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Structural studies of a carbohydrate-containing immunoglobulin-λ-light-chain amyloid-fibril protein (AL) of variable subgroup III

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The amino acid sequence of the variable region of a carbohydrate-containing amyloid-fibril protein MOL of immunoglobulin-light-chain type (AL) was elucidated. The sequence determination involved cleaving the protein with CNBr, BNPS-skatole, thermolysin and trypsin. The sequenced protein consisted of about 130 amino acid residues; however, gel-filtration and *N*-terminal analysis studies revealed AL proteins ranging in M_r from about 10000 to 25000. The oligosaccharide chain was found to be bound in the hypervariable region. By sequence homology to other λ chains the AL protein MOL was shown to be of the V λ III subgroup.

INTRODUCTION

It is well established that the term amyloidosis represents a group of diseases rather than a single disease entity (Husby & Sletten, 1986). It is also evident that the amyloid fibril can be made up by different proteins or differently sized fragments of these proteins (Husby & Sletten, 1986; Glenner, 1980*a*,*b*). The various amyloid proteins appear to have in common that they can change their configuration and aggregate into fibrils with unique ultrastructural, tinctorial and optical properties (Husby & Sletten, 1986; Glenner, 1980*a*,*b*). Monoclonal immunoglobulin light chains or fragments thereof (AL proteins) make up the fibrils in primary amyloidosis, and protein AA is the chief fibril protein in the secondary form of the disease (Husby & Sletten, 1986; Glenner, 1980*a*,*b*). The first amino acid sequence data of AL proteins (Glenner *et al.*, 1971) as well as of AA protein (Benditt *et al.*, 1971) were reported in 1971. However, until now, few amyloid fibril proteins of AL type have been characterized by complete amino acid sequencing.

The reason why AL-type amyloid fibrils are produced only in some individuals with plasma-cell dyscrasias is not known. A possible mechanism is the presence of specific amino acid substitutions in the variable region of a monoclonal light chain that prime the formation of the fibrils. Particular interest has been paid to the λ VI subgroup of light chains because of its close association with AL proteins (Husby & Sletten, 1980; Sletten *et al.*, 1981; Solomon *et al.*, 1982). Recently we have also reported the amino acid sequence of the variable regions of two AL proteins of light-chain subgroups λ I and λ II, which contained some characteristic amino acid substitutions, and in addition were found to be glycosylated in the complementarity-determining regions (Toft *et al.*, 1985; Tveteraas *et al.*, 1985). The significance of these structural characteristics for making the light-chain proteins more 'amyloidogenic' is still obscure. In order to increase the available body of data concerning the structure of AL proteins, we now report the amino acid sequence of the variable region of a glycosylated AL protein derived from another patient with primary amyloidosis.

MATERIALS AND METHODS

Source of amyloid fibrils

Amyloid fibrils were obtained from the spleen of a 74-year-old male (MOL) with primary amyloidosis. Systemic amyloidosis was found at autopsy, with massive involvement of the heart, spleen and tongue, and less pronounced in the lungs, endocrine glands and liver.

Extraction and purification of the amyloid-fibril proteins from the spleen was performed as described in detail previously (Pras *et al.*, 1968; Sletten *et al.*, 1974). Briefly, amyloid fibrils were extracted from the tissue with distilled water after the soluble proteins had been removed by repeated washings with saline (0.9% NaCl). The amyloid-containing water supernatant was freeze-dried. Amyloid fibrils were treated with 0.5 M-Tris/HCl buffer, pH 8.5, containing 6 M-guanidinium chloride, 0.05 M-dithiothreitol and 1.0 mM-EDTA and thereafter subjected to two successive gel filtrations on a Sephadex G-100 column (3.2 cm × 99 cm) eluted with 5 M-guanidinium chloride in 0.1 M-Tris/HCl buffer, pH 7.6. Selected fractions were gel-filtered on a Sephadex G-75 column.

The fractions containing AL protein MOL were pooled, dialysed against water and freeze-dried. SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970).

Amino acid analysis

Amino acid analyses of protein and peptide samples were performed as described by Sletten *et al.* (1981).

Chemical cleavage

Chemical cleavage of the protein with CNBr and with BNPS-skatole [3′-bromo-3-methyl-2-(2-nitrophenylsulphenyl)indolamine] was as described previously (Fontana, 1972; Sletten & Husby, 1974).

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Proteolytic digestion

Tryptic digestion of reduced and carboxymethylated protein and digestion with thermolysin were performed as described by Sletten *et al.* (1981).

Carbohydrate analysis

The compositions of the carbohydrate moiety of protein MOL and of glycopeptides were determined by g.l.c. analysis according to Bolton *et al.* (1965).

Purification of peptides

CNBr-cleaved protein was gel-filtered on a Sephadex G-75 (fine grade) column (1 cm × 139 cm) equilibrated with 10% (v/v) formic acid. The flow rate was 4.1 ml/h. The effluent was monitored at 280 nm with an LKB 8300A Uvicord II photometer. Further purification was performed by gel filtration and by ion-exchange chromatography on CM-Sephadex C-25 resin (Pharmacia Fine Chemicals) (Fowler, 1978). The material was applied to a column (1.8 cm × 15 cm) of CM-Sephadex C-25 in 0.02 M-ammonium acetate buffer, pH 5.0, containing 8 M-urea. The peptides were eluted with a linear gradient of 0–0.5 M-NaCl in the same buffer. The total volume was 110 ml.

BNPS-skatole-cleaved protein was initially gel-filtered on a Sephadex G-50 (fine grade) column (1 cm × 139 cm) equilibrated with 10% (v/v) formic acid, and was further purified by several gel filtrations. Separation and purification of peptides obtained from the digestion with thermolysin was done by h.p.l.c. and t.l.c. The h.p.l.c. system used was an isocratic elution with 0.1% H_3PO_4 on a LiChrosorb RP-column and a linear gradient of 0–50% (v/v) acetonitrile in 0.1% H_3PO_4 (Fullmer & Wasserman, 1979). Trypsin-digested carboxymethylated protein was gel-filtered on a Sephadex G-50 (fine grade) column equilibrated with 0.2 M-NH_4HCO_3 and was further purified by gel filtration and by t.l.c. (Sletten *et al.*, 1981).

N-Terminal analysis

Automatic Edman degradation (in a JEOL JAS-47K instrument) and analysis of the phenylthiohydantoin derivatives were performed as described by Sletten *et al.* (1981). Dansylation (5-dimethylaminonaphthalene-1-sulphonylation) of peptides and separation of the dansyl-amino acids were performed as described by Gray (1972).

Nomenclature

Peptides are numbered on the basis of their occurrence in the deduced sequence, starting from the *N*-terminus. Partial-digestion products are labelled with consecutive numbers. As an example Th-(8+9+10) is one peptide covering the positions 31–59. The following abbreviations are used: CB, peptides obtained after cleavage with CNBr; BNPS, peptides obtained after cleavage with BNPS-skatole; T, tryptic peptides; Th, thermolytic peptides.

RESULTS

Characterization of isolated polypeptides

AL protein MOL was isolated from the spleen of a patient with primary amyloidosis. Gel filtration of the isolated amyloid fibrils revealed five distinct peaks (Fig. 1). *N*-Terminal analyses for from 12 to 28 steps of the material in the four retarded peaks showed that all four peaks consisted of an immunoglobulin λ light chain of subgroup III (Fig. 2). The material in peak 1 (V_0) was not analysed. The M_r of the light-chain material ranged from about 10000 (peak 5) to 25000 (peak 2). The main peak (peak 3) was shown by gel filtration on a Sephadex G-75 column to have an M_r of about 16000. SDS/polyacrylamide-gel electrophoresis of the latter material, performed as described by Laemmli (1970), showed one major band corresponding to an M_r of about 22000. The amino acid composition of the AL protein from the main peak was determined on several batches, and the mean values are shown in Table 1. The composition is based on one residue of methionine/molecule, which makes up a total of about 130 residues. As the amino acid analyses revealed glucosamine, the complete carbohydrate composition was determined. The results are shown in Table 2.

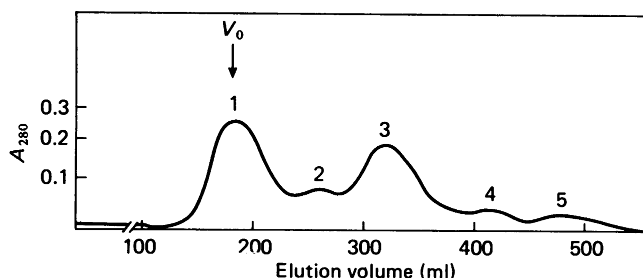

Fig. 1. Gel filtration on Sephadex G-100 of amyloid fibrils obtained from the spleen of patient MOL

The column (3.2 cm × 99 cm) was equilibrated and eluted with 5 M-guanidinium chloride in 0.1 M-Tris/HCl buffer, pH 7.6.

N-Terminal analysis of the CNBr-cleaved protein gave the derivatives of aspartic acid and tyrosine. As the amino acid sequence of the first 28 residues of the protein was already established, a part of this CNBr-cleaved protein fraction was used without further purification for automatic Edman degradation. The results confirmed the already established *N*-terminal sequence of the protein and elucidated the sequence of 26 amino acid residues of the *C*-terminal fragment (CB-2) shown in Fig. 2. The CNBr-cleavage fragments, CB-1 and CB-2, proved to be very difficult to separate from each other. After several gel filtrations and ion-exchange chromatography in the presence of 8 M-urea, a preparation of CB-1 was obtained with a purity of about 80%, as checked by *N*-terminal analysis. This purified CB-1 fragment was then used for digestion with thermolysin. The amino acid composition of these peptides purified by h.p.l.c. and by t.l.c. are shown in Table 3. The peptides confirmed the already established sequence, and from comparison with other V λ III chains the amino acid sequences of residues 31–40, 45–55, 60–70 and 71–79 were elucidated (Figs. 2 and 3). The three dipeptides corresponding to residues 1–2, 9–10 and 12–13 were not isolated.

From a tryptic digest of reduced and carboxymethylated protein seven peptides were isolated, comprising the residues 1–128 (Table 4). Automatic Edman degradation was performed on peptides T-6 and T-7. Peptides T-5 and T-(4+5+6+7) were found to contain glucosamine. A sample of peptide T-5 was used for determining the complete carbohydrate composition (Table 2). The peptide was found to contain, per molecule, three

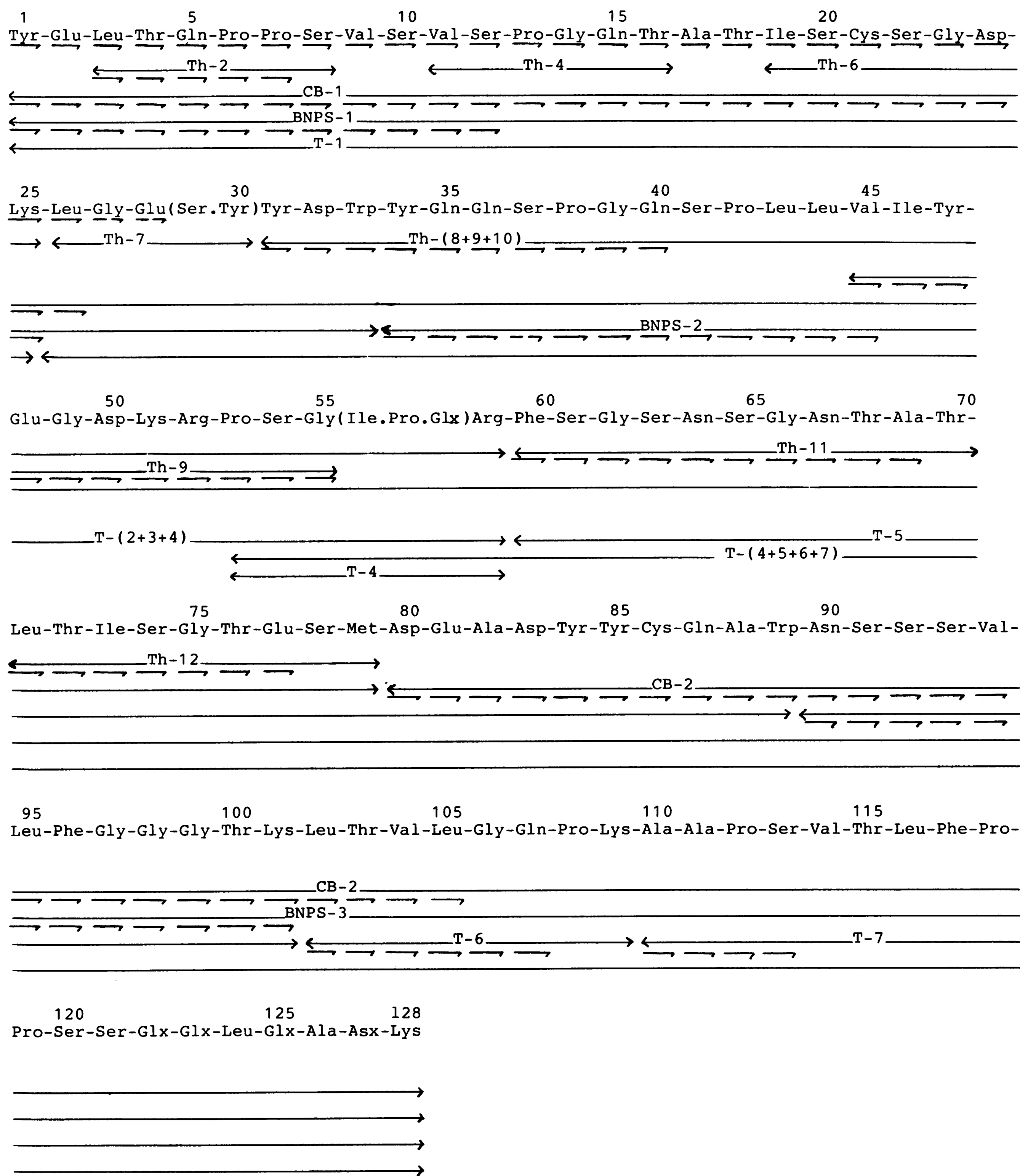

Fig. 2. Amino acid sequence of amyloid-fibril protein MOL

The various peptides are indicated by double-headed arrows. Sequences obtained by Edman degradation with direct identification of amino acid phenylthiohydantoin derivatives are shown by half-arrows. Broken half-arrows indicate residues not directly identified by Edman degradation. T and Th denote peptides obtained after digestion with trypsin and thermolysin respectively. CB and BNPS are peptides obtained after cleavage with CNBr and BNPS-skatole respectively. The presence of tryptophan in position 37 is based on u.v. absorbance and of cleavage with BNPS-skatole. The residues in positions 29–30, 56–58 and 114–128 are based on homology of other λ light chains.

Table 1. Amino acid composition of AL protein MOL

Abbreviation: N.D., not determined.

Amino acid or amino sugar	Composition (residues/molecule) Found	From proposed sequence
Asp	9.24	9
Thr	8.78	10
Ser	16.5	20
Glu	15.2	16
Pro	9.6	11
Gly	11.2	13
Ala	8.00	7
Cys	2.0	2
Val	7.06	6
Met	1.00	1
Ile	3.06	4
Leu	9.44	10
Tyr	5.70	7
Phe	3.32	3
His	0.16	0
Lys	4.80	5
Arg	3.10	2
Trp	N.D.	2
Total		128
GlcN	3.3	3–4

residues of mannose, two residues of galactose, two to four residues of glucosamine, one residue of fucose and only traces of *N*-acetylneuraminic acid and glucose. The numbers of residues of glucosamine, *N*-acetylneuraminic acid and glucose deviate from those found in the protein. The content of glucose is most probably due to contamination from the Sephadex columns. The variation observed with glucosamine and *N*-acetylneuraminic acid could be due to a partially degraded carbohydrate chain (Toft *et al.*, 1985).

Table 2. Carbohydrate compositions of AL protein MOL and of tryptic peptide T-5 derived from the protein

Monosaccharide	Composition (residues/molecule) AL protein	Peptide T-5
Man	2.9	2.6
Gal	2.3	1.8
GlcN	4.2	2.2
NeuAc	0.6	Trace
Fuc	0.7	0.6
Glc	2.1	Trace

Cleavage of the protein with BNPS-skatole resulted in the three peptides BNPS-1, BNPS-2 and BNPS-3, comprising the residues 1–33, 34–89 and 90–130 approximately. *N*-Terminal analysis of a partially purified fraction of BNPS-2 revealed a contamination by BNPS-1 and BNPS-3. However, as the *N*-terminal amino acid sequences of these fragments were already established from structural studies of the fragments CB-1 and CB-2, the amino acid sequence of residues 34–45 could be elucidated. A small amount of a BNPS-skatole-cleavage fragment starting with Lys-Ala-Asp was also found, which corresponds to the *N*-terminal sequence of a BNPS-4 fragment starting with residue 149.

Deduction of the sequence

The amino acid sequence of AL protein MOL is shown in Fig. 2.

Table 3. Amino acid compositions of peptides obtained from thermolysin-digested CB-1 of carboxymethylated AL protein MOL

Analyses were made on ninhydrin-stained fractions from t.l.c. Numbers in parentheses are from sequence determination. Cysteine was measured as carboxymethylcysteine after carboxymethylation. Abbreviation: N.D., not determined.

Amino acid	Composition (residues/molecule) Peptide . . . Th-2	Th-4	Th-6	Th-7	Th-(8+9+10)	Th-9	Th-11	Th-12
Asp	—	0.3	1.0 (1)	0.1	1.9 (2)	1.4 (1)	2.0 (2)	—
Thr	0.6 (1)	0.9 (1)	—	0.1	—	—	1.9 (2)	2.0 (2)
Ser	0.9 (1)	1.2 (1)	1.8 (2)	1.0 (1)	3.0 (3)	0.9 (1)	2.6 (3)	1.9 (2)
Glu	1.1 (1)	1.2 (1)	—	1.0 (1)	4.9 (5)	1.0 (1)	—	1.0 (1)
Pro	2.3 (2)	1.1 (1)	—	—	4.2 (4)	1.4 (1)	—	—
Gly	—	0.9 (1)	1.2 (1)	1.2 (1)	3.0 (3)	2.1 (2)	1.9 (2)	1.1 (1)
Ala	—	—	—	—	—	—	0.9 (1)	—
Cys	—	—	0.8 (1)	—	—	—	—	—
Val	—	0.8* (1)	—	0.1	0.9 (1)	0.9* (1)	—	—
Ile	—	0.1	1.1* (1)	—	1.3 (2)	1.4 (1)	—	0.9 (1)
Leu	0.6* (1)	—	—	0.8* (1)	2.6 (2)	—	—	0.3* (1)
Tyr	—	—	—	0.5 (1)	1.6* (3)	0.3 (1)	—	—
Phe	—	—	—	—	—	—	0.2* (1)	—
Lys	—	—	1.0 (1)	—	1.3 (1)	1.0 (1)	—	—
Arg	—	—	—	—	3.0 (2)	1.1 (1)	—	—
Trp	N.D.	N.D.	N.D.	N.D.	N.D. (1)	N.D.	N.D.	N.D.
Residue nos. . . .	3–8	11–16	19–25	26–30	31–59	45–55	60–70	71–78

* *N*-Terminal residue.

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          1                 10                  20                  30                 40
AL MOL    Y E L T Q P P S V S V S P G Q T A T I S C S G D K L G E(S,Y)Y D W Y Q Q S P G Q

BJ BAU      G             L                 S   T                 Q   V C         K

BJ KERN     A                               V   T         N   E K T F V S   F     R


          41                50                  60                  70                  80
AL MOL    S P L L V I Y E G D K R P S G(I,P,Z)R F S G S N S G N T A T L T I S G T E S M D

BJ BAU        V         H D S               E                 T                   Q A M

BJ KERN                 H T S E       E     E           S     A                 A Q S V D


          81                90                 100             108
AL MOL    E A D Y Y C Q A W N S S S V L F G G G T K L T V L G Q P

BJ BAU                      D   Y T   I

BJ KERN           F     T   D T I T A I                     S
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Fig. 3. Comparison of the sequence of the AL protein MOL with those of other λ chains of subgroup III

The sequence data are as listed by Kabat *et al.* (1983). In the sequences of BJ BAU and BJ KERN only those residues that are not identical with the sequence of AL protein MOL are shown.

Table 4. Amino acid compositions of tryptic peptides obtained from carboxymethylated AL protein MOL

Numbers in parentheses are from sequence determination. Cysteine was measured as carboxymethylcysteine after carboxymethylation. Abbreviation: N.D., not determined.

		Composition (residues/molecule)						
Amino acid or amino sugar	Peptide . . .	T-1	T-(2+3+4)	T-4	T-(4+5+6+7)	T-5	T-6	T-7
Asp		1.3 (1)	1.8 (2)	0.2	6.9 (6)	5.0 (5)	0.1	0.8 (1)
Thr		2.9 (3)	—	0.1	5.8 (7)	5.0 (5)	0.8 (1)	1.2 (1)
Ser		4.5 (5)	3.7 (4)	1.0 (1)	10.6 (12)	7.8 (8)	—	2.3 (3)
Glu		3.2 (3)	5.7 (6)	1.2 (1)	8.7 (8)	3.5 (3)	1.1 (1)	2.6 (3)
Pro		3.2 (3)	3.3 (4)	1.8 (2)	6.3 (6)	—	1.1 (1)	2.6 (3)
Gly		1.9 (2)	4.1 (4)	1.2 (1)	8.0 (8)	6.3 (6)	1.1 (1)	0.1
Ala		1.1 (1)	—	0.2	5.3 (6)	3.1 (3)	—	2.3 (3)
Cys		1.0 (1)	—	—	0.8 (1)	0.9 (1)	—	—
Val		2.2 (2)	1.7 (1)	0.3	3.3 (3)	0.7 (1)	1.2 (1)	1.1 (1)
Met		—	—	—	0.3* (1)	0.9 (1)	—	—
Ile		0.6 (1)	1.5 (2)	0.7 (1)	2.3 (2)	1.0 (1)	—	—
Leu		0.9 (1)	1.4 (3)	0.3	4.1 (6)	2.1 (2)	1.8 (2)	1.8 (2)
Tyr		0.4 (1)	1.8 (4)	—	3.2 (2)	1.3 (2)	—	—
Phe		—	0.1	—	1.6 (3)	1.3 (2)	—	0.9 (1)
His		—	—	—	—	—	—	—
Lys		0.5 (1)	0.6 (1)	0.2	2.5 (3)	1.0 (1)	0.7 (1)	0.3 (1)
Arg		—	2.2 (2)	1.1 (1)	0.9 (1)	—	—	—
Trp		N.D.	N.D. (1)	N.D.	N.D. (1)	N.D. (1)	N.D.	N.D.
Residue nos. . . .		1–25	26–59	53–59	53–128	60–101	102–109	110–128
GlcN		—	—	—	2.5 (3–4)	3.4 (4)	—	—

* Methionine was partially oxidized.

Residues 1–28. *N*-Terminal analysis of AL protein MOL revealed the amino acid sequence of residues 1–28. These residues were verified by Edman degradation of peptide Th-2, *N*-terminal analyses of peptides CB-1 and BNPS-1, and the amino acid compositions of peptides, Th-4, Th-6 and T-1.

Residues 29–79. Residues 29–30 were based on the amino acid composition of peptide Th-7 and on the homology with other λ chains of subgroup III (Fig. 3). The amino acid sequence of residues 31–55 was elucidated by *N*-terminal analysis of peptides Th-(8+9+10), BNPS-2 and Th-9. Residues 56–59 were aligned from the amino acid composition of peptide T-4, the specificity of thermolysin and trypsin, and homology with other human λ light chains of subgroup III (Fig. 3). The amino acid compositions of peptides T-(2+3+4) and Th-(8+9+10) are in agreement with the residues in positions 26–59. Automatic Edman degradation of peptides Th-11 and Th-12 revealed the amino acid sequences of residues 60–69 and 71–77. Residues 70 and 78 were determined indirectly on the basis of the compositions of peptides Th-11 and Th-12. The alignment of peptides Th-11 and Th-12 was based on the composition of peptide T-(4+5+6+7) and homology with other light chains of subgroup III (Fig. 3).

Residues 79–128. Residue 79 was established by the specificity of cleavage with CNBr. The amino acid sequence of residues 80–105 was elucidated by Edman degradation of peptide CB-2. Residues 90–101 were verified by *N*-terminal analysis of peptide BNPS-3, and peptides T-6 and T-7 gave the amino acid sequence of residues 102–113 except for residues 108 and 109. These residues were determined from the amino acid compositions of peptides T-6 and T-(4+5+6+7) and on the specificity of trypsin. Residues 113–128 were aligned from the amino acid composition and from comparison with the constant region from other λ light chains.

Analyses of carbohydrate-containing polypeptides

Amino acid analyses of acid-hydrolysed samples of the AL protein and of the tryptic peptides T-(4+5+6+7) and T-5 revealed glucosamine. As none of the other tryptic and thermolytic peptides containing residues 1–79 gave glucosamine, the carbohydrate has to be linked to the polypeptide between positions 80 and 101. Automatic Edman degradation of peptide CB-2 showed no drastic change in the yield that could indicate a glycosylated amino acid derivative. However, the amino acid sequence found in positions 90, 91 and 92 (-Asn-Ser-Ser-) is an appropriate acceptor sequence for *N*-glycosylation (Marshall, 1974; Sox & Hood, 1970). *N*-Terminal analysis of a partially purified fraction BNPS-3 showed the phenylthiohydantoin derivative of asparagine in addition to that of tyrosine from peptides BNPS-1 and BNPS-2. No determination of amino acid composition of fraction BNPS-3 was made.

DISCUSSION

Gel-filtration studies of amyloid fibrils obtained from the spleen of the patient revealed four retarded peaks ranging in M_r from about 25000 to 10000. The protein material in all four peaks had an identical *N*-terminal amino acid sequence, showing that the *C*-terminal constant region varied in length and that these light chains have been degraded to different states. Other studies of AL proteins have also indicated a ragged *C*-terminal (White *et al.*, 1973; Husby *et al.*, 1981; Eulitz & Linke, 1982; Toft *et al.*, 1985; Tveteraas *et al.*, 1985); however, the present paper contains the first report where all the retarded gel-filtration fractions of AL amyloid fibrils have been checked by *N*-terminal analyses.

The protein material in the main peak was found to have an M_r of about 16000. The amino acid composition of this AL protein, based on one residue of methionine/molecule, gave a total number of about 130 residues. This number of residues is, together with the carbohydrate content, in good agreement with the M_r obtained from gel filtration. Additional information about the length of the polypeptide came from the isolation of tryptic peptides, where no peptide beyond position 128 could be obtained (Table 4).

Elucidation of the primary structure of the protein is based on the characterization of peptides isolated after cleavage of the protein with CNBr, of digesting the *N*-terminal CNBr-cleavage fragment with thermolysin, of tryptic digestion of the protein and of cleavage with BNPS-skatole. In positions 29, 30, 56, 57, 58 and 114–128 the amino acid residues were not fully proved, but were deduced from homology with other λ light chains of the same subgroup (Fig. 3).

A few of the residue numbers obtained from amino acid composition of the protein deviate from that found by sequence determination. A reason for this deviation could be due to the heterogeneity seen in the length of the polypeptide.

Comparison with the sequence of other human λ chains shows that the AL protein MOL can be placed in variable subgroup III (Fig. 3). In fact, the highest, homology seen was to Bence Jones protein BAU (Baczko *et al.*, 1974) (Table 5). Comparison of the FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4 segments of AL protein MOL and other λ chains, including the AL proteins AR (Sletten *et al.*, 1981); EPS (Toft *et al.*, 1985) and Es492 (Tveteraas *et al.*, 1985), shows that there is indeed a higher degree of homology with the Bence Jones protein BAU than with these AL proteins. The studies revealed some unusual amino acid substitutions in the variable region of AL protein MOL. In positions 31, 32 and 37 tyrosine, aspartic acid and serine respectively were found. These residues have not previously been observed in these positions in λ chains (Kabat *et al.*, 1983). The significance of this sequence for the formation of the amyloid fibrils is not known. However, it may be noted that unusual amino acid residues are often observed in AL proteins (Sletten *et al.*, 1981, 1983; Toft *et al.*, 1985; Tveteraas *et al.*, 1985).

Characterization of tryptic peptides T-(4+5+6+7) and T-5, as well as the AL protein MOL by amino acid analyses, revealed glucosamine (Tables 1 and 4). Analyses for total carbohydrate gave a composition similar to that found in AL proteins EPS and Es492 (Toft *et al.*, 1985; Tveteraas *et al.*, 1985) and to the oligosaccharide type II described by Winkelhake (1978). The final proof for the glycosylation site could not be obtained, but an appropriate acceptor sequence for *N*-glycosylation was found in positions 90, 91 and 92 (-Asx-Ser-Ser-). This would correspond to the same position as that found in AL protein Es492 (Tveteraas *et al.*, 1985), and where the acceptor sequence -Asx-Thr-

Table 5. Sequence homology between AL protein MOL and some human λ-chains including AL proteins

The numbers of amino acid residues that are identical between AL protein MOL and the Bence Jones protein sequences listed are shown. Sequences are divided into framework segments (FR-1 etc., residues 1–23 etc.) and complementarity-determining regions (CDR-1 etc., residues 24–34 etc.). The sequence data for Bence Jones proteins HA, VOR, WEIR, TOG, BAU, DEL, SH, BO, MCG, NIG are as listed by Kabat *et al.* (1983), and those for the AL proteins AR, EPS and Es492 are as shown by Sletten *et al.* (1981), Toft *et al.* (1985) and Tveteraas *et al.* (1985).

Bence Jones protein	No. of residues that are identical							Total no. of identical residues
	FR-1 1–23	CDR-1 24–34	FR-2 35–49	CDR-2 50–56	FR-3 57–88	CDR-3 89–97	FR-4 98–107	
HA (I)	15	4	10	5	20	3	9	66
VOR (I)	14	3	9	3	20	2	10	61
WEIR (II)	14	1	8	3	24	1	8	59
TOG (II)	15	3	8	3	21	1	8	59
BAU (III)	17	8	13	4	28	5	11	86
DEL (III)	16	3	10	3	27	2	11	72
SH (IV)	13	3	13	3	23	4	11	70
BO (V)	15	2	10	4	22	0	10	63
MCG (V)	14	3	8	5	23	1	9	63
NIG (VI)	14	1	8	2	19	3	10	57
AL AR (VI)	14	2	10	3	22	4	10	65
AL EPS (I)	15	3	9	4	21	1	7	60
AL Es492 (II)	16	0	8	3	23	1	10	61

Thr- was determined. In AL protein EPS the glycosylation site was in position 104, corresponding to position 101 in AL protein MOL. In this case the acceptor sequence -Asn-Val-Thr- was found (Toft *et al.*, 1985). Recent reports on the amino acid sequence of an amyloid-fibril protein of the AL type (Eulitz & Linke, 1985) and of two Bence Jones proteins from patients NIG-77 and NIG-88 with myeloma-associated amyloidosis (Tonoike *et al.*, 1985*a*,*b*) did not mention carbohydrate.

We have now isolated AL proteins from amyloid-laden organs of 15 different patients with plasma-cell dyscrasias and analysed them for hexosamines by amino acid analyses (Sletten *et al.*, 1986). Nine of these AL proteins contained hexosamine, and three of them (AL EPS, AL Es492 and AL MOL) have been characterized by amino acid sequencing. If this number of carbohydrate-containing AL proteins is representative, it is more than 4 times higher than that reported for light chains isolated from patients with multiple myeloma and supposedly without amyloidosis (Sox & Hood, 1970). However, as with the presence of unusual amino acid residues in AL protein MOL, the significance of its carbohydrate content for fibril formation and deposition remains to be elucidated.

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