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Amyloid formation from Immunoglobulin chains

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Amyloid[1] was first discovered during the examination of tissue by Rudolf Virchow in 1854. Because it could be stained in a similar manner as starch with iodine in Lugol's solution it was named "amyloid" by Virchow [1]. The deposition of this substance occurs extracellularly in small blood vessels and various organs. Examination under the light microscope reveals a homogeneous eosinophilic substance with hyaline appearance. Nowadays the diagnosis of amyloidosis still depends mainly on the examination of bioptic material from involved organs. Main criteria are the staining of amyloid with Congo red [2] and a characteristic green birefringence in polarized light. The formation of amyloid is associated with many different disorders and impairs the normal organ function when the deposits are severe. There have been controversial discussions about the nature of this substance for more than one hundred years. The observation by electron microscopy that the deposited material is composed of fibrils was a first step toward the unraveling of the mysteries of this disease [3]. A further major breakthrough happened when it became possible to extract amyloid fibrils from the tissue [4]. It was proven by serological and chemical methods that proteins are the main constituents of extracted amyloid fibrils and not, as supposed for a long time, starchlike polysaccharides. Particularly amino acid sequence analysis was applied successfully to identify the individual proteins which participate in the formation of amyloid deposits. These studies revealed that amyloidosis is not a single entity but is connected to a still growing range of various disorders and diseases. The current state of research in this field has been summarized by an international nomenclature committee at the VIth International Symposium on Amyloidosis [5], which was held in Oslo in 1990.

Because several excellent reviews on amyloidosis have been published [6-10], this paper concentrates only on amyloid formation from immunoglobulin chains. Magnus-Levy (in 1931 [11]) and Apitz (in 1940 [12]) were the first authors to report on correlations between multiple myeloma

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1 Abbreviations: Protein A = amyloid associated protein; apoSAA = serum precursor of protein AA; AL-type = amyloid derived from immunoglobulin light chains; VH_{III} = V-regions of immunoglobulin heavy (H) chains, subgroup III; CH = constant regions of H-chains

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and amyloidosis. Direct evidence that immunoglobulin light chains are involved in the process of amyloid formation was first gained by amino acid sequence studies on amyloid fibril proteins by Glenner et al. [13]. They found that the amino acid sequence of the fibril protein corresponds to the N-terminus of immunoglobulin æ-light chains. Similarly, it was proven that also λ-immunoglobulin L-chains take part in the process of amyloid formation [14]. Now it is agreed upon that all amyloids derived from immunoglobulin light chains are named as AL-type. Amyloids of AL-type are classified into two forms. Either they are associated with multiple myeloma or macroglobulinemia or occur as de novo deposits of amyloid (primary amyloidosis), although in the latter cases a meticulous examination of serum and urine revealed M-components in about 80% [6,10,15]. Not all myeloma patients develop systemic amyloid depositions. The incidence was calculated to be about 10 to 15% [6,15,16]. Contrary to the patterns found in serum, λ-L-chains predominate over κ-L-chains at a rate of about 2:1 in amyloid deposits. One subgroup of human λ-L-chains, i.e. subgroup λVI, seems to be especially prone to form amyloid [17]. Characteristically, the examination of an amyloid fibril protein led to the discovery of these subgroups [18]. Amyloid deposition was most often found to occur in the heart, tongue (macroglossia), skin, gastrointestinal tract and in peripheral nerves, but also the spleen, liver, kidneys and lungs may be involved [6,10,15,16].

Amyloid fibrils have some characteristic features. By means of electron microscopy, it was shown that the fibrils have a diameter of 70-100Å, are not branching and are of indefinite length [3,19]. X-ray diffraction analysis of the fibrils revealed that the composing polypeptide chains are ordered perpendicular to the axis of the fibrils and have a ß-pleated sheet conformation [20]. This structure applies for every type of amyloid. Amyloidosis was also designated as ß-fibrillosis by Glenner [6].

Once it was first discovered that immunoglobulin light chains participate in the formation of amyloid deposits, more sequence studies were performed on amyloid fibril proteins of the AL-type. In all cases studied, either complete L-chains [21] or more often fragments thereof have been found as main constituents of the amyloid fibrils [22-35]. With few exceptions, the fragmented L-chains contain the complete V-Region [26,33,36] and extend more or less far into the C-region. When sequence studies were performed simultaneously on the amyloid fibril proteins and the Bence Jones proteins from the urine of the same patient, sequence identity was found in most cases. Exceptions were the proteins BAN [28] and MAL [32] where amino acid substitutions occurred between the sequence of the fibril protein and the Bence Jones protein. Nevertheless, it could not be excluded that the patient who produced the MAL amyloid protein hosted a second active clone secreting an unrelated Bence Jones protein. Attempts to characterize the C-terminal end of amyloid proteins revealed almost uniformly a ragged end, so that exact cleavage points could not be easily defined. In this way the cleavage points given in figure 1 describe only predominant C-terminal ends and do not exclude other split points around the marked position.

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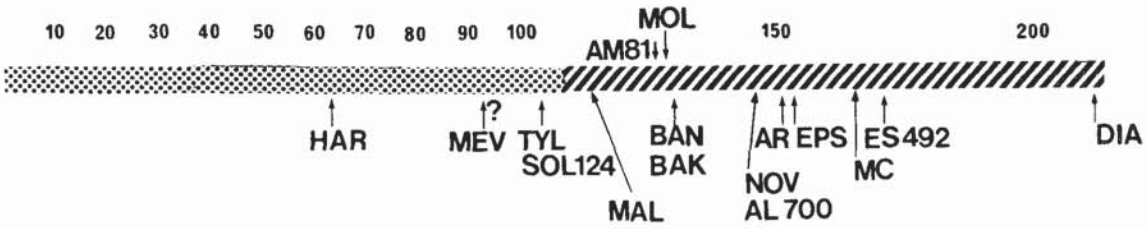

Fig.1. Localisation of split points along an immunoglobulin L-chain. The dotted area marks the V-region, while the C-region is hatched. Arrows point to cleavage sites. Information about the C-terminal ends are given in the following references: HAR [26],MEV [23], TYL [36], SOL124 and AL700 [33], MAL [32], AM81 [31], MOL [27], BAN [28], BAK [29], NOV and MC [34], AR [22], EPS [24], ES492 [25] and DIA [35].

Although it seems that the split points are scattered along the N-terminal part of the C-region, there is nevertheless some accumulation around the residues 110, 130 and 150. These areas are exposed on the surface of the molecule according to three dimensional models calculated from the x-ray diffraction patterns of crystallized antibody molecules [37,38]. It is obvious that these areas represent vulnerable sites for proteolytic action of enzymes, but no individual proteases responsible for this cleavage have, as yet, been identified. Serine proteases are thought to play a role in the formation of protein AA from the precursor apoSAA [39], but again there are no experiments reported which allow the conclusion that these enzymes also participate in the formation of amyloid fibrils of the AL-type.

Bence Jones proteins have been digested by in vitro incubation with trypsin, pepsin and lysosomal enzymes from human kidney [40-42]. Some, but not all proteins responded by forming precipitates in the digestion mixture. When these precipitates were studied by electron microscopy the formation of amyloid-like fibrils had been seen. This phenomenon occurred regardless whether the Bence Jones proteins belonged to the κ- or to the λ-class. Some of the precipitates stained positive with Congo red and exhibited the green birefringence characteristic for amyloid. Chemical studies revealed that in one case the amyloid-like material was composed of a small fragment (4600 Dalton) from the N-terminal part of the V-region [40]. This observation has led to the hypothesis that "amyloidogenic" and "non-amyloidogenic" immunoglobulin L-chains may exist. Thus, conformational properties may influence the processes which lead to the progression of amyloid in individual patients with plasma cell dyscrasias. It is interesting in this context that tryptic digestion of reduced and alkylated L-chains often results in the formation of precipitates. Positive staining with Congo red was exhibited by some precipitates as also the green birefringence in polarized light [43]. Fibril formation was seen in these precipitates by electron microscopy [44]. We produced the precipitates by tryptic digestion of immunoglobulin κ- and λ-light chains. The peptides forming them were separated by high performance liquid chromatography and subsequently subjected to amino acid sequence analysis. In this way we determined their positions within the chains. In almost

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all cases studied so far, the peptides derived from position 62-103 of the V-region and were uniformly accompanied by a peptide, which included residues 127-142 in κ-chains and 130-149 in λ-chains. Because the formation of ß-pleated sheets is one of the prerequisites to the formation of amyloid fibrils, the peptides mentioned above must have refolded in this conformation, although the parent chains were completely unfolded under the conditions used to reduce and alkylate the sulfhydryl groups. Therefore, it has been concluded that the ability to reform ß-pleated sheet structures is an intrinsic property of certain immunoglobulin light chain stretches. This ability may be further pronounced by the attachment of carbohydrate side chains, as was noted to occur more frequently in AL-type amyloid proteins [24,25,27,33,34]. Other authors recognized that in some amyloidogenic L-chains hydrophilic residues were exchanged against hydrophobic amino acids, which also may enhance their disposition to form amyloid fibrils [28-30]. However, some steps in the pathogenesis of AL-type amyloids from immunoglobulin L-chains still await clarification.

Heavy chains of immunoglobulins are not known to form amyloid fibrils. A few papers report about the occurrence of amyloidosis in the course of γ-heavy chain disease [45,46], but it was not proven in these cases that the amyloid derives from H-chains. We recently had the opportunity to study the amyloid fibrils of a patient with primary amyloidosis. The amyloid fibrils extracted from the spleen were examined by amino acid sequence analysis. These studies revealed that in this case the amyloid fibrils were composed of a VH_{III} domain directly connected to a CH3-domain. The complete hinge region as well as the CH1- and CH2-domains were found to be deleted in this protein [47]. Thus, it is clearly established that also immunoglobulin heavy chains may be involved in the formation of amyloid. The suggestion that this occurs rarely only remains to be clarified by further studies.

Generally, many factors may contribute to the formation of amyloid from immunoglobulin chains. There is no doubt that proteolytic degradation of the L-chains or mutations during the assembly of H-chains take part in the pathogenesis of amyloidotic lesions. Despite the progress which has been achieved during the last years, further efforts are necessary before the goal of preventing amyloidosis can be reached.

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