Complementary DNA sequence of human amyloidogenic immunoglobulin light-chain precursors.

Pierre Aucouturier, Ahmed Khamlichi, Jean-Louis Preudhomme, Marc Bauwens, Guy Touchard, Michel Cogné

To cite this version:


HAL Id: inserm-01876794
http://www.hal.inserm.fr/inserm-01876794
Submitted on 18 Sep 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Complementary DNA sequence of human amyloidogenic immunoglobulin light-chain precursors

Pierre AUCOUTURIER,*‡ Ahmed A. KHAMLICHI,* Jean-Louis PREUD'HOMME,* Marc BAUWENS,‡
Guy TOUCHARD‡ and Michel COGNE*

*Laboratories of Immunology and Immunopathology and of Molecular Immunology, CNRS URA 1172, F-86021 Poitiers, France, and ‡Department of Nephrology, University Hospital, F-86000 Poitiers, France

The primary structure of three amyloid precursor light chains was deduced from the sequence of complementary DNA (cDNA) from bone marrow cells from patients affected with classical λ (patient Air) or κ (patient Arn) amyloidosis and from a patient (Aub) in whom λ amyloid deposits were unusual by their perimembranous location in the kidney glomerulus. All three RNAs were of normal size, as estimated by Northern blotting, and encoded normal-sized light chains. The deduced light-chain sequence from patient Arn was related to the V\(\lambda_1\) subgroup, and included ten residues that had not been previously reported at these positions, only one of which (Leu-21) was located in a β-sheet (4-2). The unusual presence of Asn-70 determined a potential N-glycosylation site. The sequence of the light chain from patient Air belonged to the V\(\lambda_2\) subgroup, and included three unusually located amino acid residues, one of which had already been reported in an amyloidogenic λ-chain. The sequence of the light chain from patient Aub was related to the V\(\lambda_5\) subgroup, and contained five amino acid residues that had not previously been described at the corresponding positions; two of them (His-36 and Ser-77) were located in β-sheets (3-1 and 4-3 respectively). This sequence was also peculiar because of the presence of numerous acidic residues in the complementarity-determining regions. Such unusual primary structures might be responsible for the amyloidogenic properties of these light-chain precursors.

INTRODUCTION

The propensity of certain proteins to form fibrillar insoluble extracellular deposits in tissues leads to amyloidosis, a serious disease which often affects multiple organs (Glenner, 1980; Stone, 1990). A variety of amyloidogenic precursors of different origins have been identified; a common structural property is their predominant β-pleated sheet conformation, which is responsible for the general structural organization of amyloid fibrils.

Monoclonal immunoglobulin light chains are responsible for amyloid light chain (AL)-type amyloidosis. In more than 80% of cases of AL primary amyloidosis, a monoclonal light chain can be detected by sensitive methods in the patient’s urine and/or serum (Gertz & Kyle, 1989), and in every case careful study of the bone marrow cells demonstrates a significant monoclonal plasma cell population (Buxbaum, 1986; Preud’homme et al., 1988). Proof of the relationship between plasma cell-secreted and tissue-deposited monoclonal light chains has long been established (Glenner et al., 1971). Several observations suggest that structural peculiarities of the precursor light chain may play a critical role in the pathogenesis of AL amyloidosis: the λ isotype (especially C\(\lambda_\lambda\)) is largely over-represented (Walker et al., 1988; Gertz & Kyle, 1990), as is the rare V\(\lambda_4\) variability subgroup (Solomon et al., 1982), and aberrant immunoglobulin biosynthesis patterns were found in bone marrow cells from patients with primary or myeloma amyloidosis (Buxbaum, 1986; Preud’homme et al., 1988).

Although complete primary structures of several AL-type amyloid fibrils have been published in the last decade (Sletten et al., 1981; Eulitz & Linke, 1985; Toft et al., 1985; Tveteraas et al., 1985; Dwulet et al., 1985, 1986; Holm et al., 1986; Benson et al., 1989; Liepniks et al., 1990), no specific common sequence could be identified. Unusual structural features of light chains include N-glycosylation (Toft et al., 1985; Tveteraas et al., 1985; Dwulet et al., 1986; Holm et al., 1986), the presence of numerous hydrophobic residues (Dwulet et al., 1986) and insertion of acidic amino acids (Sletten et al., 1981). However, these findings do not provide a general explanation for the mechanisms of AL amyloid fibril formation.

Since most amyloid fibrils mainly contain light-chain fragments, often with a length heterogeneity, relevant information might be better obtained from a knowledge of complete structures of amyloidogenic precursor proteins. Only a small number of circulating light-chain amyloid precursors have been studied so far at the molecular level; the three completely determined sequences belonged to the V\(\lambda_1\) subgroup (Takahashi et al., 1980; Tonoike et al., 1985; Eulitz et al., 1987). Further study of a number of amyloid light chains hence would appear to be potentially useful. Large-scale studies are hardly feasible at the protein level and a cDNA cloning strategy would appear to be more suitable. We present the complete primary structures, deduced from cDNA sequences, of one κ and two λ amyloid light-chain precursors; one of the latter was associated with an unusual form of AL-type amyloidosis featuring an unusual location of kidney deposits.

MATERIALS AND METHODS

Cell samples

Bone marrow cells were collected by aspiration from three patients with AL amyloidosis. After sedimentation in 0.8% gelatin for 30 min at 37 °C, a sample was analysed by immunofluorescence with specific anti-immunoglobulin heavy and light
chain antibodies; the remainder was used for RNA extraction. Bone marrow from patient Arn contained 9% monoclonal plasma cells with intracytoplasmic κ chain and no detectable heavy chain, in the absence of clinical evidence of myeloma; a κ-type Bence–Jones protein was detected in the urine. Patient Air presented with Waldenstrom’s macroglobulinemia; bone marrow smears showed a lymphoplasmaeytic proliferation including 3% plasma cells containing an IgM(λ) which was detectable in the serum. Small amounts of urinary free λ chains were also detectable. Patient Aub had 3% bone marrow plasma cells stained by anti-κ and anti-λ fluorescent antibodies; a monoclonal IgA(λ) was found in the serum and a λ-type Bence–Jones protein in the urine. In all three patients pathological, immunofluorescent and ultrastructural studies of kidney biopsy specimens showed typical fibrillar deposits stained by Congo Red, thioflavine T and anti-κ (Arn) or anti-λ (Air and Aub) antibodies. In patient Aub, glomerular deposits predominantly involved the epithelial aspect of the capillary walls.

RNA preparations

Cells from the patients’ bone marrow were washed with 0.01 M-phosphate-buffered saline, pH 7.4 (PBS; 0.15 M-NaCl) and lysed in 4 M-guanidine isothiocyanate. After a 30 s homogenization with a blender, total RNA was separated by ultracentrifugation for 18 h at 170000 g on 5.7 m-cesium chloride. Northern blot analyses were performed by electrophoresis on 1% agarose/20 mM-Mops/5 mM-sodium acetate/1 mM-EDTA/0.7 M-formaldehyde gels, transfer on to Nyal sheets and hybridization with appropriate DNA probes (Davies et al., 1986). The κ probe was a 2.5 kb EcoRI genomic fragment including the entire constant-region exon; the λ probe was a 3.5 kb EcoRI/HindIII fragment containing the Cκ exon (Cogné et al., 1991). Northern blots were hybridized with heavy chain constant-region probes to evaluate the relative amount of RNA from polyclonal immunoglobulin-secreting cells: the Cκ probe was a 1.5 kb PstI fragment of the Cκ gene segment including the hinge, CHκ and CHκ exons (Cogné et al., 1991); the

Fig. 1. Northern blot analysis of RNA (5 μg per lane) extracted from bone marrow cells of AL-amyloidosis patients

(a) Blot was hybridized with a Cκ probe; (b) blot hybridized with a Cλ probe. Lane 1, Burkitt’s lymphoma cell line Ly67 producing a normal-sized (1.2 kb) λ-chain mRNA (control); lane 2, RNA from patient Air; lane 3, RNA from patient Aub; lane 4, Burkitt’s lymphoma cell line J1 producing a normal-sized κ-chain; lane 5, RNA from patient Arn.

RNA from patients’ biopsies or peripheral blood cells was extracted from bone marrow or peripheral blood mononuclear cells using standard procedures (Davies et al., 1986). The κ probe was a 2.5 kb EcoRI genomic fragment including the entire constant-region exon; the λ probe was a 3.5 kb EcoRI/HindIII fragment containing the Cκ exon (Cogné et al., 1991). Northern blots were hybridized with heavy chain constant-region probes to evaluate the relative amount of RNA from polyclonal immunoglobulin-secreting cells: the Cκ probe was a 1.5 kb PstI fragment of the Cκ gene segment including the hinge, CHκ and CHκ exons (Cogné et al., 1991); the

![Northern blot analysis of RNA (5 μg per lane) extracted from bone marrow cells of AL-amyloidosis patients](image)

Leader

-22

ATG GAC ATG AGG GTC GCT GAG CAG TTC TCT GTG Met Asp Met Arg Val Pro Ala Gin Leu Leu Gly Leu Leu Leu Trp

Vκ region

-4

CTC TCA GGT GCC AGA TGT GAC ATC AGT ACC CAC TCT CCA TCT TCC

Leu Ser Gly Ala Arg Cys Asp Ile Gin Met Thr Gin Ser Pro Ser Ser

+11

CTG TCT GCA TCT GTA GGA GAG GGA GTC ACC CTC ACT TGC CAG GCG AGT

Leu Ser Ala Ser Val Gly Asp Gly Val Thr Leu Thr Cys Gin Ala Ser

+31

CAG GAC ATT AGC GAC TAT TTA AAT TGG TAT CAG CAG AAA GTA GGG GAA

Gln Asp Ile Ser Asp Tyr Leu Asn Trp Tyr Gln Gln Lys Val Gly Glu

+41

G GCC CCT AAG CTC ATG ATT TAC GAT GCA TCA TAC TTG GAA ACA GGC GTC

Ala Pro Lys Leu Leu Met Tyr Asp Ala Tyr Ser Tyr Leu Thr Gly Val

+51

CCA TTA AGA TGC AGT GAG GGA TGT GCG ACA TAT TAC TTG GCG ACA CAG Cyg ATT

Leu Ser Leu Ala Gin Ser Gin Tyr Asp Leu Pro Phe Ser Gly Ser Thr Arg Leu Ile

Cκ region (Km(3) allele)

AAA CGA ACT GTC GGA CCA TCT...

Lys Arg Thr Val Ala Pro Ser...

Translation is indicated below the nucleotide sequence. Numbering is according to Kabat et al. (1987). The sequence of the constant segment corresponded to a normal Km(3) allele (not shown). Codons corresponding to S' ends of segments Vκ1, Jκd and Cκ are indicated.

Cλ probe was a 1.2 kb PsI genomic fragment containing the 3' end of CHλ, the CHλ and S' part of CHλ exons (Cogné & Preud’homme, 1990).

Poly(A) mRNA was isolated from total RNA preparations by affinity chromatography on oligo(dT)-cellulose (Pharmacia, Upsala, Sweden) according to the manufacturer’s instructions.

cDNA cloning and sequencing

cDNA libraries were constructed using standard procedures (Davis et al., 1986). Briefly, single-stranded cDNAs were synthesized with reverse transcriptase by extending oligo(dT) primers on poly(A) mRNA from each patient (Amersham International, Amersham, Bucks., U.K.). Double-stranded cDNA was obtained by adding RNAase H and DNA polymerase I and cloned in the Agt10 vector using EcoRI adaptors (Amersham); recombinant phages were screened with the appropriate light-chain probes. All cDNA clones were sequenced on both strands by cloning full-length CDNA and their restriction fragments in mp18 and mp19 M13 vectors. Sites used for subcloning were a SacI restriction site in the constant region of the light chain from patient Arn, two EcoRI sites in the V-C segment of the light chain from patient Air and two BamHI sites in the variable and 3' flanking regions of the light chain from patient Aub. In addition, synthetic primers complementary to the S' end of the Cκ and Cλ exons allowed us to obtain sequences overlapping variable-region restriction sites. Sequencing was performed by the dyeoxy termination method (Sanger et al., 1977) with T7 polymerase using an automated laser fluorescence DNA sequencer (ALF; Pharmacia).
RESULTS

Analyses of total RNA extracted from the patients’ bone marrow samples by Northern blotting with light-chain constant-region probes showed essentially normal-sized transcripts of the respective light chain type (Fig. 1). In every case, a study of the cDNA library showed more than 1% of the Agt10 cDNA clones hybridizing with the corresponding light-chain probe. Identification of the predominant monochromic light chain was achieved by sequencing different cDNA clones; in two cases (patients Air and Arn) three out of three clones studied were identical. In patient Aub, one cDNA clone out of four appeared to originate from the polyclonal B-cell population. These sequences confirmed the normal size of the three amyloid precursors, and revealed overall normal structures but original substitutions in all cases.

The sequence from patient Arn included a variable segment related to the V₃ subgroup, rearranged with J₃ and a normal C₃ segment of the Km(3) allotype (Fig. 2). Comparisons of the deduced peptide sequence with complete V₄ sequences (Kabat et al., 1987) showed ten previously unreported amino acid residues: two in framework region (FR1) (Gly-18, Leu-21), three in FR2 (Val-40, Glu-42, Met-48), one in complementarity-determining region (CDR2) (Tyr-53), two in FR3 (Leu-60, Asn-70) and two in CDR3 (Ser-92, Phe-96); the sequence Asn-Tyr-Ser determined a potential N-glycosylation site at position +70. Among these residues, only Leu-21 was located in a β-sheet (β-sheet 4-2, Edmundson et al., 1975).

The sequence from patient Air included a V₃ segment rearranged with J₃ and a normal C₃ exon (Fig. 3). Three aminoacids, Val-52 (CDR2), Glu-85 (FR3) and Glu-97 (CDR3), had never been found at the corresponding locations in previously described V₃ sequences; all were located in loops between β-sheets.

The sequence from patient Aub was made up of a V₄ segment, a J₃ and a C₃ segment (Fig. 4). It included five amino acid residues which had not been reported at these positions: Ser-30 and Asp-31 in CDR1, His-36 in FR2, Ser-77 in FR3 and Glu-92 in CDR3; His-36 and Ser-77 were located in β-sheets 3-1 and 4-3 respectively.

DISCUSSION

AL-type amyloidosis is a structurally heterogeneous entity. Indeed, a feature of each case is visceral deposits of a homogeneous monochromatic light chain and/or its fragments with a unique variable region. This makes the study of structure–pathogenicity relationships complex, since large series of experiments are required to be able to draw conclusions. The present results show that cDNA sequencing of the precursor light chain at the secretion clone level is a possible approach, having the following advantages over protein studies: (1) circulating light chains are not always detectable in biological fluids; (2) obtaining fresh tissues containing amyloid proteins is restricted by a number of practical factors; (3) purification of amyloid substances in sufficient amount without proteolysis is tedious and sometimes difficult.

Several structural features of the amyloidogenic light chains reported here are worth noting. Previously unreported or infrequent amino acid residues were found at certain locations. As already suggested by others (Dwuetal., 1985), it is probable that these residues present in the framework regions, especially in portions corresponding to β-sheets, are more likely to influence the conformation of the domain than amino acids located in the CDRs.

Amyloidogenic light chain from patient Arn was related to the V₃ subgroup, which is the most frequent subgroup among monochromic κ-chains and is slightly over-represented in amyloidosis (Solomon & Weiss, 1988). Only Leu-21 appeared to be an unusual residue inside a β-sheet (most sequenced V₄ regions bear an isoleucine at this position) and it is unlikely that
it could markedly modify the folding of the light chain. None of the other rare amino acid residues of the sequence from patient Arn was found in the two known V_{41} amylloid proteins (Dwulet et al., 1986; Liepniets et al., 1990). The potential glycosylation site at position +70 has not previously been described in V_{41} light chain. In a patient with light chain deposition disease and a deposited κ-light chain of the V_{41} subgroup, we have demonstrated that Asn-70 was actually glycosylated (Cogné et al., 1991); N-glycosylation could play some role in tissue deposition in both light chain deposition disease and amyloidosis. Indeed, an important proportion of sequenced amyloid light chains, including one of the V_{41} subgroup (Dwulet et al., 1986), proved to be N-glycosylated (Toft et al., 1985; Tveteraas et al., 1985; Holm et al., 1986; Dwulet et al., 1986).

The sequence from patient Air belonged to the V_{41} subgroup. Three V_{41} subgroup amylloidogenic Bence–Jones proteins [NIG51 (Takahashi et al., 1980), NIG77 (Tonoike et al., 1985) and ZIM (Eulitz et al., 1987)] and one amyloid substance [EPS, (Toft et al., 1985)] have already been sequenced. Several rare amino acids (Ala-13, Gly-74 and Gly-89 in β-sheets 4-1, 4-3 and 3-2 respectively and Thr-80 and Gly-81 in peptide loops) were also present in the amyloid protein EPS. Such similarity between the variable regions of the light chain of patient Air and the protein EPS, especially in the β-sheets, might possibly lead to delineation of an ‘amyloidogenic family’ inside the V_{41} subgroup. However, strong similarities were also found to proteins NIG64 (Tonoike et al., 1985), New (Langer et al., 1968) and BL2’ CL (Tsujimoto & Croce, 1984), which are not known to be associated with amyloidosis. Finally, it is noticeable that the presence of alanine at position +42 has been reported in the amyloidogenic Bence–Jones protein NIG51 only (instead of a threonine in 12 out of 13 other V_{41} light chains and lysine in the other one) (Takahashi et al., 1980).

The sequence from patient Aub belonged to the V_{33} subgroup. Only one such AL-type amyloid variable region had been completely analysed so far (protein Mol; Holm et al., 1986). None of the infrequent amino acids found in the light chain of patient Aub was present in protein Mol. A striking difference was the absence of an N-glycosylation site in the light chain of patient Aub, whereas protein Mol was glycosylated on Asn-90. The sequence from patient Aub demonstrated a high density of acidic residues (Asp-31, -50, -51 and -53, Glu-92) in the CDRs. Insertion of two aspartic acid residues between positions 66 and 67 was noted in the V_{34} amyloid protein AR (Sletten et al., 1981), and a possible role for interactions between charged amino acids in amyloid fibril formation has been suggested (Solomon & Weiss, 1988). This is in accordance with the finding of a low mean isoelectric point for urinary Bence–Jones proteins in amyloidosis (Bellotti et al., 1990). Patient Aub presented with an unusual type of AL-amyloidosis with predominant perimembranous deposits, as already described in a few cases for which molecular studies had not been carried out (Shiiki et al., 1989). The study of further patients might allow us to delineate special structural features of perimembranous-type amyloidosis.

In conclusion, our results confirm that the structural abnormalities of immunoglobulin light chains that could play a role in amyloidosis are multiple. cDNA sequencing seems to be a powerful strategy for accumulating molecular data and defining more precisely the complex relationship between light-chain structure and pathogenicity.

We thank Ms. F. Buisson for editorial assistance and Dr. C. Giraud for her help in this study. This work was supported by grants from INSERM (CRE 893012) and the Association pour la Recherche sur le Cancer. A. A. K. is a recipient of a fellowship from the Ligue Nationale Centre de Cancer.

REFERENCES

Stone, M. J. (1990) Blood 75, 531-545

Received 12 July 1991/23 October 1991; accepted 28 October 1991

P. Aucouturier and others