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Review

Immunoglobulin light chains, glycosaminoglycans, and amyloid

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Abstract. Immunoglobulin light chains are the precursor proteins for fibrils that are formed during primary amyloidosis and in amyloidosis associated with multiple myeloma. As found for the approximately 20 currently described forms of focal, localized, or systemic amyloidoses, light chain-related fibrils extracted from physiological deposits are invariably associated with glycosaminoglycans, predominantly heparan sulfate. Other amyloid-related proteins are either structurally normal, such as β2-microglobulin and islet amyloid polypeptide, fragments of normal proteins such as serum amyloid A protein or the precursor protein of the β peptide involved in Alzheimer's disease, or are inherited forms of single amino acid variants of a normal protein such as found in the familial forms of amyloid associated with transthyretin. In contrast, the primary structures of light chains involved in fibril formation exhibit extensive mutational diversity rendering some proteins highly amyloidogenic and others non-pathological. The interactions between light chains and glycosaminoglycans are also affected by amino acid variation and may influence the clinical course of disease by enhancing fibril stability and contributing to resistance to protease degradation. Relatively little is currently known about the mechanisms by which glycosaminoglycans interact with light chains and light-chain fibrils. It is probable that future studies of this uniquely diverse family of proteins will continue to shed light on the processes of amyloidosis, and contribute as well to a greater understanding of the normal physiological roles of glycosaminoglycans.

Key words. Glycosaminoglycans; immunoglobulin light chains; amyloidosis; amyloid AL; heparan sulfate; heparin.

Glycosaminoglycans and proteoglycans

Glycosaminoglycans (GAGs) are unbranched, long, heteropolysaccharides composed of repeating disaccharide units consisting of a uronate (either glucuronate or iduronate) and a glycosamine (either glucosamine or galactosamine) [1, 2]. The structures of the repeating disaccharides, illustrated in figure 1, determine the class of GAG, of which there are fundamentally five. These include heparin/heparan sulfate (HS), chondroitin sulfate, dermatan sulfate, hyaluronan, and keratan sulfate. The latter (not depicted in fig. 1) lacks uronate and is composed of a disaccharide consisting of galactose and glucosamine. With the exception of hyaluronan, which is found as a free polysaccharide, most of the other GAGs are usually found covalently linked to a protein backbone. This protein:GAG complex is termed a proteoglycan [2]. More than one protein is known to act as the backbone for a given class of GAGs, giving rise to families of, for example, HS proteoglycans (HSPGs). Furthermore, in some cases, a single protein backbone may provide sites for the synthesis of, and contain more than one class of, GAGs.

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Heparin and HS differ from the other GAGs in that they are the only GAGs which may be N-sulfated at the C-2-position of the glucosamine, and the alternating uronate and glucosamine are linked in an α1,4 arrangement [3]. Heparin and HS are closely related structurally and synthetically. In relation to amyloidogenesis, HS may be the most important GAG; HS and the HSPG perlecan are the best-studied GAG and proteoglycan, respectively [4, 5]. Although heparin is the better known entity of the two, because of its clinical use, physiologically it is synthesized primarily by mast cells. HS is the more physiologically relevant GAG, found ubiquitously in the body. For these reasons, heparin and HS will be used to illustrate the mechanism of GAG synthesis, as well as the differences in heparin and HS structure.

GGA synthesis

Heparin and HS synthesis take place in the endoplasmic reticulum and the Golgi on a serine hydroxyl group within specific domains of the proteoglycan protein backbone [6, 7]. This domain contains within it a sequence consisting of the amino acids:

-(amino acids)$_n$-Ala-Asp-Ser-Gly-Glu-Tyr-(amino acids)$_n$-.

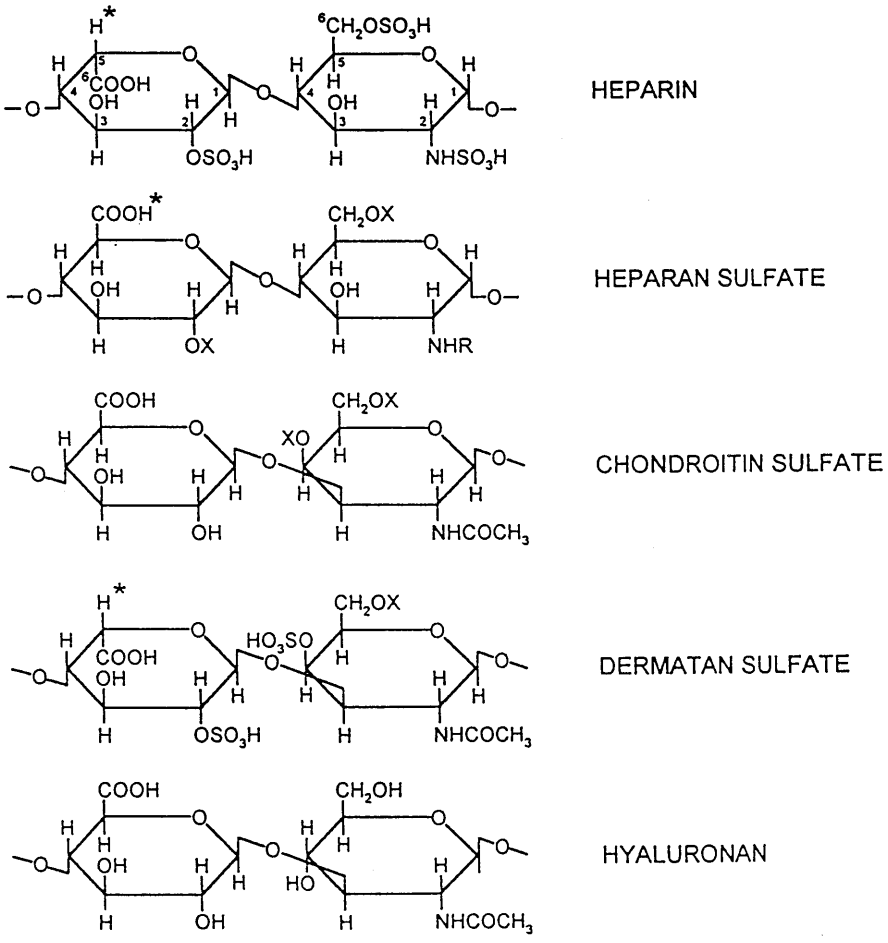

Figure 1. Representative structures for the disaccharide repeating units of the different GAGs. The asterisks indicate variable amounts of epimerization for the carboxyl at C-5 of the hexuronic acid, and X = H or SO_3. Heparin is composed of $\geq$80% iduronic acid (IdoA)-2-O-SO_3, N-acetylglucosamine (GlcN)-N-SO_3,6-O-SO_3. HS contains large regions ($\geq$50%) of glucuronic acid (GlcA)-GlcN with little or no sulfation separating heparin-like regions with highly variable sulfation patterns and about 50% of the GlcN remaining acetylated. Chondroitin sulfate is composed of GlcA-N-acetylgalactosamine (GalN)-4-O-SO_3 or 6-O-SO_3. For dermatan sulfate, some of the GlcA is converted to IdoA and the GalN is sulfated at C-4 and C-6. Hyaluronan consists of GlcA-GlcN and is the only GAG that does not contain sulfate. The amino group for a small proportion of each of the GAG chains remain deacetylated, leaving a free amine.

Using nucleotide sugars and the corresponding transferases, in sequential order a tetrasaccharide stem of xylose-galactose-galactose-glucuronate- is added to the serine. This polysaccharide sequence is a common primer for the synthesis of heparin/heparan, chondroitin, and dermatan sulfates. The class of GAG synthesized on this primer is presumably determined, in part, by the nature of the proteoglycan backbone. In the case of heparin/HS, this stem serves as the site for the elongation of the polysaccharide, which grows by the sequential and alternate addition of N-acetyl-glucosamine and glucuronate. Corresponding UDP-hexoses and transferases serve as the substrates and enzymes, respectively. At this stage, heparin and HS synthesis follow the same path. It is during the elongation of the GAG chain but at sites behind the growing end that structural modifications of the nascent polysaccharide take place. These structural modifications are fairly uniform with heparin, but, in ways that are not understood, occur in an intermittent fashion during the synthesis of the HS polysaccharide.

In the case of heparin, the N-acetyl-glucosamine is deacetylated and sulfated by a single enzyme, heparan sulfate N-deacetylase:N-sulfotransferase, using 3′-phosphoadenylylsulfate (PAPS) as the sulfate donor. This modification must occur to allow an epimerase to convert the preceding glucuronate into an iduronate and which in turn permits 2-O-sulfation of the iduronic acid and 6-O-sulfation of the preceding N-sulfated glucosamine. These modifications result in the fairly uniform heparin disaccharide structure illustrated in figure 1.

In the case of HS, the deacetylation and the sulfation of N-acetyl-glucosamine does not occur in as regular a manner as during heparin synthesis. This in turn prevents the operation of the epimerase and subsequent sulfations of the 2-O-position of the uronate and 6-O-position N-acetyl-glucosamine. As a result, HS contains stretches of heparin-like structure where the N-deacetylase:N-sulfotransferase, epimerase, and O-sulfotransferases have been active, but also has regions in which the N-acetyl-glucosamine is unaltered, and therefore where glucuronate persists and where there is relatively little sulfation of the uronate and N-acetyl-glucosamine. The resulting disaccharide structures in HS are much more heterogeneous (see fig. 1).

GAGs and amyloids

Amyloid is a generic term for the primarily extracellular accumulation of fibrillar protein deposits which have unique tinctorial and structural properties. At least 20 unrelated, normally non-fibrillar proteins are known precursors of amyloid [8, 9]. Each is associated with a specific disease such as Alzheimer's disease (Aβ), chronic hemodialysis (β2-microglobulin), adult-onset diabetes [amylin, also called islet amyloid polypeptide (IAPP)], inflammation-associated amyloid [serum amyloid A (SAA)], plasma cell dyscrasias and B-cell lymphomas (immunoglobulin light chains), and certain malignancies. Regardless of the underlying amyloid fibril protein/peptide or associated disease, isolated amyloid fibrils when examined by negative staining or high-resolution electron microscopy are composed of multiple filaments or protofibrils twisted around each other forming non-branching fibrils typically 7–10 nm in diameter, with a crossed β-pleated sheet conformation [10–12]. They stain with Congo red, and when so stained and viewed under polarized light exhibit a red/green birefringence, a property diagnostic for amyloid.

Amyloid, as the name indicates, means 'starchlike,' being derived from 'amylose' or 'amylon.' The coining of the term amyloid, and the presence of a carbohydrate in amyloid, was established by Virchow in 1854 [13]. The general nature of this carbohydrate was known to be a GAG as early as 1927 [14]. The relationship of GAGs and amyloid was a subject of only intermittent interest for the next 50 years. The potential significance of GAGs in amyloidogenesis did not emerge until 15 years ago when the temporal appearance of GAGs relative to the appearance of SAA protein in inflammation-associated amyloid (AA-amyloid) was examined [15]. The availability of conventional and rapid AA-amyloid induction protocols allowed one to determine whether the appearance of GAGs in amyloid preceded, was coincident with, or followed the deposition of the AA protein and, furthermore, whether the presence of the GAGs was an epiphenomenon of the inflammation required for this type of amyloid induction. It was shown that GAGs were deposited coincidentally with the AA protein no matter how the protocol was varied, what inflammatory stimulus was used, and what tissue was involved. Inflammation on its own without the other components of the rapid AA induction protocol failed to elicit GAG deposition and amyloid. It therefore became of interest to characterize the presence of the GAGs morphologically, their chemical nature, and what influence they had on amyloidogenic protein/peptide conformation.

Immunohistochemical and histochemical techniques established that the GAG was likely HS, and that this HS was part of perlecan [16, 17]. The HS nature of the amyloid-associated GAG has also been confirmed chemically [16]. In mouse models of AA-amyloid induction, the quantity of HS increases in tissue in parallel with the AA protein. Furthermore, in experimental animal models of AA-amyloid, the deposition of perlecan coincides anatomically and temporally with the appearance of the AA fibrils. Ultrastructural, histo-

chemical, and immunogold studies have revealed that HS and perlecan are constituents of the amyloid fibril in situ, as is a chondroitin sulfate proteoglycan [18, 19]. High-resolution electron microscopy has suggested that the organization of the amyloid fibril in situ may be different from that seen in vitro, and a preliminary model of the in vivo organization of the various AA-amyloid structural components has been proposed [18, 19].

Of particular importance to the pathogenesis of amyloid is the influence of HS on amyloid protein/peptide conformation. Using circular dichroism to assess β-sheet structure, HS and/or heparin incubated in vitro with the Aβ protein or SAA rapidly increases their β-sheet content [20–23]. In the case of the Aβ protein, this is rapidly followed (minutes) by the generation of Aβ fibrils [22]. More striking yet are the observations with SAA. In the mouse, only the SAA isoform 2 is amyloidogenic and is deposited as amyloid. The amino acid sequence of isoforms 1 and 2 are 91–92% homologous. But, HS when incubated with isoforms 1, 2, or a non-amyloidogenic hybrid of the two, confers a marked increase in β-sheet structure only on isoform 2 [20, 21]. Furthermore, no other GAG examined (dermatan sulfate, chondroitin sulfate, or hyaluronan) possesses this property. The precise HS-binding site on SAA proposed previously has been substantiated [24].

With the possible exception of the β2-microglobulin form of amyloid seen in patients undergoing chronic hemodialysis, HS or HS-like GAGs are part of every form of amyloid so far examined, and perlecan has been found in forms as diverse as AA (inflammation associated), Aβ (Alzheimer's disease), ATTR (familial amyloid polyneuropathy), and AIAPP (pancreatic amyloid in adult-onset diabetes). Where studied, very high affinity binding (in the nM range) occurs between individual extracellular basement membrane (ECBM) proteins (such as perlecan or laminin) and amyloidogenic proteins such as SAA or the Aβ protein precursor (AβPP) [25–27]. Furthermore, consensus HS binding sequences have been identified in SAA, AβPP, and in the Aβ protein segment of AβPP, as well as in many other amyloidogeneic proteins, such as apoA-I and pro-IAPP.

HS-binding consensus sequences

HS binding to proteins has become a topic of interest in a broad spectrum of research areas ranging from hemostasis [28] to lipoprotein metabolism and atherosclerosis [29–31], growth factors [32], chemokine and viral binding to cell surfaces [33–35], and amyloidosis [36].

By comparing 12 heparin-binding sequences from a series of heparin-binding proteins, Cardin and Weintraub in 1989 [37] identified two consensus sequences, XBBBXXBBX and XBBXBX (where X is any amino acid and B is a basic residue). A third consensus sequence was reported by an additional group (XBBXXBBBXXBBX) [38]. For murine SAA, an XBBXBX sequence is present between residues 82–87. However, inspection of other available SAA sequences (from 12 species) revealed that this consensus sequence was not conserved [24]. There are many examples of heparin/HS-binding protein or peptides which also lack these apparent 'consensus' sequences, including antithrombin III (residues 124–145) [39], platelet factor 4 (residues 46–70) [40], apoE (residues 202–242) [41], and basic fibroblast growth factor (residues 25–46 and 111–141) [42]. Thus, the primary sequence alone cannot define the heparin/HS-binding site. Protein conformation may also play a role in placing critical basic residues into energetically favorable positions, juxtapositioning them with cationic groups on the GAG chain. Supporting this view, Margalit and colleagues [43] have found, in a comparison of the spatial distribution of the basic residues for 18 known heparin-binding domains, for which three-dimensional structures were available, that two basic residues were always about 20 Å apart. In binding sites with an α-helical or β-strand conformation, the two basic residues were separated by 13 and 7 residues, respectively, without a discernable consensus sequence. Furthermore, it was demonstrated that a distance of 20 Å between the two outermost basic residues of a heparin-binding site could accommodate docking of the binding site to a heparin pentasaccharide, the size sequence of heparin that binds to antithrombin III.

The SAA domain containing the HS/heparin-binding site is found at the carboxy-terminal end of this protein and possesses the following sequence of residues:

residue 77 102

-ADQEA**NRHGRSG<u>K</u>**DPNYY**<u>R</u>**PPGLPA**<u>K</u>**Y

The residues in bold represent the XBBXBX consensus sequence. Nevertheless, it can be shown that the histidine (H) within this sequence is unimportant, but the basic residues in bold and underlined outside this sequence are important in HS binding [24]. The hierarchy of importance of the individual basic amino acids in SAA required for HS binding is:

R86 > K89 > R83 > R95 > K102.

The secondary structure for SAA is unknown. Interestingly, an H or R residue is found at position 93 in all species, except mouse, and it is possible that R/H93 functionally replaces the mouse R83. Furthermore, it can be shown that this peptide region will bind to

Congo red covalently linked to a column matrix. This binding appears to depend upon the Congo red sulfonates with a separation of approximately 20 Å; no binding is found to a column bearing randomly distributed sulfonates [24].

Other ECBM proteins in amyloidogenesis

Many aspects of the work described for HS and HSPG perlecan are also true for several other ECBM proteins, such as laminin, entactin, and collagen IV. These ECBM proteins are also deposited coincidentally with the AA protein in models of AA-amyloid [44], and have been identified immunohistochemically in other forms of amyloid, such as Aβ [45]. Furthermore, high-affinity binding can also be demonstrated between these ECBM proteins and amyloidogenic protein precursors [25–27]. Moreover, evidence implicating ECBM proteins, in addition to perlecan, in amyloidogenesis comes from reverse transcription-polymerase chain reaction analyses of perlecan, laminin β and λ chains, and the α_1 (IV)-collagen chain splenic mRNA levels during rapid AA-amyloid induction. In mice, the spleen is the first organ to manifest AA-amyloid during persistent inflammation. Other than laminin β, the aforementioned splenic mRNAs all begin to increase within 18–24 h of the commencement of the amyloid induction protocol [46, 47]. Amyloid is not visible histologically until 18–24 h later and occurs only in those animals receiving the full amyloid induction protocol. None of the appropriate controls manifests the increases in mRNA, nor amyloid, suggesting that these changes in ECBM protein gene expression are closely tied to the onset of AA amyloidogenesis. Furthermore, the induction of these genes is not likely a response to the deposition of amyloid. The purposeful intravenous injection of AA-amyloid fibrils into mice leads to the clearance of the fibrils and their splenic perifollicular localization, the same site as natural AA-amyloid deposits, without increase in perlecan, laminin β and λ chains, and the α_1 (IV)-collagen chain splenic mRNA levels [47].

The foregoing review provides a strong argument that ECBM proteins are involved in amyloidogenesis, and that HS and perlecan in particular play an important role. If this perspective is substantially correct, then molecules that interfere with HS:amyloidogenic protein interactions should prove useful as anti-amyloid agents. To this end, several series of compounds have been synthesized which mimic aspects of HS structure and their effect on amyloid fibril formation assessed in vivo and in vitro. Some of these compounds proved useful in inhibiting AA-amyloid induction in vivo, and also in causing regression of established deposits. Many of these same compounds have also proved effective in inhibiting, and reversing, HS-induced Aβ fibril formation in vitro [22].

Proteoglycans, GAGs, and HS in immunoglobulin light-chain amyloid

The role of ECMB proteins, proteoglycans, GAGs, and HS in the genesis of immunoglobulin light-chain amyloid deposits (AL amyloid) has yet to be examined in any significant way. HS and several other GAGs have been identified in AL amyloid fibrils isolated from human tissues [4, 48], as well as in situ [S. Inoue and R. Kisilevsky, unpublished data]. Nevertheless, the techniques used to liberate the AL fibrils from tissue may have allowed for the spurious association of tissue GAGs with the amyloid; these data should be interpreted with caution.

Though light chains can bind to heparin and other sulfated polysaccharides [49–51], the influence of GAGs, and HS in particular, on light-chain conformation has yet to be explored in detail. Nor is it apparent what aspects of light-chain structure are responsible for the observed binding properties of HS and light chains. Jiang et al. [49] surveyed a number of human antibody light chains that had been obtained from patients with multiple myeloma for interactions with high- and low-molecular-weight heparin, heparin sulfate, chondroitin sulfate B, and chondroitin sulfate C. Light chains were of both κ and λ origin and included representatives of amyloid- and non-amyloid-forming nature. All light chains tested showed some degree of interaction with the glycosaminoglycans; high-molecular-weight heparin exhibited the strongest interaction and heparin sulfate the weakest. Interaction was evaluated by the loss of protein in solution following addition of GAG; hence, 'consequence' may be a more appropriate term than 'interaction.' The experiment effectively measured the solubility of the protein-GAG complex rather than the affinity between protein and GAG. Interestingly, the strongest interaction, or consequence of incubation, with heparin was found for the amyloid-related proteins, which in several cases showed almost complete precipitation.

In contrast to other proteins typically associated with amyloidosis, not all patients who overproduce light chains during myeloma experience development of clinically significant deposits of AL amyloid fibrils. Some patients exhibit cast formation (apparently amorphous precipitation) in nephron tubules. This precipitation may result from overconcentration of protein via the water recovery function of the tubules and may be facilitated by interactions of the light chains with Tamm Horsfall protein [52, 53], a ubiquitous urinary constituent. Other patients experience non-fibrillar deposi-

tion of light chains systemically or preferentially in the kidney or other organs [50]. Yet other individuals can produce large quantities of protein with no clinically evident physiological consequences [54, 55]. This diversity in clinical expression arises from the extreme diversity of light-chain primary structure that results from multiple germline genes of origin [56, 57] and somatic mutation that occurs during the maturation of the B cell.

The amino acid sequences of light chains produced by myeloma patients may differ at many sites; to date, no examples of light chains that differ at only a single amino acid position have been found in a database that now includes sequences from more than 300 patients [F. Stevens, Argonne National Laboratory, unpublished results]. This structural diversity has frustrated attempts to identify those variations that account for different pathological consequences. However, a recent study examined the fibril-forming properties of a series of recombinant variable domains that differed at positions at which variations were associated with amyloidogenic and non-amyloidogenic proteins. These proteins originated from the same germline gene in different patients [58], thus minimizing the number of potential amino acid differences that could account for different pathological properties. A clear correlation was found that related in vitro fibril formation to the thermodynamic stability of the variable domain. Thus, as experimentally demonstrated for human lysozyme [59] and transthyretin [see ref. 60 for a review] and as previously suggested by Wetzel and co-workers [61–65], stability is a major enabling element rendering certain light chains amyloidogenic. As such, a virtually unlimited number of variations, both inherited and acquired through somatic mutation, can account for fibril formation potential. The conformation of the hypothesized partially folded intermediate involved in fibril assembly, as well as the identity of amino acids involved with intersubunit contacts in the fibril, remain unknown.

The potential existence of a vast repertoire of potential amino acid substitutions that render light chains susceptible to amyloidosis (amyloid AL) is illustrated by the fact that in the limited study of Raffen et al. [58], six single amino acid replacements (at five sites) converted the control, non-fibrillogenic, protein into variants that readily formed fibrils in vitro. None of the variations involved replacement of an amino acid involved in packing of the core of the domain. The fibril-rendering replacements were scattered throughout the domain, underscoring the susceptibility of the β-sandwich domain to destabilization by amino acid replacements at many loci within its structure.

This vulnerability may be rationalized by the high degree of cooperativity characteristic of the β-domain structure. In addition to the backbone-mediated interactions characteristic of β-sheets, many of the amino acid side chains participate in hydrogen bonds involving donor or acceptor atoms in the backbone or other side chains, or participate in core-packing van der Waals interactions. As such, amino acid substitutions that deplete hydrogen bond interactions, as illustrated by the histidine replacement of an aspartic acid in an amyloidogenic variant of human lysozyme [59], or van der Waals contacts, remove an element of structural rigidity with results that propagate throughout the structure. Thus, replacements that increase the population of partially folded intermediates involved in fibril assembly need not be contiguous to the structural elements, which remain to be identified, that are involved in the interactions of non-native forms responsible for fibril construction.

Surprisingly, the Raffen study also identified a number of mutations in the amyloidogenic light chains that increased the stability of the protein by introducing hydrogen bonds or improving internal hydrophobic contacts. This finding may also reflect the high cooperativity of the β-domain structure but also reveals that the light chain encoded by inherited germline genes is only marginally stable. Thus, it is poised to incur diminished stability, resulting in fibril-forming tendency, as a result of single or limited amino acid substitutions. The suboptimal stability of variable domains, from the perspective of vulnerability to fibril formation, may suggest that flexibility of the structure contributes to immunological diversity. The lambda-six subgroup, which is highly associated with amyloidosis [66], appears to be particularly unstable [67, 68]. The recently determined structure (fig. 2) of recombinant representatives of lambda-six variable domains demonstrated conventional conformations [68]. However, one of the structures exhibited a unique ionic interaction between somatically introduced Asp and Arg residues that may account for increased stability and lack of amyloidosis in the patient.

The amino acid diversity of antibody light chains, while frustrating facile attempts to identify causal relationships with amyloidogenesis and other light chain-related pathologies, has implications with respect to the interactions of AL fibrils with GAGs. None of the Arg and Lys residues of light-chain variable domains is absolutely conserved throughout the approximately 100 amyloid-associated amino acid sequences that have been obtained to date. Although certain basic residues are found in most light chains, and may account for some degree of interaction between free variable domains and GAGs, as observed in the study of Jiang et al. [49], it seems likely that interactions of fibrils and GAGs may result from a less specific mechanism. It is probable that the interaction involves a multitude of contacts between the fibril and the basement membrane.

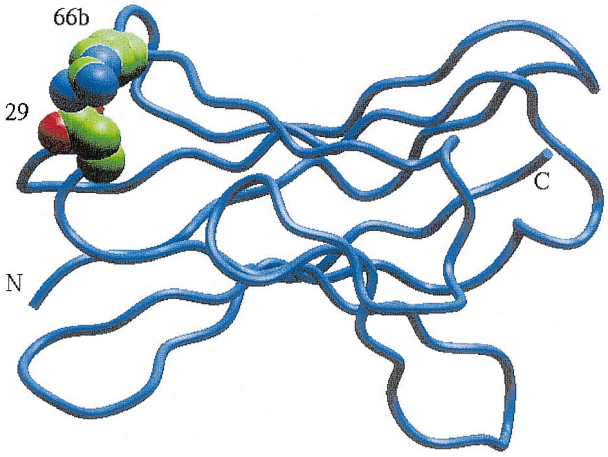

Figure 2. Backbone structure of the variable domain of a human lambda-six light chain [68]. The N and C termini are indicated. The proximity of the side chains of positions 29 and 66b is also illustrated. Charged residues, Asp29 and Arg66b, replaced germline-encoded serines at both positions in patient JTO. Ionic interaction between Asp and Arg may increase the stability of protein JTO and account for lack of amyloidosis in the patient.

Thus, spacings of charged groups observed in crystallographic studies may not pertain to the interactions of fibrils with the basement membrane, both because of the coparticipation of groups in adjacent subunits, as well as the probable involvement of a non-native conformation in the assembly of the fibril itself.

Do GAGs contribute to the physiological fate of amyloid?

In vitro systems now exist for the formation of fibrils by several amyloid-associated proteins, including antibody light chains, transthyretin, β-peptide, and amylin. These in vitro-produced fibrils share physicochemical features associated with physiologically derived amyloid, including the characteristic morphology observed by electron microscopy, binding of Congo red with typical tinctorial and birefringent properties, and a helical arrangement of β-sheets parallel to the fibril axis as revealed by fiber diffraction study [69]. In vitro systems are an important tool for understanding the mechanism of fibril formation and the physicochemical characteristics of certain proteins that make them prone to fibrillogenesis. Moreover, it is quite possible that these systems can lead to screening assays that will eventually allow the development of drugs that will halt or attenuate the fibril formation process of amyloidogenesis. However, it is appropriate in closing this review to emphasize that none of the homogeneous fibrillar in vitro assemblies produced to date is equivalent to physiologically produced amyloid. While the proteinaceous fibril may be the key to the genesis of amyloid, we should perhaps consider the constituent GAGs and other ancillary components to be intrinsic parts of the physiological definition of amyloid itself, rather than simply materials that are associated with amyloid [36]. These non-protein components undoubtedly contribute much to the clinical sequellae of fibril formation, including proteolysis resistance [70] and tissue destruction. Much remains to be learned concerning the post-fibril formation processes of amyloidogenesis. The structurally diverse and well-characterized antibody light chain should be a powerful tool in studies directed to elucidating the morphogenesis of protein fibrils into amyloid.

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