The primary structure of the variable region of an immunoglobulin IV light-chain amyloid-fibril protein (AL GIL)

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The primary structure of the variable region of an amyloid-fibril protein GIL of immunoglobulin λ -light-chain origin (AL) was determined. The AL protein obtained from the fibrils in the spleen of a 54-year-old man with primary systemic amyloidosis could be assigned to subgroup IV of the lambda variable-region sequence. About 50% of the protein was found to be truncated in the *N*-terminus and lacked the first six amino acid residues. The polypeptides consisted of about 146 amino acid residues and contained traces of carbohydrate. An acceptor site for *N*-glycosylation was found in positions 90–93, but no glycopeptide could be isolated. Comparison of the amino acid sequence of AL protein GIL with that of the only Bence–Jones protein of subgroup IV previously studied revealed a sequence homology of 89%. A similar comparison made with other AL proteins gave sequence homologies below 66%.

INTRODUCTION

There are three major types of systemic amyloid-fibril proteins (Glennner, 1980*a,b*; Husby & Sletten, 1986). These are monoclonal immunoglobulin-light-chain (AL) proteins found in idiopathic (primary) and myeloma-associated amyloidosis (Husby & Sletten, 1986); amyloid A (AA) protein associated with reactive (secondary) amyloidosis (Benditt & Eriksen, 1977); and prealbumin variants seen in familial amyloid polyneuropathy (FAP) or cardiomyopathy (FAC) (Glennner, 1980*a,b*; Husby & Sletten, 1986) and senile systemic amyloidosis (Pitkänen *et al.*, 1984).

Among the AL proteins there is a predominance of λ -light chains, in contrast with what is seen among Bence–Jones proteins in myelomatosis and macroglobulinaemia, where κ -chains are more frequent (Sletten *et al.*, 1981). Light-chain proteins of the A^{VI} type appear to be most commonly associated with amyloid (Glennner, 1980*a,b*; Sletten *et al.*, 1981; Husby & Sletten, 1986). However, nothing is known about the possible specific properties of the amyloid-related light chains which make them more likely to form amyloid fibrils than others. Structural studies of a larger number of AL proteins are needed to elucidate this problem. We here report the covalent structure of an AL protein, GIL, of variable subgroup ^{IV} derived from a patient with idiopathic amyloidosis. To our knowledge, no AL protein and only one Bence–Jones protein of this subgroup has thus far been characterized with respect to its complete amino acid sequences (Kabat *et al.*, 1987).

MATERIALS AND METHODS

Amyloid fibrils

Amyloid fibrils were obtained from the spleen of a 54-year-old man (GIL) with primary systemic amyloidosis. He underwent surgical removal of a pancreatic mass suspected to be cancer, and at the same time the spleen

and several abdominal lymph nodes were removed. Biopsies were taken from kidneys and liver. All of these tissues contained rich amounts of amyloid. The spleen was chosen for extraction of amyloid fibrils. Extraction and purification of the amyloid-fibril proteins were performed as described by Pras *et al.* (1968) and Sletten *et al.* (1974). The 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 successive gel filtrations on a column of Sephadex G-100 eluted with 5 M-guanidinium chloride in 0.1 M-acetic acid. Selected retarded fractions were further gel-filtered on a column of Sephadex G-75 with 10% (v/v) formic acid. The major retarded protein fraction reacted with an antisera raised against a lambda AL protein in double immunodiffusion. The main fraction containing AL protein GIL was dialysed against water and freeze-dried and used for the structural studies. The protein was reduced and carboxymethylated as described by Crestfield *et al.* (1963). SDS/polyacrylamide-gel electrophoresis was performed using a 15%-(w/v)-polyacrylamide gel in a vertical chamber as described by Laemmli (1970). The protein markers used were those in the Electrophoresis Calibration Kit obtained from Pharmacia Fine Chemicals.

Proteolytic digestion

The reduced and carboxymethylated protein was digested with trypsin in 0.2 M-NH₄HCO₃, pH 8.5, containing 2 M-urea. Before use, the urea solution was made up as a 10 M-solution and deionized on a column of AG 501-X8 mixed-bed resin. The enzyme/substrate molar ratio was 1:50 and the digestion lasted for 8 h at 37 °C (Sletten *et al.*, 1981; Tveteraas *et al.*, 1985). Digestion with thermolysin was performed on reduced and carboxymethylated protein in 0.1 M-NH₄HCO₃, pH 8.0, and with an enzyme/substrate molar ratio of 1:400. The digestion was performed at 40 °C for 2 h. Peptide

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Th-17 was digested with *Staphylococcus aureus* V8 proteinase in 0.1 M-NH₄HCO₃, pH 8.0, for 16 h at 40 °C. The enzyme/substrate molar ratio was 1:20 (Austen & Smith, 1976). Digestion with carboxypeptidase A and B was performed in 0.2 M-N-ethylmorpholine/acetate buffer, pH 8.5, at 37 °C (Ambler, 1972). Samples of the digestion mixture were taken at intervals over a 0–120 min period and analysed on an automatic amino acid analyser.

Chemical cleavage

The protein was cleaved with BNPS-skatole [3'-bromo-3-methyl-2-(2-nitrophenylsulphenyl)indolamine] as described by Fontana (1972).

Purification of peptides

Thermolytic (thermolysin-cleaved) peptides were purified by ion-exchange chromatography on Aminex A-5 (Toft *et al.*, 1985) and selected fractions were further purified by t.l.c. on precoated plates (Polygram SIL G/UV₂₅₄ from Macherey-Nagel) using the solvent system I described by Sletten *et al.* (1981). Some of the thermolytic peptides were also purified by h.p.l.c. The h.p.l.c. system was an Altex model 312 instrument equipped with a variable u.v. detector (Uvikon LCD 725) and an Ultrapore TM RPSC column (4.6 mm × 75 mm) obtained from Beckman Instruments. Flow rates in all experiments were 1 ml/min, and the temperature was 23 °C. The following separation systems were employed: system I: solution A was 0.1 % trifluoroacetic acid, and solution B was acetonitrile with 30 % (v/v) of solution A; system II: solution A was ammonium formate, pH 6.5 (1.0 ml of 98 % formic acid/litre of water adjusted to the correct pH with dilute NH₃), and solution B was propan-2-ol with 20 % (v/v) solution A. A linear gradient from 0 to 70 % of solution B over 45 min was used in both systems.

The tryptic peptides were purified by gel filtration on a column (1.7 cm × 92 cm) of Sephadex G-50 (Fine grade) eluted with 0.2 M-NH₄HCO₃ at a flow rate of 11 ml/h. The tryptic peptides were further purified by h.p.l.c. in system II and by t.l.c. with solvent system I of Sletten *et al.* (1981).

Peptides resulting from cleavage with BNPS-skatoles were purified by gel filtration on a column (1 cm × 108 cm) of Sephadex G-50 (fine grade) eluted with 10 % formic acid at a flow rate of 3.9 ml/h, and by precipitation in 10 % formic acid. Peptides obtained after digesting thermolytic peptide Th-17 with *S. aureus* V8 proteinase were separated by h.p.l.c. in system I.

Amino acid analysis

The amino acid compositions of the polypeptides were determined as described by Sletten *et al.* (1981). Approx. 0.5–5 nmol was applied to a Biotronik LC 5000 amino acid analyser.

N-Terminal analysis

Sequence analysis was performed automatically on a JEOL JAS-47K liquid-phase sequence analyser by using the protein program (Sletten *et al.*, 1981). Samples of 10–200 nmol were used. The phenylthiohydantoin derivatives were analysed by g.l.c. and by t.l.c. (Sletten *et al.*, 1981).

Peptide nomenclature

The following prefixes were used to denote the origin

Table 1. Amino acid compositions of AL protein GIL and of fragments obtained after cleavage with BNPS-skatoles

Cysteine was determined as carboxymethylcysteine. Values in parentheses are from sequence determination.

Amino acid	Composition (residues/molecule)		
	AL protein GIL	BNPS-1	BNPS-2
Asp	13.8 (14)	3.3 (3)	10.5 (11)
Thr	9.1 (9–10)	2.7 (3)	6.9 (7)
Ser	17.0 (18)	4.1 (4)	11.8 (14)
Glu	15.4 (13)	4.3 (4)	9.7 (9)
Pro	10.1 (10)	1.2 (1)	9.1 (9)
Gly	13.6 (13)	3.1 (2)	9.6 (11)
Ala	11.9 (13–14)	3.8 (4)	7.9 (9–10)
Cys	1.8 (2–3)	0.2 (1)	0.9 (2)
Val	6.7 (10)	3.3 (4)	4.1 (6)
Ile	5.5 (6)	0.7 (1)	3.9 (5)
Leu	10.1 (12)	2.7 (3)	7.6 (9)
Tyr	5.8 (6)	0.8	3.3 (6)
Phe	5.6 (5)	1.1 (1)	4.5 (4)
His	1.8 (1)	–	1.4 (1)
Lys	5.8 (5)	0.8	4.8 (5)
Arg	6.3 (6)	1.8 (2)	4.0 (4)
Trp	(1)	(1)	
Residue nos. ...	1–146 (7–146)	1–33	34–146

of the various peptides: BNPS, peptide obtained after cleavage with BNPS-skatoles; T, tryptic digestion; Th, thermolytic (thermolysin) digestion; Pa, digestion with *S. aureus* V8 proteinase. The peptides are numbered in order of their position in the final sequence, and a letter is added to the number whenever several peptides were obtained from the same region. However, longer peptides derived from partial digestion are labelled with consecutive numbers.

RESULTS

Analysis of the material in the main retarded fraction of AL protein GIL by SDS/polyacrylamide-gel electrophoresis revealed several bands ranging in *M_r* from about 15000 to 18500. The major band was estimated to have an *M_r* of 16500 and to amount to two-thirds of the stained material on the slab gel. N-Terminal analyses of the carboxymethylated AL protein released two amino acid residues, starting with serine and proline. After 22 degradation cycles, it was evident that the differences between the two polypeptides were attributed to the lack of the N-terminal six amino acid residues in one of the chains (Fig. 1), and that the ratio was about 1:1.

The amino acid composition of AL protein GIL is presented in Table 1. The composition is based on 146 amino acid residues. Only trace amounts of glucosamine were observed.

Cleavage of the protein with BNPS-skatoles resulted in the two fragments, BNPS-1 and BNPS-2. The amino acid compositions of the two fragments are shown in Table 1. N-Terminal analysis of BNPS-1 confirmed the already established N-terminal sequence and also that about 50 % of the fragment lacked the first six amino acid residues (Fig. 1). Twenty steps of Edman degra-

Table 2. Amino acid composition (residues/molecule) of peptides obtained from thermolysin-digested carboxymethylated AL protein GIL

Values in parentheses are from sequence data. Cysteine was determined as carboxymethylcysteine. All values are from ninhydrin-stained material, except for Th-(13+14). Abbreviations used: I.c., ion-exchange chromatography.

Amino acid	Th-2	Th-3	Th-4a	Th-4b	Th-(5+6)	Th-(5+6+7)	Th-(6+7)	Th-7
Asp	1.0 (1)				1.1 (1)	2.5 (2)	1.9 (2)	1.0 (1)
Thr	0.9 (1)		1.0 (1)	0.9 (1)	1.1 (1)	1.0 (1)	0.9 (1)	0.1
Ser	0.2	1.0 (1)	0.2		1.3 (1)	1.1 (1)	1.1 (1)	0.2
Glu	1.2 (1)		1.2 (1)	1.2 (1)	1.3 (1)	1.4 (1)	1.1 (1)	
Pro	1.0 (1)							0.4
Gly	0.2		1.0 (1)	0.9 (1)	1.3 (1)	1.2 (1)	1.0 (1)	0.2
Ala	1.0 (1)		0.8 (1)		0.1	0.2	0.1	0.1
Cys					0.9 (1)	1.0 (1)	0.8 (1)	
Val	0.2	0.9 (1)			0.7 (1)	1.1 (1)		
Ile					1.0 (1)	1.2 (1)	0.8 (1)	
Leu	0.8 (1)		0.8 (1)	0.7 (1)		1.3 (1)	1.0 (1)	0.9 (1)
Tyr								0.2
Phe					0.1	0.1		0.2
His						0.2		
Lys								
Arg					0.7 (1)	1.9 (2)	0.9 (1)	1.0 (1)
Residue nos. ...	3-8	9-10	12-16	13-16	17-25	17-28	19-28	26-28
Purification procedure ...	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.
Yield (%)...	21	19	14	35	6	3	15	10

Amino acid	Th-9	Th-(9+10)	Th-(11+12)a	Th-(11+12)b	Th-(11+12+13+14)	Th-(13+14)	Th-14	Th-15
Asp	0.2		3.2 (3)	3.0 (3)	4.4 (4)	1.0 (1)	0.2	
Thr	0.2				1.1 (1)	1.0 (1)		1.0 (1)
Ser	1.1 (1)	1.1 (1)	1.1 (1)	1.0 (1)	6.4 (6)	4.4 (5)	1.0 (1)	0.1
Glu	3.4 (3)	3.2 (3)	0.4		0.3	0.3	0.2	
Pro	1.8 (2)	1.8 (2)	1.9 (2)	2.2 (2)	2.1 (2)			
Gly	1.5 (1)	1.0 (1)	2.6 (2)	2.2 (2)	4.1 (4)	2.3 (2)	0.3	0.1
Ala	1.8 (2)	1.7 (2)	0.2	0.1	1.2 (1)	0.9 (1)	1.0 (1)	
Cys								
Val	0.2	0.5 (1)	0.1	0.1	0.1	0.2	0.1	
Ile		0.9 (1)	1.8 (2)	1.7 (2)	1.7 (2)	0.1		
Leu	0.2	1.8 (2)	0.2			0.2	0.2	0.8 (1)
Tyr	0.8 (1)	0.8 (1)	0.8 (1)	0.7 (1)	1.1 (1)	0.3		
Phe				1.3 (1)	1.0 (1)	0.9 (1)		
His			0.2					
Lys	0.8 (1)	0.7 (1)	1.0 (1)	0.8 (1)	0.9 (1)	0.1		
Arg			2.0 (2)	1.9 (2)	2.0 (2)			
Trp	(1)	(1)						
Residue nos. ...	31-42	31-46	46-59	46-60	46-70	60-70	69-70	70-71
Purification procedure ...	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., h.p.l.c.	H.p.l.c.	I.c., t.l.c.	I.c., t.l.c.
Yield (%)...	3	32	5	3	18		17	21

dation on BNPS-2 gave the amino acid sequence of residues 34-53.

Digestion of the AL protein with thermolysin followed by ion-exchange chromatography and t.l.c. or h.p.l.c. results in 26 pure peptides. The amino acid compositions are shown in Table 2 together with the yield and the purification procedures used. The peptides comprised the residues 3-10, 12-28, 31-96, 99-112 and 135-144. The yield of the different peptides varied from about 10-35%. Characterization of Th-(6+7) by Edman degradation and carboxypeptidase A revealed the amino acid residues

in positions 19-28. Edman degradation of Th-(9+10) gave the residues in positions 31-40, and twelve degradation cycles on Th-(11+12)a, the residues in positions 46-57. Digesting Th-(11+12)a and Th-(11+12)b with carboxypeptidase A and B revealed the residues in positions 59 and 60. Nine degradation cycles on Th-(13+14) gave the residues in positions 60-68. The amino acid sequences of the residues in the two dipeptides Th-14 and Th-15 are based on the amino acid composition and on the specificity of thermolysin. Two degradation cycles of Th-16 resulted in the amino acid

Table 2. (continued)

Amino acid	Th-16	Th-17	Th-19a	Th-19b	Th-20a	Th-20b	Th-20c	Th-27	Th-28	Th-(27 + 28 + 29)	Th-29
Asp		5.1 (5)							1.1 (1)	1.0 (1)	
Thr	1.0 (1)		1.8 (2)	0.9 (1)	0.8 (1)			0.1			
Ser		3.3 (3)			1.2 (1)	1.0 (1)	0.9 (1)		1.0 (1)	0.9 (1)	
Glu		2.4 (2)			1.1 (1)	1.2 (1)	1.3 (1)		0.2	0.3	
Pro					1.0 (1)	1.1 (1)	1.1 (1)			1.1 (1)	
Gly	1.1 (1)	1.1 (1)	4.1 (3)	0.2	0.1		0.3	0.2	0.3	1.3 (1)	
Ala		2.7 (3)		0.1		0.2			0.1	0.3	
Cys		0.9 (1)						1.1 (1)			
Val		0.3	0.8 (1)	1.1 (1)	0.8 (1)	0.9 (1)		1.1 (1)		0.2	
Ile	0.8 (1)								1.1 (1)	1.0 (1)	
Leu			0.9 (1)	0.7 (1)	1.7 (2)	1.0 (1)	0.8 (1)	1.0 (1)	0.9 (1)	0.9 (1)	
Tyr		2.8 (3)								0.9 (1)	1.1 (1)
Phe			1.1 (1)							1.0 (1)	0.9 (1)
His		1.0 (1)								0.1	
Lys			0.9 (1)		0.7 (1)	1.0 (1)	0.9 (1)				
Arg		2.0 (2)									
Trp											
Residue nos. ...	73-75	76-96	99-107	105-107	105-112	107-112	108-112	135-137	137-140	137-144	141-142
Purification procedure ...	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.	I.c., t.l.c.
Yield (%)...	15	16	25	9	2	2	8	21	8	7	14

sequence of this peptide. Peptide Th-17 was run through 15 cycles of Edman degradation. The results were confirmed by digesting the same peptide with *S. aureus* proteinase, which yielded the two peptides Pa-1 and Pa-2 (Table 3 and Fig. 1). A characteristic composition of Th-19a, Th-19b, Th-20a, Th-20b, Th-20c, Th-27, Th-28, Th-29 and Th-(27+28+29) placed these peptides in positions (Kabat *et al.*, 1987).

From a tryptic digest of the AL protein, seven peptides were isolated and characterized. The peptides comprised the residues in positions 28-49, 60-77, 78-89, 90-104, 105-112, 113-131 and 132-146 or 149. The amino acid compositions, together with the isolation procedures used and the yield of the peptides, are shown in Table 4. The yields of all tryptic peptides obtained are very low, owing to poor solubility of the AL protein.

Five degradation cycles of T-(3+4) made the overlap between Th-(6+7) and Th-(9+10), and the composition confirmed the residues found in positions 28-49. The amino acid compositions of T-7 and T-8 confirmed the residues in positions 60-89. The sequence of peptides and Th-15 and Th-16 are placed in positions according to their homology with other lambda chains (Kabat *et al.*, 1987). Thirteen cycles of Edman degradation of T-9 made an overlap to Th-17 and elucidated the amino acid sequence from positions of 90 to 104. The results were confirmed from the amino acid compositions of Pa-2 and Th-19a, which extended the sequence of T-10 to position 112. Peptide T-11 was aligned according to its characteristic composition by homology with other light chains (Kabat *et al.*, 1987). Four degradation cycles of T-12 yielded one amino acid residue at each cycle and confirmed the amino acid sequence in positions 132-135 in the constant region of light chains (Kabat *et al.*, 1987).

The amino acid composition of T-12 revealed a low yield of threonine, alanine, valine and lysine, which would indicate that a part of the peptide lacks these C-terminal residues.

Table 3. Amino acid composition of peptides obtained after digestion of peptide Th-17 with *S. aureus* V8 proteinase

Values in parentheses are from sequence determination.

Amino acid	Composition (residues/molecule)		
	Pa-1	Pa-2	Th-17
Asp	1.1 (1)	4.0 (4)	5
Thr		0.2	
Ser	0.3	3.7 (3)	3
Glu	2.2 (2)	0.1	2
Pro			
Gly	0.5	1.2 (1)	1
Ala	1.8 (2)	1.0 (1)	3
Cys		1.0 (1)	1
Val			
Ile			
Leu			
Tyr		2.6 (3)	3
Phe			
His	0.1	1.0 (1)	1
Lys			
Arg	0.8 (1)	0.8 (1)	2
Residue nos. ...	76-81	82-96	76-96

DISCUSSION

The AL protein GIL obtained from the spleen of a 54-year-old man (GIL) with primary systemic amyloidosis was found to consist of approx. 146 amino acid residues. This number corresponds well with the estimated M_r of 16 500 found by SDS/polyacrylamide-gel electrophoresis and represents about 70% of an immunoglobulin λ -light chain. N-Terminal analyses of the AL protein

Table 4. Amino acid compositions (residues/molecule) of tryptic peptides obtained from carboxymethylated AL protein GIL

Values in parentheses are from sequence data. Cysteine was determined as carboxymethylcysteine. The data for peptides T-(3+4), T-9 and T-12 are from ninhydrin-stained material. Abbreviation used: SG-50F, Sephadex G-50 (Fine grade).

Amino acid	T-(3+4)	T-7	T-8	T-9	T-10	T-11	T-12
Asp	1.1 (1)	0.8 (1)	2.7 (3)	1.8 (2)	0.2	1.1 (1)	1.1 (1)
Thr	0.4	1.7 (3)	0.4	1.0 (1)	0.9 (1)	1.1 (1)	1.5 (1-2)
Ser	1.2 (1)	4.7 (5)	1.1 (1)	2.2 (2)	1.1 (1)	2.6 (3)	1.4 (1)
Glu	3.5 (3)	0.4	2.5 (2)	0.2	1.1 (1)	3.1 (3)	0.4
Pro	1.6 (2)	0.3			1.1 (1)	2.9 (3)	1.4 (1)
Gly	2.7 (2)	2.9 (3)	0.4	5.2 (4)	0.2	0.8	1.7 (1)
Ala	2.0 (2)	1.9 (2)	1.9 (2)	0.2	0.2	2.4 (3)	2.2 (2-3)
Cys			0.8 (1)				0.6 (1)
Val	1.7 (2)	0.3	0.3	0.7 (1)	0.8 (1)	1.1 (1)	2.2 (2-3)
Ile	1.4 (1)	0.6 (1)	0.2	0.9 (1)		0.2	1.0 (1)
Leu	1.9 (2)	0.7 (1)	0.3		1.8 (2)	1.9 (2)	2.0 (2)
Tyr	1.8 (2)		1.5 (2)	0.8 (1)		0.3	0.9 (1)
Phe	0.9 (1)	1.1 (1)		0.9 (1)	0.1	0.9 (1)	1.0 (1)
His	0.2			1.0 (1)		0.1	0.3
Lys	1.1 (2)	0.2	0.2	0.8 (1)	1.0 (1)	0.8 (1)	0.4
Arg	0.2	0.9 (1)	1.2 (1)				
Trp	(1)						
Residue nos. ...	28-49	60-77	78-89	90-104	105-112	113-131	132-146 (149)
Purification procedure ...	SG-50F, t.l.c.	SG-50F, h.p.l.c.	SG-50F, h.p.l.c.	SG-50F, t.l.c.	SG-50F, h.p.l.c.	SG-50F, h.p.l.c.	SG-50F, t.l.c.
Yield (%) ...	5	4	4	8	5	5	2

revealed a truncated terminus. About half of the protein started with serine in position 1, whereas the other half started with proline, in position 7. Serine is the usual amino acid residue found in position 1 of the human immunoglobulin λ -light chain of subgroup IV (Kabat *et al.*, 1987). The amino acid sequence was elucidated from *N*-terminal analyses of the protein and from peptides obtained after cleaving the AL protein with BNPS-skatole and digestion of the protein with trypsin and thermolysin. The alignment of Th-15 and Th-16, corresponding to positions 71-76 is uncertain, as the amount of T-7 was insufficient for *N*-terminal analysis. This region (FR 3) seems, however, to be fairly constant in all λ -chains (Fig. 2). The alignment of peptides T-11 and T-12 and Th-27, Th-28 and Th-29, corresponding to residues 113 to about 144, is based on the homology with the constant region of human λ -chains (Kabat *et al.*, 1987). From the thermolytic digest a peptide [Th-(11+12)b], corresponding to residues 46-60 (Fig. 1), was obtained which, after treatment with carboxypeptidase A, yielded only phenylalanine. The results indicate that an unexpected cleavage had occurred between a phenylalanine residue and a serine residue or that a small part of the AL protein had this residue as the *C*-terminus. *N*-Terminal analysis of the protein as well as of the BNPS-1, BNPS-1a and peptides T-12 and Th-29 provide evidence for a truncated protein at both ends. Although the results obtained seem quite clear, we cannot completely exclude the possibility that mild acid cleavage may have occurred at the Asp-Pro bond between position 6 and 7.

To our knowledge, this is the first example of an AL protein in which about half of the polypeptides were truncated in the *N*-terminus; this phenomenon is very commonly observed, however, at the *C*-terminal end of

such proteins. So far there is only one report of an example of a Bence-Jones protein from a patient with amyloidosis which showed a 'ragged' *N*-terminus (Eulitz & Linke, 1982). In this case approx. 40% of the Bence-Jones protein lacked an *N*-terminal tripeptide. However, *N*-terminal analysis of the amyloid-fibril protein (AL) isolated from the heart and the bone marrow from the same patient revealed only one *N*-terminus, corresponding to the longer sequence (Eulitz & Linke, 1982). In another report (Eulitz & Linke, 1985) the AL protein HAR of the V λ II subgroup was found to be heterogeneous. In this case 70% of the polypeptide chains commence with a serine residue, whereas the other chains start with an alanine residue, corresponding to positions 9 and 8 respectively in light chains of subgroup II. All of these observations are hard to understand, but other proteins from amyloid fibrils show heterogeneities in both *N*- and *C*-terminal regions (Møyner *et al.*, 1980; Cornwell *et al.*, 1987). The *N*-terminal truncated proteins seem to be more uncommon, and could possibly result from deterioration of the tissue at the time of fibril extraction.

Amino acid analyses of acid-hydrolysed AL protein GIL showed trace amounts of glucosamine, but no glycopeptide could be isolated. However, the protein has an acceptor site for *N*-glycosylation in positions 90-92 (Asn-Ser-Ser-), a region where we have found carbohydrate in other AL proteins (Toft *et al.*, 1985; Tveteraas *et al.*, 1985; Holm *et al.*, 1986). Glycosylated AL proteins appear to be quite common and more frequent than among Bence-Jones proteins (Sletten *et al.*, 1986), but whether glycosylation plays a role in the formation of AL fibrils is not known.

The sequence here determined was compared with the variable region of Bence-Jones protein SH of subgroup

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