Current concepts on the pathogenesis of systemic amyloidosis

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Abstract. Amyloidosis is a pathological condition in which protein is deposited extracellularly in the form of insoluble fibrils that lead to organ dysfunction and death. Many different types of proteins are known to form amyloid and cause a heterogeneous array of clinical conditions. The unifying aspect of these conditions is the common structural entity resulting from the assembly of a primarily \( \beta \)-structure protein into 5–10 nm wide non-branching insoluble fibrils displaying the characteristic green birefringence of bound Congo red dye when viewed under polarized light. Several factors contribute to amyloid assembly. Certain biophysical characteristics of the amyloidogenic precursor influence amyloidogenicity. Any mutation that sufficiently decreases protein stability favours the formation of a partially folded state under physiological conditions. This intermediate exposes other key sequence elements to the solvent, i.e. hydrophobic or charged residues that decrease solubility and promote aggregation and ultimately amyloid formation. In addition to primary protein structure, which confers a susceptibility to amyloid formation, other elements are probably important for the initiation, development and persistence of amyloid deposits: proteoglycans, amyloid P component, apolipoprotein E and others, most of which are normal constituents of basement membranes. The role of these factors in amyloidogenesis has been studied in two major systemic amyloidoses with prominent renal involvement: light-chain and \( \beta \)-2-microglobulin. A detailed understanding of the molecular processes leading to amyloid deposition is required for the development of effective therapies.

Key words: amyloid protein; \( \beta \)-2-microglobulin; immunoglobulin light chains; pathogenesis; protein folding/unfolding

Introduction

Amyloidosis is a rare disorder of protein conformation and metabolism that results in tissue deposition of insoluble fibrils that cause organ dysfunction and death. Amyloid fibrils present a common ultrastructure: rigid non-branching fibrils of variable length, 5–10 nm wide. Each fibril is composed of two or more filamentous subunits \( \sim 3 \) nm in diameter, running in parallel and twisting about each other. Polypeptide subunits in the \( \beta \)-pleated-sheet conformation are polymerized within each filament with an antiparallel orientation [1,2]. Many different types of proteins are known to form amyloid and the \( \beta \)-pleated-sheet conformation is widely represented in several amyloid precursor proteins such as immunoglobulins, transthyretin (TTR) and \( \beta \)-2-microglobulin. The ordered structure of the fibrils forms the basis of the diagnostic criteria currently used to define amyloid: (i) birefringent staining with Congo red [3,4]; (ii) unbranched fibrillar morphology as determined by electron microscopy [5]; and (iii) observation of a characteristic X-ray fibre diffraction pattern (spacing and orientation of the major reflections: 4.7 \( \AA \) on the meridian and 5–10 \( \AA \) on the equator) [6,7].

Numerous, apparently non-related, proteins and peptides are a major component of amyloid fibrils in clinically diverse conditions. A steadily increasing number of such proteins have been characterized and classified by structural analysis of the fibril proteins and/or the genes coding for them. An exhaustive classification and listing of these proteins has been published [8]; an abridged version is listed in Table 1, which includes the two recently reported forms caused by lysozyme and fibrinogen Aa chain variants. The clinical and biochemical diversity of amyloidosis is evident from this table.

Despite the heterogeneity of the clinical presentation, biochemical composition and functional aspects of the various protein precursors, the unifying aspect of these conditions is the common structural entity we call amyloid: the 5–10 nm wide non-branching fibrils composed of polypeptide in the \( \beta \)-pleated-sheet conformation. This final end-product is probably the result of diverse pathogenetic mechanisms in view of
This assumption derives from several observations. Very few individuals with inflammation actually develop reactive amyloid. The Swedish form of the familial amyloidotic polypeuropathy caused by the TTR Val30 → Met variant has a late onset [12,13], while the Portuguese [14] and Japanese [15] forms of the disease caused by the same TTR variant present an earlier age of onset (by ~25 years) with a more rapid progression; the clinical phenotype of apolipoprotein AI variants Arg26 [16–18] and Arg60 [19] may vary considerably. This confirms that although the amyloidogenic variant protein is necessary for disease expression in hereditary amyloidosis syndromes, additional, as yet undetermined, genetic and/or environmental factors are clearly important determinants of the timing, distribution and effects of amyloid deposition.

Besides the fibrillar protein, there are other common constituents in the amyloid deposits, namely serum amyloid P (SAP) component [20], glycosaminoglycans (GAGs) and proteoglycans [21,22], which interact with amyloidogenic precursors. These components have been found to be normal constituents of basement membranes [23]. Several other basement membrane proteins, namely laminin, entactin, collagen IV and fibronectin, have been shown to be deposited coincidentally with AA amyloid protein [24]. Amyloid P component accounts for up to 15% of the amyloid deposit and is identical to and derived from the normal circulating plasma protein, SAP component, a member of the pentraxin family of proteins to which C-reactive protein belongs [20,25]. Amyloid P component is synthesized by the liver; it is composed of 10 glycosylated polypeptide subunits, each with a mol. wt of 23 500 daltons, arranged as two pentamers. It is a normal component of the basement membrane [26] and is a

### General mechanisms of amyloidogenesis

An adequate pool of amyloidogenic protein is clearly necessary. If the precursor has a very low amyloidogenic potential, it will require considerable time before triggering amyloid deposition (this may be the case of TTR in senile cardiac amyloidosis, or will require a significant increase in concentration (as is the case of β2-microglobulin in chronic haemodialysis and certain monoclonal light chains). Point mutations resulting in amino acid substitution can create or increase the amyloidogenic potential of the precursor; this is the case for the numerous TTR variants and for immunoglobulin light chains. Most of the information regarding the structure of amyloid subunit proteins has been generated with hereditary syndromes. For example, the TTR model has been extensively investigated from biochemical, genetic and structural (10,11) points of view and has provided important insights into amyloidogenesis.

In addition to the amyloidogenic primary protein structure that confers susceptibility for determining amyloidosis, other elements are probably important for the initiation and development of amyloid deposits. This assumption derives from several observations. Very few individuals with inflammation actually develop reactive amyloid. The Swedish form of the familial amyloidotic polypeuropathy caused by the TTR Val30 → Met variant has a late onset [12,13], while the Portuguese [14] and Japanese [15] forms of the disease caused by the same TTR variant present an earlier age of onset (by ~25 years) with a more rapid progression; the clinical phenotype of apolipoprotein AI variants Arg26 [16–18] and Arg60 [19] may vary considerably. This confirms that although the amyloidogenic variant protein is necessary for disease expression in hereditary amyloidosis syndromes, additional, as yet undetermined, genetic and/or environmental factors are clearly important determinants of the timing, distribution and effects of amyloid deposition.

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### Table 1. Nomenclature and classification of human amyloid and amyloidosis

<table>
<thead>
<tr>
<th>Amyloid protein</th>
<th>Protein precursor</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>SAA</td>
<td>Reactive (secondary), familial Mediterranean fever, familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome)</td>
</tr>
<tr>
<td>AL</td>
<td>κ and λ light chains</td>
<td>Idiopathic (primary), myeloma- or macroglobulinemia-associated</td>
</tr>
<tr>
<td>AH</td>
<td>Ig heavy chain</td>
<td>Idiopathic (primary)</td>
</tr>
<tr>
<td>ATTR</td>
<td>Transthyretin and transthyretin variants</td>
<td>Familial amyloid polyneuropathy, familial amyloid cardiomyopathy, systemic senile amyloidosis</td>
</tr>
<tr>
<td>APO</td>
<td>apoA1 variants (Arg26 and Arg60)</td>
<td>Familial amyloidosis, Finnish</td>
</tr>
<tr>
<td>AGel</td>
<td>Gelsolin variants (Asn187 and Tyr187)</td>
<td>Hereditary cerebral hemorrhage with amyloidosis, Icelandic</td>
</tr>
<tr>
<td>ACys</td>
<td>Cystatin-C variant (Gln188)</td>
<td>Hereditary amyloidosis, Finnish</td>
</tr>
<tr>
<td>Aβ2M</td>
<td>β2-microglobulin</td>
<td>Associated with chronic dialysis</td>
</tr>
<tr>
<td>AB</td>
<td>b protein precursor, β protein variant (Gln618)</td>
<td>Alzheimer’s disease, Down’s syndrome</td>
</tr>
<tr>
<td>AP2M</td>
<td>Prion proteins and variants</td>
<td>Hereditary cerebral haemorrhage with amyloidosis, Dutch</td>
</tr>
<tr>
<td>AIAPP</td>
<td>Islet amyloid polypeptide (amylin)</td>
<td>Creutzfeldt–Jacob disease</td>
</tr>
<tr>
<td>AANF</td>
<td>Atrial natriuretic factor</td>
<td>Gerstmann-Sträussler-Scheinker syndrome, etc.</td>
</tr>
<tr>
<td>ACal</td>
<td>(Pro)calcitonin</td>
<td>In islets of Langerhans, diabetes type II, insulinoma</td>
</tr>
<tr>
<td>ALys</td>
<td>Lysozyme variants (Thr56 and His67)</td>
<td>Isolated atrial amyloid</td>
</tr>
<tr>
<td>APrP</td>
<td>In medullary carcinomas of the thyroid</td>
<td></td>
</tr>
<tr>
<td>AANF</td>
<td>Lysozyme variants (Thr56 and His67)</td>
<td>Hereditary non-neuropathic systemic amyloidosis</td>
</tr>
<tr>
<td>APrP</td>
<td>Lysozyme variants (Thr56 and His67)</td>
<td>Hereditary non-neuropathic renal amyloidosis</td>
</tr>
</tbody>
</table>

the clinical and biochemical diversity of amyloidosis; however, some common elements are recognizable in the midst of this variability and are derived from lessons learned about one form of amyloid that have been found to hold true for other forms of amyloid as well [9].

### General mechanisms of amyloidogenesis

An adequate pool of amyloidogenic protein is clearly necessary. If the precursor has a very low amyloidogenic potential, it will require considerable time before triggering amyloid deposition (this may be the case of TTR in senile cardiac amyloidosis, or will require a significant increase in concentration (as is the case of β2-microglobulin in chronic haemodialysis and certain monoclonal light chains). Point mutations resulting in amino acid substitution can create or increase the amyloidogenic potential of the precursor; this is the case for the numerous TTR variants and for immunoglobulin light chains. Most of the information regarding the structure of amyloid subunit proteins has been generated with hereditary syndromes. For example, the TTR model has been extensively investigated from biochemical, genetic and structural (10,11) points of view and has provided important insights into amyloidogenesis.

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major constituent of the microfibrillar network of elastic connective tissue [27]. The recent definition of the three-dimensional structure of SAP [28] suggests that binding to amyloid deposits is calcium mediated, possibly via sulphated polysaccharides, and that the coating of the deposits by SAP, which is remarkably resistant to proteolytic degradation in the presence of calcium, may protect the amyloid deposits from degradation, thus contributing to their persistence [28].

GAGs are deposited concomitantly with murine amyloid A protein [29,30]. They consist mainly of heparan sulphate, which is part of a large proteoglycan, the heparan sulphate proteoglycan, of the basement membrane type (31,32). The latter has been demonstrated in several distinct forms of amyloid (21). High mol. wt glycosaminoglycans, including chondroitin sulphate, dermatan sulphate and heparin/heparan sulphate, have been specifically co-purified or localized ultrastructurally with amyloid fibrils of various biochemical composition (33-35). Recent studies have implicated apolipoprotein E in the pathogenesis of Alzheimer’s disease, with the protein accumulating extracellularly in the senile plaque and in the congophilic angiopathy of Alzheimer’s disease, and intracellularly in the neurofibrillary tangle (36,37); there is also good evidence that apolipoprotein E is associated with amyloid plaques in virtually any disease in which amyloid forms (38). The role played by these common constituents in amyloidogenesis is undetermined. Some evidence indicates that they may interact with amyloidogenic precursors favouring the nucleation stage of amyloidogenesis. Very high specific affinity interactions have been shown to occur between heparan sulphate proteoglycan and the various forms of the Alzheimer amyloid precursor proteins [31,39-41], as well as with SAA [42,43] and β2-microglobulin [44]; it has also been demonstrated that heparan sulphate influences SAA and Alzheimer β-protein to undergo a marked increase in β-pleated sheeting [40,43], thus promoting amyloid formation. Heparan sulphate proteoglycan has also been implicated in the aggregation and stabilization of already assembled fibrils [40,45]. AP and heparan sulphate proteoglycan can also prevent the immediate resorption of fibrils before bundle formation and accumulation of amyloid deposits. Most of the common elements of amyloid deposits are constituents of the basement membrane: a disturbance in this structure’s metabolism in certain organs may provide the necessary microenvironment for triggering fibril formation (46), thus contributing to the distinctive organ distribution of amyloid. For instance, it has been hypothesized that a local acute phase reaction in the brain can be a concurring event in Alzheimer’s disease (47). However, in all probability, in addition to common elements, there is a set of as yet unidentified specific factors which ensure that the amyloid in distinct clinical entities retains its exclusivity, as well as its specific anatomic sites [21].

The tropism of an amyloid protein for a particular organ remains one of the most relevant and unsolved aspects of amyloidogenesis. The anatomic site of a specific form of amyloid is probably determined by the concurrence of several elements involving both the amyloid precursor (its affinity for a certain tissue can be increased by mutations and/or proteolysis) and tissue factor (local enrichment of interactive components such as GAGs, disturbance in basement membrane metabolism or extracellular matrix elements at these locations, tissue complement of proteases) [46].

The induction time for AA amyloid development can be dramatically shortened to 36–48 h by ‘amyloid enhancing factor’ (AEF) [48,49]. This is a non-inflammatory, non-amyloidogenic substance that, when co-administered with a single inflammatory stimulus, synchronizes experimental animals such as mice to deposit AA amyloid within 2–3 days [50]. The precise characterization of AEF has so far been elusive [51–53], but it has been reported that a specific amyloid-enhancing factor preparation has the metabolic effect of increasing heparan sulphate synthesis [24]. It has also been reported that fibril AEF in the hamster is not dependent on mononuclear phagocytes [54]. Ubiquitin has been observed to possess AEF activity [55]. AEF activity has now been isolated from at least four forms of amyloid: AA, AL, TTR [56] and Alzheimer’s amyloid [57]. Functionally AEF is very similar to prion protein in that both act by accelerating a slow process. It has been suggested that these two agents act as nucleating agents, as was postulated for fibril AEF in hamsters [58] and for infectious prion diseases [59,60].

These basic concepts are best illustrated in two systemic forms of amyloidosis with prominent renal involvement: AL and Aβ2M.

**AL amyloidosis**

While the causative role may be different, the general mechanisms of amyloidogenesis described in non-immunoglobulin amyloidogenic proteins are probably valid in AL amyloidosis as well. Fibrillogenesis can be considered the result of interactions of light chain Vλ monomers that lead to the formation of dimers, followed by the interaction of these dimers to form filaments and, finally, between filaments to form fibres [61]. A causative role for dimerization was particularly emphasized by Benson [62] and increased dimerization was noted in an amyloid-associated kIV protein [63]. Nevertheless, the dimerization that primes fibrillogenesis is probably structurally different from the self-limiting type that creates Vλ–Vλ dimers in Fab fragments or Vλ–Vλ dimers in functionally active light-chain dimers, and in all probability these structural differences hold the key to the molecular basis of the disease.

**Primary structure**

The partial or complete amino acid sequencing of >60 tissue-extracted amyloidogenic light chains has not
defined a 'disease-associated' paradigmatic primary structure. Therefore a 'consensus sequence' cannot be found in any of the light chains extracted from amyloid deposits partially or completely sequenced. However, an extensive survey and analysis of the published sequences of amyloidogenic proteins has identified critical amino acid positions involved in amyloidogenesis [64].

The results of efforts to sequence so many amyloidogenic light chains have yielded the following conclusions. All light chain classes can cause AL amyloid; however, $\lambda$ light chains are involved in amyloid deposition two to three times as often as $\kappa$ chains [65,66]. One of the most intriguing findings is that $\lambda$ VI light chains are unequivocally associated with the disease [67]. The strong pathogenic impact of this light chain class has not been explained, although a one-residue insert in positions 68 and 69 was hypothesized to be critical for subgroup amyloidogenicity, probably via a proteolytically mediated event. An attempt to understand the reasons for the amyloidogenic potential of $\lambda$ VI light chains was made by Dvulet et al. [68] by comparing proteins WLT-SUT and AR (both $\lambda$ VI) with the myeloma protein NIG48. This study demonstrated that amino acid replacements in the framework regions are more effective at disturbing light chain folding than those located in the hypermutated complementary determining region (CDR). This is understandable given the crucial role of the framework regions in light chain scaffolding. A disturbing effect on the tertiary structure was found in the protein BAN [69], where a single base change enables two hydrophobic amino acids to replace two hydrophilic residues at a surface position (residues 36 and 72: Phe for Tyr, Ile for Thr). Such changes were considered to be capable of creating self-aggregation sites. In the same molecule at residue 61, Asn was found in place of Arg, an extremely conserved residue in non-amyloidogenic light chains. This replacement of Arg with other residues was observed by our group in the amyloidogenic light chain Inc [70] and by the Benson group [71] in light chains Coz (Gly instead of Arg) and Mum, where Arg is substituted by Asn. These amino acid replacements at residue 61 prevent the formation of a salt link between Arg61 and Asp82 and leave the unbalanced negative charge of Asp in a rather hydrophobic neighbourhood, with consequent domain destabilization. The crucial role of this substitution (asparagine for aspartate) in destabilizing the domain structure and in favouring the generation of an aggregation-prone non-native state was recently supported by site-directed mutagenesis studies on the VL domain [72]. The mutation identified in BAN, and evidence that the same mutation reproduced in other light chains was able to create amyloid, suggested that replacement of polar residues with hydrophobic ones at the surface of the molecule and the loss of hydrogen bonding within the domain can be considered mutations that favour light chain amyloidogenicity. The observation that amyloidogenic light chains have an unusual negative charge was first made by Isebe and Osserman [65]. Our group supported these data in 1990 [73] by determining light chain isoelectric points. We reported that the mean isoelectric point (pI) of amyloidogenic Bence Jones proteins was 4.8 $\pm$ 1.1 versus 6.2 $\pm$ 1.6 for non-amyloidogenic proteins ($P < 0.001$). This finding confirmed that many amyloidogenic light chains expose an unusually high number of unbalanced mono-amino-dicarboxylic residues to the solvent. In a few instances, determination of the primary structure permits elucidation of insertions or mutations responsible for the low pI; this is the case for $\lambda$ VI light chain AR because the insert residues 68 and 69 are both Asp [74], and for light chain Aub [75] in which acidic residues are unusually present in positions 31, 50, 51, 53 and 92. The lower pI may contribute to the decreased solubility of these molecules. In 1990 Benson's group [71] presented the sequence of an amyloidogenic light chain xen AND in which the mutations were not as prototypic as in BAN. Here the authors recognized a few significant mutations: Ser45 for Lys, Phe49 for Tyr, Phe57 for Tyr; however, all these mutations were located in the $\beta$-strand around the contact area of the dimer and therefore could influence dimer formation. The kinetics of dimer association was probably affected in our light chain Inc as well, where a Trp was found in position 96 [70]. Computer modelling analysis considered such a bulky residue in this position to be incompatible with a correct dimerization process. Disturbances in the physiological tendency of light chains to create dimers have been created in light chain REI by site-directed mutagenesis [76]. Introduction of the dimer interface of amino acids not compatible with monomer–monomer interaction was strictly correlated with a strong propensity to polymerize in vitro.

The techniques of genetic engineering and site-directed mutagenesis [63] will probably clarify the relevance of single mutations in preventing or causing the disease. Furthermore, the expression of recombinant amyloidogenic light chains could provide the amount of protein necessary to accomplish crystallization studies such as Benson's group recently presented [77]. However, even if this structural analysis could visualize several pathological folding modifications, the chemical and physical conditions (pH, ion strength, solvation, limited proteolysis) in which these proteins crystallize are generally far from those under which the same proteins form fibrils in vitro.

**Role of light-chain fragments in amyloidogenesis**

Extraction of light chains from amyloid deposits, according to the Pras method [78], even when conducted with mixtures of protease inhibitors, demonstrates the presence of light chain fragments associated with different percentages of complete light chains. In general, light chain fragments are almost always detected in the extracted material. The limited proteolysis of particular light chains, whose products are the light chain fragments found in amyloid deposits, could have the particular effect of making them less stable and of rendering the conditions for the formation...
of self-limiting dimers less favourable. Determination of the precise COOH-terminal position of light chain fragments is not always easy and has rarely been reported. However, Eulitz [79] reviewed the localization of split points along amyloid light chains: there is an extreme heterogeneity in these points, and it is not possible to determine a specific protease that is responsible for the cleavage. The same author also studied the possibility of forming amyloid fibrils from fragments proteolytically obtained from reduced and carboxymethylated pathogenic light chains [80]. In these experiments chemical denaturation and fragment formation do not correspond to the in vivo event, but rather they represent an interesting model for studying molecules that interfere with the folding and unfolding process. The origin of the light chain fragments present in amyloid fibrils is still uncertain. It has been demonstrated that light chain fragments circulate, in small amounts, in the serum of these patients [81] and that bone marrow plasma cells of patients with AL amyloidosis secrete different mol. wt light chains in vitro [82,83]. The post-translational role of monocyte endoproteases was also emphasized by data obtained in myeloma stem cell cultures [84]. However, Solomon's group [61] reported that in some cases light chain amyloid fragments isolated from different organs of the same patient have different mol. wts. This heterogeneity implies that light chains can be deposited in a relatively intact form as amyloid and that degradation is, at least partially, dependent on tissue-specific endoproteases. Evidence gathered from AA amyloidosis also suggests that proteolytic enzymes trim the protein after it enters an amyloid deposit [85]. The role of the COOH-terminal portion of the light chain in conferring stability on the globular dimer is particularly relevant for the role of Cys214, which forms the only one covalent bridge in the dimer. The study conducted by Hilschmann's group [86] on light chain DIA is particularly significant. This light chain was sequenced from both urine and amyloid deposits. In the urine it is present mainly as a complete light chain, while in the deposits it is present both as a complete light chain and as an almost complete light chain fragment. In the fragment the only residues missing are the last three amino acids of CL, including Cys214. The role of this cysteine in preserving the globular nature of the light chain, and above all in stabilizing the self-limiting dimer, has already been emphasized. Klafki and co-workers [87] demonstrated that Cys reduction determined a strong propensity in the intact light chain to polymerize into amyloid fibers under physiological conditions. Amyloid extraction from tissues can yield surprises, as in the case of patient MAL, in whom a difference between circulating light chain (serum-urine) and deposited light chain was demonstrated [88]. This patient suffered from a biclonal plasma cell dyscrasia, and only one of the two monoclonal light chains was amyloidogenic. In this case the presence of the amyloidogenic light chain in the NaCl washing fraction of the tissue extraction is surprising. This probably means that a large portion of the light chains is in an intermediate transition state between the soluble and the fibrillar form (unfolding intermediates) and this fraction can be overlooked during the extraction procedure.

Anti-idiotypic antibodies

Using anti-idiotypic monoclonal antibodies, we have documented that fibrillar light chains lose reactivity [89]. The loss of idioype specificity in the fibrillar light chains has been confirmed with polyclonal antibodies [90]. This phenomenon corresponds to a process of protein unfolding, which is considered to be a necessary step towards aggregation and subsequent amyloid formation. Even following the amyloidogenic mutation the protein preserves its function in the soluble state; it loses this function as soon as polymerization starts [91]. These data suggest that presumed light chain antibody activity [92] in determining tissue specificity is probably unlikely in the context of AL amyloidosis. The affinity of some AL amyloid fibres for hydrophobic haptens is probably due to hydrophobic interactions more than to specific antibody activity [92]. The tissue specificity of certain amyloid deposits is probably better correlated with the fulfilment, in certain tissues, of particular physicochemical conditions capable of priming fibrillogenesis, such as pH, ionic concentration, the presence of certain glycosaminoglycans, and over-representation of certain matrix proteins and certain proteolytic enzyme complements.

Glycosylation and amyloidogenesis

A pathogenic effect for glycosylation has been hypothesized on the basis of the high frequency of glycosylated amyloidogenic light chains: >50% of them seem to be sugar linked as compared with 15% of non-amyloidogenic Bence Jones proteins [93]. Glycosylation has been observed in κ as well as in λ light chains in regions corresponding to the CDR, as in Es492 [93], EPS 103 [93] and MOL 92 [94] and in the framework region, as in BAN 61 [69], MAL 106 [95] and ARN, where a consensus sequence was found in position 70 [75]. In functionally active immunoglobulins, a carbohydrate chain in the combining site can modify and in some cases increase the affinity constant of the antibody [96]. In isolated light chains, the presence of carbohydrate in the interior of the molecule, i.e CDR regions, or at the dimer interface could make these areas more solvated in aqueous solutions, causing destabilization of correct monomer assembly. In general, when carbohydrates are found in regions exposed to the solvent they are located at the edges of the β-pleated-sheet structure (BAN) or in hairpin bends (ARN), and they do not disturb the β-sheet conformation, which is a prerequisite for fibril formation. The possibility that these carbohydrates can have a stabilizing effect on the growing fibril has been postulated [97,98].
Aβ2M amyloidosis

β-2-Microglobulin (B2M) is the protein responsible for the amyloidosis of haemodialysed patients; it is a 99 amino acid protein that participates in the formation of the MHCI complex [99]. Serum B2M is correlated with the expression of MHCI on the cell surface, decomposition of the heterodimeric protein and kidney clearance. In haemodialysis increases in B2M are due to the absence of kidney clearance. Increased plasma concentrations favour aggregation and amyloid deposition. Uraemia per se, which is associated with increased plasma B2M, can cause this type of amyloidosis, but the haemodialysis procedure itself may accelerate amyloid deposition. The use of biocompatible membranes could probably delay the appearance of this complication [100].

Cases of very early appearance of amyloidosis during haemodialysis [101] and the fact that B2M concentration is not always correlated with the extent of amyloid deposition suggest that other factors are involved in the kinetics of B2M aggregation and fibril formation. Mature B2M is an intrinsically amyloidogenic protein because it is capable of spontaneously creating fibrils in vitro at high concentrations and low ion strengths [102]. However, in vivo other as yet undefined factors are involved in making the transition from native protein to amyloid fibrils more productive and more efficient, and a contributing effect of peripheral blood mononuclear cells has been demonstrated in experiments on B2M fibrillogenesis in cell culture [103].

Amino acid replacements in the B2M sequence have not been reported in any patient with AH amyloidosis; only deamidation at Asn17 and/or Asn42 has recently been described [104]. However, we think it would be useful to sequence the B2M from haemodialysed patients with very early amyloidosis in order to investigate the possible occurrence of sporadic mutations. Three main post-translational modifications can be found in the B2M of patients undergoing haemodialysis: proteolysis, oxidation and glycation; all of these could modify the structure and functional characteristics of this protein, thus favouring its tendency to aggregate and make fibrils.

The role of proteolysis in B2M amyloidosis is not fully established. Fragments of B2M are present in amyloid deposits [105] and in B2M kidney stones [106]. Proteolysis can remove fragments of heterogeneous size from the amino terminus of the protein, while the COOH terminus is apparently not modified. In fact, methionine 99 is always present in the proper proportion in amino acid analysis of B2M extracted from amyloid deposits (V. Bellotti and M. Stoppini, unpublished observations). B2M oxidation has been studied by exposing the protein to defined oxygen-derived species, 'OH hydroxyl radicals, and O− anion superoxide radicals [107]. Treatment with oxygen radicals deeply modifies B2M: tryptophan is destroyed and tyrosine and methionine are altered. These chemical changes determine protein unfolding, the disappearance of the β-sheet structure and the appearance of a typical random coil spectrum [107]. Glycation has been found to be a constant post-translational modification of B2M of patients undergoing haemodialysis [108,109].

The glycated fraction of B2M, also referred to as acidic B2M, is able to enhance the chemotaxis and chemokinesis of human monocytes and could play a key role in triggering the inflammatory response that leads to bone and joint destruction [108,109]. Definition of the glycation site has established that the primary location is the α-amino group of the amino-terminal isoleucine. Other minor sites were found at the ε-amino groups of lysine 19, 41, 48, 58, 91 and 94 [104].

The role of glycation in the alteration of the folding properties and the fibrillogenesis of B2M has not been established and will probably require further investigation.

Conclusion and future perspectives

The formation of amyloid fibrils can be viewed as a peculiar folding pathway that some proteins can follow in vivo. The pathological folding pathway is schematically represented in Figure 1.

The transition from native, functionally active proteins to protein aggregates requires an intermediate protein unfolding step. The fundamental role of protein denaturation in the fibrillogenesis of insulin [110], transthyretin [111] and light chains [112] has been clearly established. Thermodynamic studies of amyloidogenic variants of proteins whose wild-type counterpart is not amyloidogenic demonstrate that the former are less resistant to unfolding [72,91]. Aggregation of unfolded proteins is a side reaction that entraps a huge amount of protein in an irreversible state. In vivo these denatured proteins, organized into a fibrillary structure, are substantially excluded from further metabolic processing.

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Conclusion and future perspectives

The formation of amyloid fibrils can be viewed as a peculiar folding pathway that some proteins can follow in vivo. The pathological folding pathway is schematically represented in Figure 1.

The transition from native, functionally active proteins to protein aggregates requires an intermediate protein unfolding step. The fundamental role of protein denaturation in the fibrillogenesis of insulin [110], transthyretin [111] and light chains [112] has been clearly established. Thermodynamic studies of amyloidogenic variants of proteins whose wild-type counterpart is not amyloidogenic demonstrate that the former are less resistant to unfolding [72,91]. Aggregation of unfolded proteins is a side reaction that entraps a huge amount of protein in an irreversible state. In vivo these denatured proteins, organized into a fibrillary structure, are substantially excluded from further metabolic processing.

Transition from the native to an unfolded, denatured...
state requires several levels of protein unfolding and the reaction is reversible. Modifying the direction of this process could be a future therapeutic target for this disease. Subsequent aggregation of partially folded or unfolded protein into amorphous aggregates and finally to amyloid fibrils also entails theoretically reversible reactions. However, these final transitions from unfolded proteins to fibrils involves dramatic energy modifications which are difficult to overcome. This implies that in vivo it is unlikely that the physiological systems dedicated to protein refolding could convert extracellular fibrillar proteins to their native state. The main means of clearing amyloid deposits remains proteolysis and protein catabolism. Recent data suggest that it may be possible to promote amyloid reabsorption by drugs that interact directly with amyloid fibrils [114,115]. Deposition of glycosaminoglycans favours stabilization of the deposit and an accumulation of SAP offers protection from proteolytic enzyme-mediated reabsorption. Ways of reducing the protective effect of GAG and SAP on amyloid fibrils are currently being investigated extensively [116,117].

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