal propeptide in green and the C-terminal propetide in blue. The middle figure shows a schematic presentation of proSP-C (right) and the mature SP-C peptide inserted in a phospholipid bilayer. The amino acid sequence of human SP-C in one-letter code is shown at the bottom. The wavy lines represent palmitoyl groups linked to Cys5 and Cys6, and the underlined part is the discordant stretch.

proSP-C. Cathepsin H has recently been implicated in this process [8], but the precise number and identity of proteases involved in maturation of proSP-C is not known. Inward budding of the limiting membrane of the multivesicular body results in the release of small SP-C-containing vesicles into the lumen [6, 9]. Fusion of the multivesicular body with a lamellar body (a secretory granule specialized for intracellular storage of surfactant) leads to incorporation of the internal SP-C-containing vesicles into the highly packed bilayer membranes of the lamellar body. The lipid bilayer contents of the lamellar body, including surfactant protein B (SP-B) and SP-C, are released into the alveolar airspace where they unravel and ultimately contribute to formation and maintenance of a phospholipid rich film (pulmonary surfactant) along the surface of the epithelium. The N-terminal propeptide, located in the cytosol or inside the internal vesicles following inward vesiculation of the multivesicular body limiting membrane, is inaccessible to lumenal proteases. In the absence of SP-B the N-terminal SP-C propeptide is not cleaved completely [10]. Thus SP-B may facilitate access of lumenal proteases to proSP-C by lysing internal vesicle membranes [9]. The membranolytic properties of SP-B in vitro are consistent with this hypothesis [11]. The clearance kinetics of SP-C from the airspaces are similar to those for SP-B, suggesting that they cotraffic in the recycling pathway [12]. Internalized SP-B has previously been shown to localize to endocytic vesicles, multivesicular bodies and lamellar bodies [13]. Collectively, these results suggest that the multivesicular body plays a critical role in surfactant homeostasis by integrating the surfactant biosynthetic and recycling pathways of the type II cell.

SP-C mutations and interstitial lung disease

Mutations in the SP-C gene have recently been linked to familial and sporadic interstitial lung disease (fig. 2). The index case was diagnosed with interstitial pneumonitis [14]. DNA sequence analysis of the SP-C gene identified a mutation of one allele that resulted in loss of 37 amino acids in the C-terminal part of proSP-C. A separate SP-C mutation, resulting in substitution of glutamine for leucine (SP-C^{L188Q}) in the C-terminal propeptide, was recently identified in an extended kindred [15]. Affected family members exhibited variable onset of disease and variable phenotype. More recently, missense, splice or frameshift mutations were identified in 11 infants with chronic lung disease of unknown etiology [16]. More than half of these infants had a family history of disease, and one infant carried a mutation not present in either parent. Therefore, both sporadic and familial interstitial lung disease are associated with mutations in the SP-C gene.

To date, all SP-C mutations except one (SP-C^{P30L}) map to the C-terminal part of proSP-C. Since removal of the C-terminal propeptide does not affect intracellular trafficking [6], it is possible that retention in the ER is caused by misfolding of proSP-C. Consistent with this hypothesis,

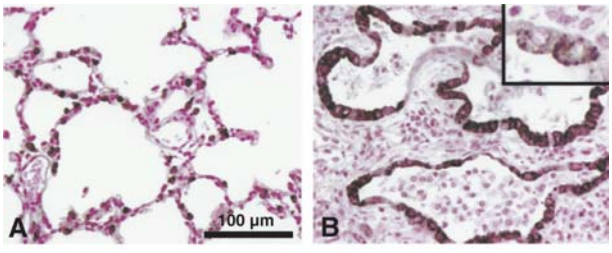

Figure 2. ProSP-C mutation and lung disease. Immunohistochemical analysis of proSP-C expression in normal human lung tissue (*A*) and in a patient with the P30L mutation in one allele of the SP-C gene (*B*). Robust immunostaining for proSP-C was detected in the alveolar type II cells of both lung samples. Inset shows a higher magnification of proSP-C staining of lamellar bodies in the cytoplasm of the alveolar type II cells found in the P30L mutation. Note that the histopathology of the lung in the P30L mutation is typical of advanced, chronic, interstitial lung disease with markedly thickened alveolar septa and hyperplastic alveolar epithelium composed of hypertrophic type II cells. Accumulation of alveolar macrophages in the airspaces is also frequently found in the SP-C mutations. Panels *A* and *B* are shown at identical magnifications. Material and figure provided by Dr Larry Nogee, Johns Hopkins University, Baltimore, MD, and Dr. Susan Wert, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.

mature SP-C was not detected in the index patient. In every case to date SP-C mutations have been detected on only one allele, and the amount of normal proSP-C is significantly reduced in association with SP-C mutations [14, 16]. This suggests that the affected allele produces a dominant-negative proSP-C that traps the product of the wild-type allele in the ER. ProSP-C forms oligomers [17], and mutant proprotein may sequester wild-type protein, leading to accumulation and degradation early in the biosynthetic pathway.

Several independent studies support a cause-effect relationship between mutations in the SP-C gene and interstitial lung disease (ILD). Cells transfected with the SP-C^{L188Q} mutant exhibited slower growth and evidence of cytotoxicity [15]. Cotransfection of cells in culture with a C-terminally truncated proSP-C and wild-type proSP-C resulted in trapping of both proteins in juxtanuclear complexes resembling aggresomes [18]. Expression of the SP-C peptide in the absence of both flanking domains in type II cells of transgenic mice resulted in a profound disruption of lung morphogenesis, leading to respiratory failure at birth [19]. A similar phenotype was observed in transgenic mice expressing C-terminally truncated proSP-C [20]. Importantly, lung dysmorphogenesis was associated with high levels of transgene expression and occurred in the presence of two wild-type SP-C alleles. These results suggest that mutations in the SP-C gene lead to aggregates of misfolded protein that induce cell death during lung development. Lower levels of mutant protein may cause a correspondingly milder phenotype, leading to ILD observed in human patients. Chronic ER stress related to retention of unfolded/misfolded protein may lead to apoptosis, for review see [21–23]. This pathway may underlie disrupted lung morphogenesis in transgenic mice expressing SP-C^{24-58} or C-terminally truncated proSP-C.

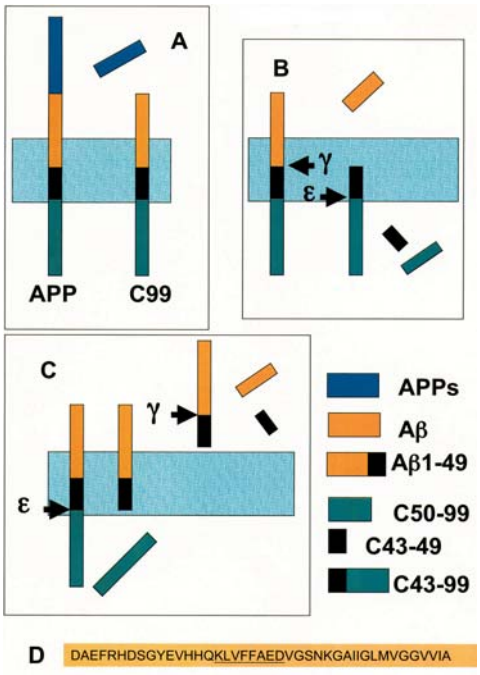

Figure 3. Generation of Aβ from APP. The type I membrane protein APP can be processed to generate Aβ, or in a non-amyloidogenic pathway (not shown). The membrane is depicted as a light-blue box, where the lumenal side faces upwards. In the amyloidogenic pathway, the first cut is mediated by BACE (*A*). This cleavage generates soluble APP (APPs) and a membrane-bound 99-residue fragment, C99. The next step, γ- or ε-cleavage, is presenilin dependent. The sequential order is not known, and at least two possibilities exist (*B* and *C*). In (*B*), C99 is first γ-cleaved in the middle of the transmembrane region, generating Aβ42 (or A40). The corresponding C-terminal fragment is then ε-cleaved, producing the AICD (C50–99) and a short fragment (C43–49). In (*C*), C99 is first ε-cleaved close to the cytosol. The scissile bond could in this case (depending on the thickness of the membrane) be exposed to water. Next, the resulting Aβ1–49 is pulled out of the membrane and γ-cleaved. No proteolysis occurs in the membrane in this alternative. (*D*) The amino acid sequence of human Aβ1–42 in one letter code, with the discordant stretch underlined.

Processing of the amyloid precursor protein (APP)

The aggregation of the 40- to 42-residue amyloid β-peptide (Aβ) into toxic species is of importance in Alzheimer´s disease (AD). Elevated levels of Aβ in the brain are associated with AD, and transgenic mice overexpressing human APP develop AD-like lesions [24, 25]. Aβ is generated from its precursor, the 695- to 770-residue APP, by the sequential activities of β-secretase and γ-secretase (fig. 3) (reviewed in [26]). APP is suggested to be involved in neurite growth, plasticity and cell adhesion, but the physiological role for Aβ is unknown [27]. β-Secretase was identified at the end of the last century and dubbed beta-site APP cleaving enzyme (BACE) [28–31]. This enzyme cleaves APP on the lumenal side, generating soluble APP and a 99-residue membrane-bound fragment called C99 (fig. 3A). The nature of the γ-secratase has been more elusive, but this year it was shown that four components are necessary and sufficient: presenilin, nicastrin, Aph-1 and Pen-2 [32, 33]. This complex cleaves not only APP, but also other type I membrane proteins such as Notch [34]. Interestingly, the cleavage appears to take place in the membrane and belongs to a new concept called regulated intramembrane proteolysis (RIP) [35]. Presenilin seems to be the component that mediates the actual cleavage generating Aβ40 and Aβ42, critically depending on two aspartyl residues [36, 37]. Recently it was shown that APP also is cleaved closer to the cytosol, between residues 49 and 50 (Aβ numbering), in a presenilin-dependent manner called ε-cleavage (fig. 3B, C) [38]. This activity generates an APP intracellular domain (AICD) that translocates to the nucleus. The Notch receptor is processed in a similar way, and the intracellular domain of Notch is a transcription factor. Therefore, it has been suggested that also AICD is a tran-

scription factor [39]. It is not known whether γ-secretase cleavage precedes ε-cleavage or vice versa. If the ε-cleavage occurs first, membrane-bound Aβ1-49 could be pulled out and then further processed to Aβ40/42 (fig. 3C). Thus, the γ-cleavage could take place in the lumen, and intramembrane proteolysis would not be required.

The processing of APP occurs at several different locations; the ER, the Golgi apparatus, at the cell surface and in the endosomal/lysosomal compartment [40–43]. It is possible that certain organelles generate more toxic Aβ species than others. For example, the ratio Aβ42/40 is high in the ER, which like lysosomes contains aggregated Aβ [44-46]. Not only Aβ, but also its precursor C99, has a strong tendency to aggregate and form fibrils. It is possible to generate Aβ-like peptides by nonspecific digestion of fibrils formed by C99 [46, 47]. Thus, toxic Aβ species might be generated in specific compartments and in a presenilin-independent pathway [48, 49]. It is possible that APP, as well as C99, to some extent can oligomerize while still inserted in the membrane and thereby affect processing [50]. The proteolytic degradation of Aβ has also been studied, and several candidate proteases are emerging. Of these, neprilysin and insulin degrading enzyme seem to be most relevant [51]. Pharmacological inhibition of neprilysin in rat, as well as knockout of the neprilysin gene in mice, result in brain amyloid accumulation [52, 53].

Amyloid fibril formation by SP-C

SP-C is the smallest of the surfactant proteins and it is very hydrophobic, not only from the presence of the two fatty acyl chains but also from the fact that 28 out of the 35 residues are aliphatic, with a pronounced dominance for valine (fig. 1). The poly-valine part forms an α helix perfectly matching the size of a fluid bilayer composed of dipalmitoylphosphatidylcholine, strongly suggesting that SP-C is a transmembrane peptide [54]. SP-C contains unexpectedly many valine and isoleucine residues for being a helical polypeptide, as these residues are branched at the β-carbon and therefore disfavour α helix formation. No explanation is available as to why SP-C has such an unusual molecular architecture; few other transmembrane helices are composed of almost exclusively a poly-valine stretch. One possibility is that the rigidity of the poly-valine helix is required for SP-C function and cannot be mimicked by amino acid sequences usually found in transmembrane helices.

Although the helical structure of SP-C is stable long term when inserted into lipid micelles or membranes, it is metastable in aqueous organic solvents [55]. Once it forms a nonhelical structure, it does not refold but forms β-sheet aggregates. 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 [55-57]. The structural conversion of SP-C from α-helix to β-sheet aggregates results in formation of amyloid fibrils [58]. A summary of our current understanding of the conversion from monomeric α-helical SP-C into fibrils is afforded in figure 4. In this model monomeric α-helical SP-C converts irreversibly to a nonhelical intermediate that rapidly aggregates into higher-order structures which eventually form fibrils. It is likely that removal of SP-C from the membrane promotes aggregation, but high concentrations of SP-C in solution can also form β-sheet aggregates in the presence of membrane lipids [59]. The irreversible nature of the first step is deduced from the lack of detectable hydrogen/deuterium exchange in the central part of the SP-C helix, as observed by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry [55, 60]. The proposal that unfolding of the helix precedes aggregation is in line with the observed first-order kinetics of disappearance of helical SP-C. Formation of SP-C fibrils is not limited to in vitro situations, but occurs in association with pulmonary alveolar proteinosis (PAP). In bronchoalveolar fluid from PAP patients, but not from healthy controls, abundant insoluble SP-C aggregates which exhibit the characteristic properties of amyloid by Congo red staining and electron microscopy can be isolated [58]. Whether these fibrils are formed as a result of altered intraalveolar metabolism in PAP, or are involved in the etiology of PAP remain to be established.

Removal of the palmitoyl groups of SP-C reduces the mechanical stability and surface activity of SP-C/lipid mixtures [61, 62]. Moreover, removal of one or both of the palmitoyl groups of SP-C in vitro destabilizes the peptide and results in faster unfolding of the α helix [63]. Dipalmitoylated SP-C forms fibrils within days, whereas SP-C in which the palmitoyl groups have been removed forms fibrils within hours. From NMR data a hydrogen bond between the carbonyl oxygen of the palmitoyl group linked to Cys6 and the amide proton of Leu10 was proposed [55]. These data together indicate that the acyl chains stabilize helical SP-C, and that small differences in helix stability can influence fibril formation in vitro.

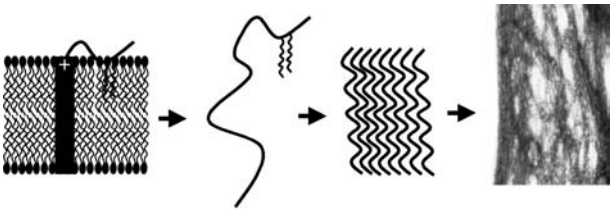

Figure 4. Fibril formation by SP-C. In its native state, SP-C is a membrane-bound α helix. After unfolding SP-C does not refold but aggregates into β-sheet polymers and amyloid fibrils. The electron micrograph shows fibrils, composed predominantly of SP-C, isolated from a patient with pulmonary alveolar proteinosis.

SP-C in the PAP-associated fibrils is partly nonpalmitoylated, while it is dipalmitoylated in bronchoalveolar lavage fluid from healthy individuals [58, 64]. The increased amount of nonpalmitoylated SP-C found in PAP-associated fibrils could be due to its increased rate of transformation from an α-helical monomeric state into aggregated β sheet. Removal of the labile thioester-bound SP-C palmitoyl groups in vivo may thus destabilize the peptide, leading to increased aggregation, which potentially could be important in a variety of lung diseases. The disease-associated mutations in the C-terminal flanking domain of proSP-C described above lead to severe alterations in protein trafficking and trapping of proSP-C in the ER. Since retention in the ER is expected to prevent palmitoylation of proSP-C, this may lead to reduced stability of proSP-C. It is likewise conceivable that the metastable nature of mature SP-C affects the stability of proSP-C, and it is possible that the flanking proSP-C domains are important for preventing aggregation of the part corresponding to the mature peptide during synthesis, folding and processing. These issues appear to be important topics for further experiments.

Aβ aggregation

Extracellular amyloid deposits of Aβ are the hallmark of AD. Aβ is expressed in most cells, but the Aβ plaques are only found in the brain. The in vitro aggregation of Aβ into fibrils is highly concentration dependent, and formation of an oligomeric seed has been suggested to be the rate-limiting step [65]. The endogenous levels of *A*β are magnitudes lower than those used for in vitro polymerization studies, and the mechanism for in vivo polymerization of Aβ is still unclear. Several different factors, such as high local concentration of Aβ, interaction with membranes, metal ions, low pH or interaction with other proteins (pathological chaperones) could promote in vivo polymerization [66].

Several different proteins have been shown by immunostaining to be associated with amyloid plaques. Of these, α1-antichymotrypsin and apolipoprotein E (ApoE) are among the most extensively studied. Their effect on Aβ aggregation is not fully understood, but studies in transgenic mice overexpressing α1-antichymotrypsin or ApoE show that they increase the amyloid burden [67, 68]. Serum amyloid P is a plaque component found in all amyloidoses. In this case, the protein seems to be important for maintenance of the plaque, rather than affecting the initial polymerization [69, 70]. The second AD plaque component to be sequenced was NAC (non-Aβ component of AD amyloid), later identified as α-synuclein [71]. This protein is the main constituent of Lewy bodies, aggregates that are found in several neurodegenerative disorders, including AD and Parkinson's disease [72]. The presence of α-synuclein in amyloid plaques is debated, and it is possible that copurification of Lewy bodies and amyloid plaques complicate interpretations.

Different proteoglycans are associated with the amyloid plaques. Negatively charged groups in the glycosaminoglycan chain seem to be more important than the core protein for the interaction with Aβ. In the case of heparan sulfate, the length of the sugar polymer, and the number and position of the sulfate groups are critical for interaction with Aβ. The saccharide moiety is sufficient for affecting Aβ polymerization, likely by interactions between the sulfate groups and positively charged side chains in Aβ. Under certain conditions, Aβ and heparan sulfate form spherical structures with the same tinctorial properties as ex vivo plaque cores [73, 74]. Possibly, heparan sulfate acts as a scaffold and affects the ordering of the amyloid fibrils, and inhibiting these interactions by short saccharides has been suggested as amyloid therapeutics [75]. Also, the interaction between Aβ and phospholipids has an electrostatic component. In addition, hydrophobic interactions are involved, and it is not clear whether the peptide inserts into the membrane or acts at the surface. There are several possible mechanisms for the increased polymerization of Aβ in the presence of the phospholipids. One is increased Aβ concentration at the lipid surface, another is that the lipids induce conformational changes in Aβ and a third possibility is that they favour the alignment of Aβ molecules in an amyloid-promoting way [66, 76].

Metal ions are found in plaques, and transgenic mice expressing human APP show a reduced amyloid burden after treatment with metal chelators [77, 78]. It is possible that metal ions coordinate the histidine residues in Aβ and induce a conformational change. Aβ aggregation is increased at low pH (as in endosomes and lysosomes), and in this case protonation of the histidines may affect aggregation. Thus, there are several possibilities for Aβ to form aggregates despite its seemingly low concentration in vivo.

Numerous studies have shown that Aβ becomes neurotoxic upon polymerization. Early studies indicated that fibrils were the toxic species [79], but recent work shows that soluble polymeric aggregates are the toxic species [80, 81]. The use of different systems for studying toxicity may contribute to the discrepancy, as could the fact that it is difficult to isolate a certain aggregation state of Aβ. Low molecular weight aggregates are in rapid equilibrium with monomers and dimers, and fibrils are also dynamic structures [82, 83]. Interestingly, soluble oligomers may be the primary toxic species of amyloids in general [84]. An antibody recognizing oligomeric, but not fibrillar or monomeric A, inhibited toxicity in vitro [85]. This antibody also recognized oligomers of several other amyloidogenic polypeptides and inhibited their in vitro toxicity. Thus, amyloidogenic oligomers have a

common sequence-independent epitope and it is possible that they also share a common mechanism of toxicity.

It is of interest to note that mutations linked to AD either increase the amount of the longer, more hydrophobic and more amyloidogenic Aβ42 [86], or give rise to a peptide with altered amino acid sequence. In the first case, the favoured hypothesis is that more Aβ42 increases the rate of aggregation. In contrast, the residue substitutions apparently alter peptide aggregation characteristics and give rise to different pathologies, in most cases with a more pronounced vascular component [87, 88]. These mutations are all located to the central part of Aβ, A21G, E22G, E22Q, E22K and D23N. The mutations either increase the flexibility (A-G, E-G), increase the hydrophobicity (E-Q and D-N) or bring the net charge closer to zero (E-G, E-Q, E-K and D-N). It is not clear why these mutations render a vascular localization of the amyloid. The arctic mutation, E22G, stabilizes a toxic intermediate structure called protofibrils [88]. Thus, single substitutions in the Aβ sequence can alter the localization of the amyloid deposits, the rate of polymerization and the type of aggregates formed. A green fluorescence protein-A fusion construct was used for an unbiased search for sequence determinants of Aβ amyloidogenesis. Amino acid substitutions that prevented aggregation of Aβ clustered mainly in the regions covering residues 17–19, 31–32, 34–36, and 39–42 [89]. Most of the mutations replaced unpolar residues with polar, suggesting that solubility is one key determinant for Aβ aggregation. However, replacements not predicted to enhance solubility, e.g. V18A, F19L, I32V, were also found to reduce aggregation. V → A and F → L may influence aggregation by decreasing the β-strand propensity (see further below).

Molecular mechanisms in amyloid fibril formation

Amyloid fibril formation is promoted under conditions where conformations present during unfolding are well populated. The process of fibril formation may include local unfolding of the native protein, or dissociation of a multimeric protein, leading to exposure of regions which are prone to self-associate, intermolecular association leading to formation of oligomers, further structural rearrangement leading to increased β-sheet content and acquisition of a cross-β-sheet structure and polymerization into fibrils.

The ability to form amyloid fibrils is not limited to the proteins that form amyloid in human and mammal disease. Similar fibrils can be formed in vitro from virtually any protein under partly denaturing conditions and at high concentrations [90, 91]. In vitro fibril formation of a globular protein and of short peptides can be modulated by changes in the amino acid sequence [92–94]. Although many peptide sequences form a cross-β-sheet structure under certain conditions, the ability to do so under physiological conditions is apparently limited to a few proteins. This raises the question of what distinguishes the small number of proteins that form amyloid under physiological conditions.

Synthetic peptides with the SP-C amino acid sequence, which has a high β-strand propensity, are inefficient in helix formation and form insoluble aggregates. Replacing the poly-valine sequence of SP-C with sequences with high helical propensities yields helical peptides [54, 95]. Thus α helices for which β strands are predicted may be prone to undergo α helix → β strand transition and amyloid formation. Sequences seven residues or longer showing discordance between experimentally determined α helices and predicted β strands were found in 3% of nonhomologous proteins [96]. These α/β discordances include helix 2 of the prion protein, Aβ (residues 16–23), and SP-C (residues 12–27). The native states of the prion protein and SP-C are metastable [55, 97]. The discordant helix of SP-C contributes to the metastability, while for the more complex prion protein it is not established to what extent helix 2 contributes to the metastability. Residues 16–20 of Aβ are essential for Aβ intermolecular contacts and fibril formation, and destabilization of a helix covering residues 11–24 (in particular residues 17–24) is critical for α-helix → β-sheet conversion and fibril formation [98, 99].

Secondary structure predictions indicate that by replacing valines in the SP-C discordant helix with leucines should give stable α-helical peptides [96]. Such a peptide forms an α helix experimentally and does not form β-sheet aggregates or fibrils [60]. Likewise, the ability of Aβ to form fibrils can be modulated by residue exchanges that favour helix formation. For example, the mutations K16A/L17A/F20A [98] and V18A [100] abrogate fibril formation in vitro and these changes reverse the discordant nature of the 16–23 region of Aβ. Moreover, placement of residues that favour β-strand conformation (F or V) in the X positions in the tetrapeptide KXXE promotes fibril formation, while L or A in the same positions give random coil structure and no fibril formation [93]. Also, in short oligopeptide segments derived from the fibril-forming peptide calcitonin, F favours fibrillation and A prevents it [94].

For both Aβ and SP-C, removal of the discordant nature by residue replacements thus abrogates fibril formation in vitro, suggesting that increasing the helical occupancy can reduce the tendencies of fibrillogenic polypeptides to form amyloid fibrils. This hypothesis is supported by the findings that optimal stabilization of helical Aβ by addition of trifluoroethanol prevents fibril formation [101]. However, partial stabilization of the helical structure of Aβ instead accelerates formation of β-sheet aggregates and fibrils, and transient formation of the α-helix structure from unstructured Aβ was observed before forma-

tion of β-sheet structure and fibrils [102]. This led the authors to suggest that partially helical forms of Aβ may be on-pathway to fibril formation. In these studies full-length Aβ peptides were used, in which both the central and the C-terminal parts can form helical structures. It should be noted that addition of trifluoroethanol will favour peptide hydrogen bonding in general. Further studies are required to determine how stabilization of different helices in Aβ affects fibril formation.

Conclusions and open questions

A growing number of proteins have been shown to be able to lose their native structure and form insoluble amyloid fibrils. This is associated with a number of severe diseases but can also occur in vitro under partly denaturing conditions. Proposed determinants underlying metastability and fibril formation include solubility, charge state and electrostatic interactions, hydrophobic interactions, destabilizing mutations, specific side-chain interactions and conflicts in tertiary structures and local secondary structure propensities. Similarities between Aβ and SP-C regarding membrane localization and processing suggest that removal of peptides from their membrane-associated states can result in loss of a stabilizing environment with concomitant aggregation and fibril formation. SP-C and Aβ clearly represent special cases and differ in amino acid sequence and site of expression, but the apparent similarities in the mechanisms underlying fibril formation of these two polypeptides suggest that loss of stabilizing interactions may be involved in fibril formation of other polypeptides as well. The loss of a stabilizing environment can be caused by different factors such as (i) proteolysis and release of fibrillogenic peptides from nonmembrane regions; (ii) exposure of hydrophobic regions and unpaired β strands as a result of local unfolding and (iii) loss of protein subunit interactions, as exemplified by dissociation of the transthyretin tetramer into monomers [103, 104]. In the latter case certain mutations shift the equilibrium from the native tetramer, resulting in a loss of stabilizing structural context for the liberated monomers, which in turn can form fibrils.

Many different factors affect fibril formation, among those the primary structure of the aggregating peptide. Since the amyloid fibrils and oligomers seem to be homogenous in terms of protein composition, the side chains are important for selection of the polypeptides to be incorporated. However, the only true common denominator of amyloid-forming peptides is the peptide backbone. Oligomers formed by several polypeptides share a common antibody epitope. Also in this case the only common denominator appears to be the polypeptide backbone. Since the antibody was reactive only to oligomers and not to fibrils or monomers, the conformation of the backbone differs between these entities. Fibrils have a common β sheet structure. Thus, both amyloidogenic oligomers and fibrils have specific and different structures which are dictated by the backbone. These features suggest that a common approach could be used for prevention and treatment of amyloid disease. Alternatively, specific stabilization of the native non-fibrillogenic conformations, rather than blocking peptide polymerization, may be explored as a means to prevent amyloid diseases.

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