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The amino acid sequence of a carbohydrate-containing immunoglobulin-light-chain-type amyloid-fibril protein

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The amino acid sequence of an amyloid-fibril protein Es492 of immunoglobulin-λ-light-chain origin (AL) was elucidated. The amyloid fibrils were obtained from the spleen of a patient who died from systemic amyloidosis. The amino acid sequence was elucidated from structural studies of peptides derived from digestion of the protein with trypsin, thermolysin, chymotrypsin and *Staphylococcus aureus* V8 proteinase and from cleavage of the protein with CNBr and BNPS-skatole. A heterogeneity in the length of the polypeptide was seen in the *C*-terminal region. The protein was by sequence homology to other λ-chains shown to be of the VλII subgroup. Although an extensive homology was seen, some amino acid residues in positions 26, 31, 32, 40, 44, 93, 97, 98 and 99 have not previously been reported in these positions of VλII proteins. The significance of these residues in the fibril formation is unclear. The protein was found to contain carbohydrate, with glycosylation sites in two of the hypervariable regions.

INTRODUCTION

Amyloidosis is a group of diseases in which a fibrillar protein is deposited in various tissues. The fibrils consist of small proteins arranged in cross β-pleated sheet conformation (Glenner, 1980*a*,*b*), which is believed to be the explanation for many properties common to the different amyloids.

Several proteins have been shown to be able to form amyloid fibrils. In plasma cell dyscrasias, both of benign and malignant type, associated with amyloidosis, the fibril subunit protein derives its origin from a monoclonal immunoglobulin light chain. This fibril protein, protein AL, is usually found as a light chain that lacks a part of the constant region (Glenner, 1980*a*,*b*).

The reasons why amyloid fibrils are produced only in some individuals with plasma cell dyscrasias are virtually unknown. There is a possibility that some light chains have a spontaneous tendency to polymerize to amyloid fibrils, i.e. that special amyloidogenic amino acid sequences occur (Sletten *et al.*, 1983). In those few partial and complete amino acid sequence studies that have been performed of AL proteins, no certain such sequences have been found, although some uncommon light-chain subgroups seem to be over-represented in amyloid fibrils (Sletten *et al.*, 1983). However, the complete primary structure of only one AL protein has been published (Kabat *et al.*, 1983). The elucidation of the primary structure of more AL proteins is necessary for the exploration of amyloidogenic amino acid sequences, if such exist.

In the present paper we report the amino acid sequence of an AL protein containing carbohydrate. Only about 15% of all immunoglobulin light chains studied have been found to contain carbohydrate, and this has not been observed among AL proteins hitherto (Sox & Hood, 1970; Garver *et al.*, 1981).

MATERIALS AND METHODS

Amyloid fibrils

Amyloid fibrils were extracted with distilled water (Pras *et al.*, 1968) from the amyloid-laden spleen of a 63-year-old patient, Es492 (material kindly provided by Dr. L. Vejlens, County Hospital, Eskilstuna, Sweden), who died from systemic amyloidosis. No underlying disorder was detected. Freeze-dried fibrils were defatted with chloroform/methanol (2:1, v/v), dissolved in 6 M-guanidine hydrochloride in 0.1 M-Tris/HCl buffer, pH 8.0, containing 0.1 M-dithiothreitol and gel-filtered on a 90 cm × 2.6 cm Sepharose 6B column, equilibrated and eluted with 5 M-guanidine hydrochloride, with monitoring of the absorbance at 280 nm. The fractions containing the main retarded peak were pooled, dialysed exhaustively against distilled water and freeze-dried, redissolved in 5 M-guanidine hydrochloride in distilled water containing 0.1 M-dithiothreitol and applied to a 90 cm × 1.6 cm Sephacryl S-300 column, other conditions being the same as above. Pooled fractions were dialysed exhaustively against distilled water and freeze-dried. SDS/polyacrylamide-gel electrophoresis was performed as described by Weber *et al.* (1973).

Amino acid analysis

The amino acid compositions of the polypeptides were determined as described previously (Sletten *et al.*, 1981). Approx. 10 nmol was applied to a BIO CAL BC-200 automatic amino acid analyser, and 0.5–5 nmol was applied to a Biotronik LC 5000 amino acid analyser. A special program was used for analyses of polypeptides containing carbohydrates.

Carbohydrate analysis

A complete composition of the carbohydrates in glycopeptides was determined after hydrolysis in 2 M-HCl

Abbreviations used: SDS, sodium dodecyl sulphate; BNPS-skatole, 3'-bromo-3-methyl-2-(2-nitrophenylsulphenyl)indolamine.

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in methanol for 18 h at 85 °C and derivative formation in trifluoroacetic acid/acetonitrile (1:1, v/v) (Bolton *et al.*, 1965). The samples were analysed by g.l.c. with a fused silica capillary column (25 m × 0.2 mm). Values from double or triple analyses were used.

Proteolytic digestions

Tryptic digestion of carboxymethylated protein was performed in 0.2 M-NH_4HCO_3, pH 8.5, containing 2 M-urea (Sletten *et al.*, 1981). Before use the urea solution was chromatographed on a mixed-bed resin (AG 501-X8). Digestion with thermolysin and chymotrypsin was performed as described previously (Sletten & Husby, 1974). Digestion with *Staphylococcus aureus* V8 proteinase was as described by Austen & Smith (1976), with an enzyme/substrate molar ratio of 1:10 for 16 h. Digestion with carboxypeptidase A was essentially as described by Ambler (1972), and the samples were analysed by an amino acid analyser.

Deblocking of the *N*-terminus

The *N*-terminal chymotryptic peptide was deblocked by incubation in 1.5 M-HCl in methanol for 12 h at 23 °C (Kawasaki & Itano, 1972).

Chemical cleavage

The protein was cleaved by CNBr and by BNPS-skatole as described previously (Fontana, 1972; Sletten & Husby, 1974).

Purification of the peptides

The peptides resulting from the cleavage with CNBr, BNPS-skatole and trypsin were separated on a Sephadex G-50 column (1 cm × 108 cm) eluted with 10% (v/v) formic acid. The flow rate was 10 ml/h. The tryptic peptides were further purified by h.p.l.c. and by t.l.c. with solvent system 1 (Sletten *et al.*, 1981). A LiChrosorb RP 18 column (250 mm × 4.6 mm; particle size 10 μm; Altex) eluted with a linear gradient of 0–50% (v/v) acetonitrile in 0.1% H_3PO_4 over 45 min was used for this separation (Fullmer & Wasserman, 1979). The flow rate was 1 ml/min. Chymotryptic peptides were separated on a Sephadex G-25 column (108 cm × 1 cm) eluted with 10% formic acid. Thermolytic peptides were separated by h.p.l.c., and selected fractions were further purified by t.l.c. with solvent system 1 (Sletten *et al.*, 1981). Peptides obtained after digestion with *S. aureus* V8 proteinase were separated by h.p.l.c. in an Altex ultrapore RPSC column (75 mm × 4.6 mm), eluted with trifluoroacetic acid/propan-2-ol (Mahoney & Hermodson, 1980).

N-Terminal analysis

Dansylation (5-dimethylaminonaphthalene-1-sulphonylation) of the protein and peptides followed by separation of the dansyl-amino acids was performed as described by Gray (1972). Sequence analysis by Edman degradation was done automatically on a JEOL JAS-47K liquid-phase sequence analyser by using the protein program (Sletten *et al.*, 1981). Samples of size about 100 nmol were used.

Peptide nomenclature

The following prefixes were used to denote the origin of the various peptides: BNPS, peptide obtained after cleavage with BNPS-skatole; C, chymotryptic digest of peptide CB-1; CB, cleavage with CNBr; CPA, digestion with carboxypeptidase A; P, digestion with *S. aureus* V8 proteinase; T, tryptic digestion; Th, thermolytic digestion. The peptides are numbered in order of their position in the final sequence.

Table 1. Amino acid composition of AL protein Es492

The values are averages for four different analyses. Hydrolysis was for 24 h. Cysteine was calculated as carboxymethylcysteine.

Amino acid or amino sugar	Composition (residues/molecule) Found	From the sequence
Asp	11.4	12
Thr	12.6	14
Ser	18.7	22
Glu	14.1	13
Pro	8.8	10
Gly	13.6	13
Ala	13.3	14
Cys	2.6	3
Val	9.8	11
Met	0.84	1
Ile	4.1	6
Leu	12.0	12
Tyr	3.7	4
Phe	5.2	7
His	1.8	2
Lys	6.2	6
Arg	3.4	2
Trp		2
Total		154
GlcN	3.5	

RESULTS

Structural studies on peptides obtained from the amyloid-fibril protein

From gel filtration of the protein an M_r of about 18000 was found. SDS/polyacrylamide-gel electrophoresis of the material revealed only one broad band, corresponding to an M_r of about 18500.

The amino acid composition of the protein is shown in Table 1, together with that found from the sequence determination. The total number of residues is in agreement with the estimated M_r found by SDS/polyacrylamide-gel electrophoresis and gel filtration. The amino acid analyses also revealed 3.5 residues of glucosamine per molecule.

The amino acid composition together with the yield of nine tryptic peptides are shown in Table 2. The yield of the peptides varied between 25% and 53%. Peptides T-9 and T-10 revealed a lower yield than the other peptides, apparently, because of a 'ragged' *C*-terminal. Peptide T-1 was not obtained in pure form. Peptide T-8 from the constant region was isolated and analysed directly on the sequencer, and no amino acid analysis exists for this peptide. Results obtained from the characterization of tryptic peptides by Edman degradation are shown in Fig. 1.

Table 2. Amino acid composition of tryptic peptides obtained from AL protein Es 492

Numbers in parentheses are from sequence determination. Cysteine was measured as carboxymethylcysteine after carboxymethylation.

Amino acid or amino sugar	Peptide ...	Composition (residues/molecule)								
		T-1	T-(2+3)	T-4	T-5	T-6	T-7	T-8	T-9	T-10
Asp		(3)	3.0 (3)	—	3.7 (4)	0.3	1.0 (1)	(1)	1.0 (1)	0.3
Thr		(4)	—	—	6.3 (6)	0.7 (1)	0.9 (1)	(2)	—	2.6 (3)
Ser		(8)	2.4 (3)	1.9 (2)	4.9 (5)	0.1	2.3 (3)	(1)	1.9 (2)	0.8 (1)
Glu		(5)	0.4	—	3.1 (4)	1.3 (1)	3.0 (3)	—	0.4	0.8 (1)
Pro		(4)	1.2 (1)	—	—	1.0 (1)	3.0 (3)	(1)	1.1 (1)	0.9 (1)
Gly		(4)	0.9 (1)	1.5 (1)	4.8 (5)	1.9 (1)	—	(1)	0.4	1.0 (1)
Ala		(5)	—	—	2.5 (3)	0.1	2.7 (3)	(3)	0.9 (1)	0.6 (1)
Cys		(1)	—	—	0.6 (1)	—	—	(1)	—	—
Val		(2)	2.0 (2)	—	1.1 (2)	0.9 (1)	1.2 (1)	(3)	1.0 (1)	1.2 (1)
Met			0.4 (1)	—	—	—	—		—	—
Ile		(3)	0.8 (1)	—	0.8 (1)	—	—	(1)	—	—
Leu		(2)	1.2 (1)	—	2.9 (3)	1.5 (2)	1.9 (2)	(2)	—	—
Tyr		(1)	—	—	1.7 (2)	—	—	(1)	—	—
Phe		(1)	0.9 (1)	1.0 (1)	1.8 (2)	—	0.9 (1)	(1)	—	—
His		(2)	—	—	—	—	—		—	—
Lys		(1)	—	1.2 (1)	0.8 (1)	0.9 (1)	0.7 (1)	(1)	1.1 (1)	1.1 (1)
Arg			2.0 (2)	—	—	0.2	—		—	—
Trp		(1)						(1)		
Residue nos.		1–47	48–63	64–68	69–107	108–115	116–134	135–154	155–161	162–171
Yield (%)			38	53	50	37	25		8	8
GlcN		+			2.2 (3)					

Peptide T-5 was digested with *S. aureus* proteinase, which yielded two peptides, P-1 and P-2 (Table 3). The cleavage was at the glutamic acid residue in position 83. Peptide P-2 contained the carbohydrate. The yield of both peptides was about 70%.

Since the protein contained only one residue of methionine, the protein was cleaved with CNBr, yielding the fragments CB-1 and CB-2. The amino acid composition of the purified peptide CB-1 is shown in Table 3. This fragment was also found to contain carbohydrate. Peptide CB-1 was treated with BNPS-skatole to obtain a cleavage at the tryptophan residue in position 37, yielding the two peptides BNPS-1 and BNPS-2a. Another portion of peptide CB-1 was taken for chymotryptic digestion, from which peptides C-1 and C-2 were isolated.

Peptide C-1 revealed a blocked *N*-terminal residue, which after methanolysis gave glutamic acid, indicating pyroglutamic acid as the *N*-terminal residue. Digestion of the tetrapeptide C-1 with carboxypeptidase A for 10 min and 30 min released leucine and alanine. Fifteen steps of Edman degradation of peptide C-2 gave the structure of this peptide.

From a thermolytic digest of the protein, 25 peptides were isolated and purified. The amino acid compositions of these peptides are summarized in Table 4. The yield of the peptides varied between 5% and 43%. Peptide Th-7 was found to contain glucosamine. No thermolytic peptide containing the second carbohydrate-attachment site could be isolated. Peptides Th-7, Th-8, Th-11 and Th-23 were characterized by Edman degradation in order to elucidate the structure in positions 23-30, 31-33, 44-47 and 99-107 (Fig. 1).

Deduction of the amino acid sequence

The complete amino acid sequence of AL protein Es 492 is shown in Fig. 1.

Residues 1 to 10. *N*-Terminal analyses of the protein revealed that the *N*-terminal amino acid residue was blocked. Cleaving the protein with CNBr resulted in isolation of the *N*-terminal fragment containing residues 1–49. Digestion of this fragment with chymotrypsin gave the peptides C-1 and C-2, which by structural studies enabled elucidation of the amino acid residues in positions 1 to 19. The sequence of this region was further verified by the amino acid composition of thermolytic peptides Th-(2+3), Th-3 and Th-4.

Residues 20 to 50. The identities of the amino acid residues in positions 20, 21 and 22 were based on the amino acid composition of Th-6, on the specificity of thermolysin and on the homology with other human λ light chains (Fig. 2). Automatic Edman degradation of peptide Th-7 resulted in the amino acid sequence of residues 23 to 30. This peptide was found to contain carbohydrate. Two steps of Edman degradation of the tripeptide Th-8 revealed the residues in positions 31 to 33. However, position 33 could be either aspartic acid or asparagine. The identities of the residues in positions 34 to 37 were based on the amino acid composition of peptide Th-9, on the specificity of thermolysin and cleavage with BNPS-skatole of peptide CB-1 and finally on the homology with other human λ light chains from subgroup II (Fig. 2). Edman degradation of peptides BNPS-2a, Th-11 and T-(2+3) elucidated the residues in

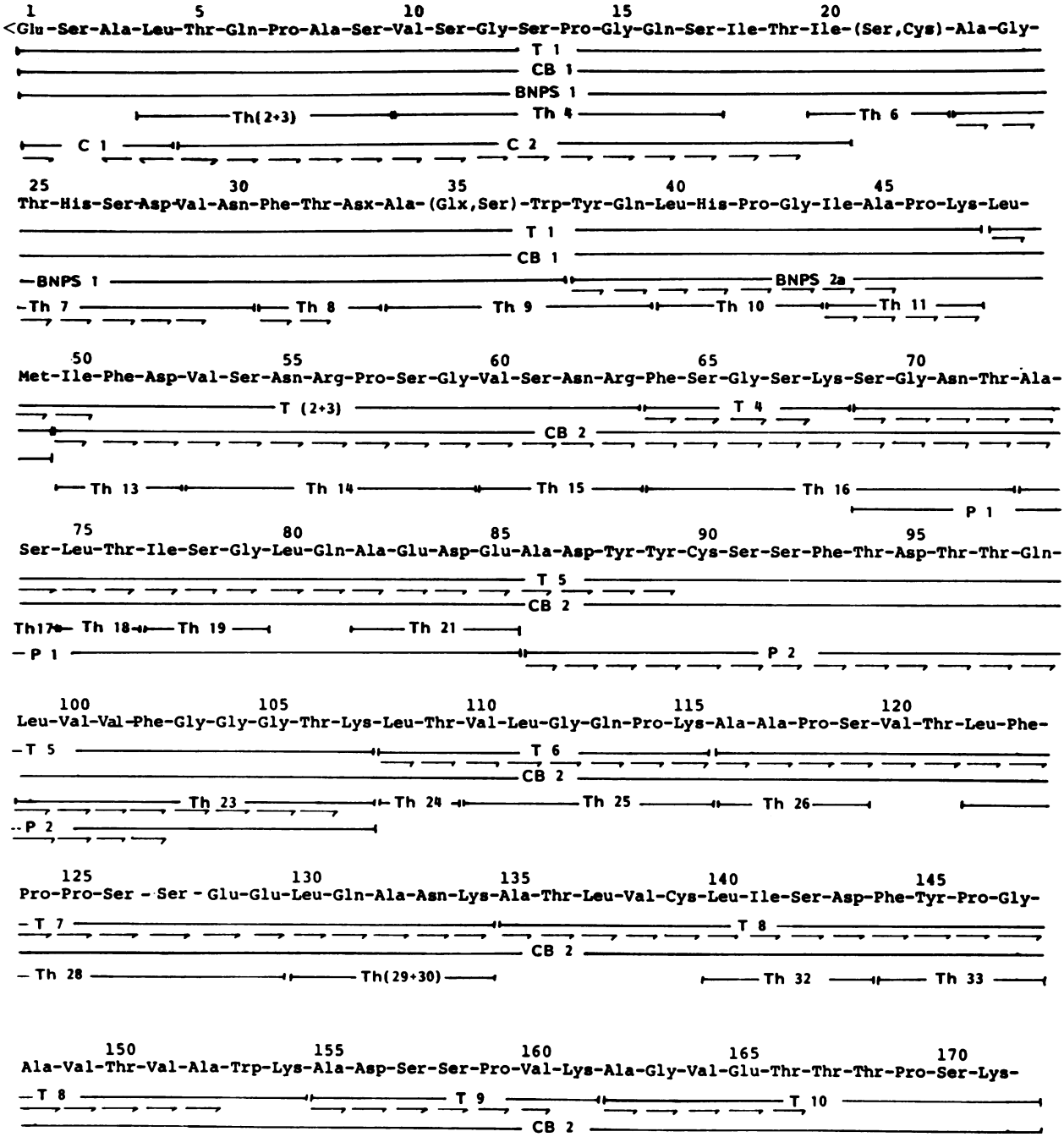

Fig. 1. Amino acid sequence of AL protein Es492

Sequence obtained by Edman degradation with direct identification of amino acid phenylthiohydantoin derivates is shown by half-arrows pointing to the right. Half-arrows pointing to the left indicate amino acids obtained after digestion with carboxypeptidase A. C, P, T and Th denote peptides obtained after digestion with chymotrypsin, *S. aureus* V8 proteinase, trypsin and thermolysin respectively. CB and BNPS are peptides obtained after cleavage with CNBr and BNPS-skatole. The presence of tryptophan in position 37 is based on u.v. absorbance and of cleavage with BNPS-skatole of peptide CB-1. The residues in positions 153 and 167–171 are based on homology with other light chains.

positions 38 to 50. The amino acid composition of peptide CB-1 is in agreement with that obtained from the sequence determination, except for the values for threonine and valine.

Residues 51 to 107. Automatic Edman degradation of peptide CB-2 gave the amino acid residues in positions 50 to 73. The identities of these residues were further verified by structural studies on peptides T-(2+3), Th-13, Th-14, Th-15, T-4 and Th-16 and a part of peptides T-5 and Th-17. Sequence analyses of peptide T-5 gave the residues in positions 69 to 89. Verification of these residues was obtained from peptides Th-17, Th-18, Th-19 and Th-21 and from two peptides P-1 and P-2 obtained

Table 3. Amino acid composition of peptides obtained after digestion of peptide T-5 with *S. aureus* V8 proteinase and of *N*-terminal fragment CB-1, obtained after CNBr cleavage

Numbers in parentheses are from sequence determination. The yield of peptides P-1 and P-2 was about 70%. Cysteine was measured as carboxymethylcysteine after carboxymethylation. Tryptophan was detected from u.v. absorbance. Glucosamine data for peptide CB-1 are from Table 5.

Amino acid or amino sugar	Composition (residues/molecule)			
Peptide ...	P-1	P-2	T-5	CB-1
Asp	2.0 (2)	1.9 (2)	(4)	2.8 (3)
Thr	1.9 (2)	3.7 (4)	(6)	3.0 (4)
Ser	3.2 (3)	2.5 (2)	(5)	8.4 (8)
Glu	3.4 (3)	0.9 (1)	(4)	5.3† (5)
Pro	—	—	—	3.8 (4)
Gly	2.4 (2)	3.6 (3)	(5)	5.7 (4)
Ala	2.1 (2)	1.2 (1)	(3)	5.4 (5)
Cys	—	0.5 (1)	(1)	Trace (1)
Val	0.1	1.1* (2)	(2)	3.3 (2)
Met†	—	—	—	(1)
Ile	1.0 (1)	0.1	(1)	2.8 (3)
Leu	2.0 (2)	1.0 (1)	(3)	3.5 (3)
Tyr	0.1	2.0 (2)	(2)	1.1 (1)
Phe	0.1	1.8 (2)	(2)	0.7 (1)
His	—	—	—	1.8 (2)
Lys	—	1.0 (1)	(1)	1.7† (1)
Arg	—	—	—	0.5 (0)
Trp	—	—	—	(1)
GlcN	—	2.5 (3)	(3)	(1)

* The low yield is due to a Val-Val- sequence.

† Homoserine and homoserine lactone were co-eluted with glutamic acid and lysine respectively.

after digestion of peptide T-5 with *S. aureus* V8 proteinase. Edman degradation of peptides P-2 and Th-23 gave the amino acid sequence of the residues in positions 86 to 107. Peptides T-5 and P-2 were found to contain carbohydrate.

Residues 108 to 171. The amino acid sequence of residues 108 to 166 was based on Edman degradation of peptides T-6, T-7, T-8, T-9 and T-10, on the amino acid composition and the specificity of thermolysin of peptides Th-24, Th-25, Th-26, Th-28, Th-(29+30), Th-32 and Th-33 and on homology. The sequences of the peptides were based on homology with the constant region from other light chains (Fig. 2) (Kabat *et al.*, 1983).

Structural studies of carbohydrate-containing polypeptides

Amino acid analyses showed that the AL protein as well as peptides obtained from the protein contained glucosamine. A complete carbohydrate composition was made on the AL protein, as well as the CB-1 fragment (residues 1–49) and the tryptic peptide T-5 (residues 69–107) (Table 5).

The numbers of residues of the monosaccharides were based on a polypeptide concentration determined from the composition. Fragment CB-1 was found to contain one residue each of *N*-acetylglucosamine and *N*-acetylneuraminic acid, and a very high content of glucose, which most probably was a contaminant from Sephadex. Peptide T-5 contained 2 residues of mannose, 1.2 residues of galactose, 3 residues of *N*-acetylglucosamine and 4 residues of *N*-acetylneuraminic acid. The total numbers of *N*-acetylglucosamine and *N*-acetylneuraminic acid found in the glycopeptides deviate from those found in the total protein.

The results obtained showed that the protein had two glycosylation sites. Thermolytic digestion of peptide material containing T-1 as well as the AL protein yielded a glucosamine-containing octapeptide, Th-7. The saccharide could in this case be attached to the residue in position 30, as the amino acid sequence in positions 30, 31 and 32 (Asn-Phe-Thr-) would fulfil the condition for attachment to protein of oligosaccharides through an *N*-glycosidic bond (Marshall, 1974; Sox & Hood, 1970). Tryptic peptide T-5 was digested with *S. aureus* V8 proteinase and yielded one *N*-acetylglucosamine-containing peptide P-2, which came out in a symmetrical peak from the h.p.l.c. separation. The amino acid sequence in positions 95, 96 and 97 (Asx-Thr-Thr-) would in this case fulfil the conditions for an *N*-glycosidic bond (Marshall, 1974; Sox & Hood, 1970).

DISCUSSION

Amyloid-fibril proteins are partially degraded proteins, a fact that makes them especially difficult to purify (Glenner, 1980*a*,*b*). The purity of the AL protein described in the present paper was checked by SDS/polyacrylamide-gel electrophoreses and by dansylation and Edman degradation. *N*-Terminal analyses revealed amino acid derivatives with a total yield of about 5% of the starting material and suggested that the protein had a blocked *N*-terminus. Characterization of the *N*-terminal CNBr-cleavage fragment CB-1 and the chymotryptic peptide C-1 revealed pyroglutamic acid as the *N*-terminal residue. The M_r of the protein was from SDS/polyacrylamide-gel electrophoreses and gel-filtration studies estimated to be about 18500. However, as the protein was found to contain carbohydrate, the M_r of the polypeptide would be somewhat smaller. The results obtained by *N*-terminal analyses, gel electrophoreses, gel filtration and from the yield of the tryptic peptides T-9 and T-10 give evidence for a 'ragged' *C*-terminal end.

An amino acid composition based on 154 amino acid residues give numbers that deviate slightly from that found from sequence determination up to position 154. The amino acid sequence elucidated from the constant region corresponds to that of protein Sh (Titani *et al.*, 1970) and has the typical constant λ region sequences. The isotypic constant-region markers, Kern (Ponstingl *et al.*, 1971) and Mcg (Fett & Deutsch, 1974), are found in peptides T-9 and Th-23. The AL protein is thus classified as $Kern^-$ and Mcg^+.

Comparison of the primary structure of the variable region of the AL protein and that of other λ light chains (residues 1–112) revealed that the AL protein had the most extensive homology to subgroup VλII (Fig. 2). There was 79% homology to VλII protein VIL (Ponstingl & Hilschmann, 1971). Although the protein has an amino acid sequence characteristic of the VλII subgroup, the AL protein revealed some peculiarities. The amino acid

Table 4. Amino acid composition of thermolytic peptides obtained from AL protein Es 492

Numbers in parentheses are from sequence determination. Cysteine was measured as carboxymethylcysteine after carboxymethylation.

Amino acid or amino sugar	Composition (residues/molecule)								
Peptide ...	Th-(2+3)	Th-3	Th-4	Th-6	Th-7	Th-8	Th-9	Th-10	Th-11
Asp					2.0 (2)	0.9 (1)			
Thr	1.0 (1)				0.8 (1)	1.1 (1)			
Ser	1.0 (1)	0.9 (1)	2.8 (3)	0.8 (1)	1.2 (1)		1.3 (1)		
Glu	1.0 (1)		1.1 (1)				2.0 (2)		
Pro	0.9 (1)		0.8 (1)					1.0 (1)	1.1 (1)
Gly			2.6 (2)		1.2 (1)			1.1 (1)	
Ala	1.1 (1)	1.1 (1)			0.8 (1)		0.8 (1)		1.5 (1)
Cys				1.0 (1)					
Val			0.7 (1)		1.0 (1)				
Ile				0.8 (1)					0.9 (1)
Leu	0.7 (1)							0.2 (1)	
Tyr							0.5 (1)		
Phe						1.0 (1)			
His					0.9 (1)			0.9 (1)	
Lys									0.6 (1)
Arg									
Trp							(1)		
Residue nos.	4–9	8–9	10–17	20–22	23–30	31–33	34–39	40–43	44–47
Yield (%)	9	29	10	12	7	21	25	11	14
GlcN					0.6 (1)				

Peptide ...	Th-13	Th-14	Th-15	Th-16	Th-17	Th-18	Th-19	Th-21
Asp	1.2 (1)	0.9 (1)	1.2 (1)	0.9 (1)				1.4 (1)
Thr				0.9 (1)		1.0 (1)		
Ser		1.8 (2)	1.1 (1)	3.2 (3)	0.9 (1)		1.2 (1)	
Glu								2.0 (2)
Pro		0.8 (1)						
Gly		1.8 (1)		2.8 (2)			0.9 (1)	
Ala					1.1 (1)			0.9 (1)
Cys								
Val		0.9 (1)	0.8 (1)					
Ile	0.8 (1)						0.6 (1)	
Leu						0.4 (1)		
Tyr								
Phe	1.0 (1)			1.1 (1)				
His								
Lys				0.8 (1)				
Arg		0.8 (1)	0.9 (1)					
Trp								
Residue nos.	50–52	53–59	60–63	64–72	73–74	75–76	77–79	82–85
Yield (%)	37	17	16	14	29	43	5	9
GlcN								

Peptide ...	Th-23	Th-24	Th-25	Th-26	Th-28	Th-(29+30)	Th-32	Th-33
Asp						1.3 (1)	1.4 (1)	
Thr	0.6 (1)	1.0 (1)						
Ser				1.1 (1)	1.8 (2)		1.0 (1)	
Glu			1.3 (1)		2.3 (2)	1.1 (1)		
Pro			0.9 (1)	1.0 (1)	1.8 (2)			1.0 (1)
Gly	3.2 (3)		1.2 (1)					1.7 (1)
Ala				1.6 (2)		0.8 (1)		
Cys								
Val	1.5 (2)		0.9 (1)					
Ile							0.9 (1)	
Leu	0.5 (1)	0.4 (1)	0.9 (1)		0.7 (1)	0.6 (1)	0.5 (1)	
Tyr								0.5 (1)
Phe	1.0 (1)				1.4 (1)			0.6 (1)
His								
Lys	1.0 (1)		0.8 (1)			1.0 (1)		
Arg								
Trp								
Residue nos.	99–107	108–109	110–115	116–119	122–129	130–134	140–143	144–147
Yield (%)	5	43	32	25	7	10	25	10
GlcN								

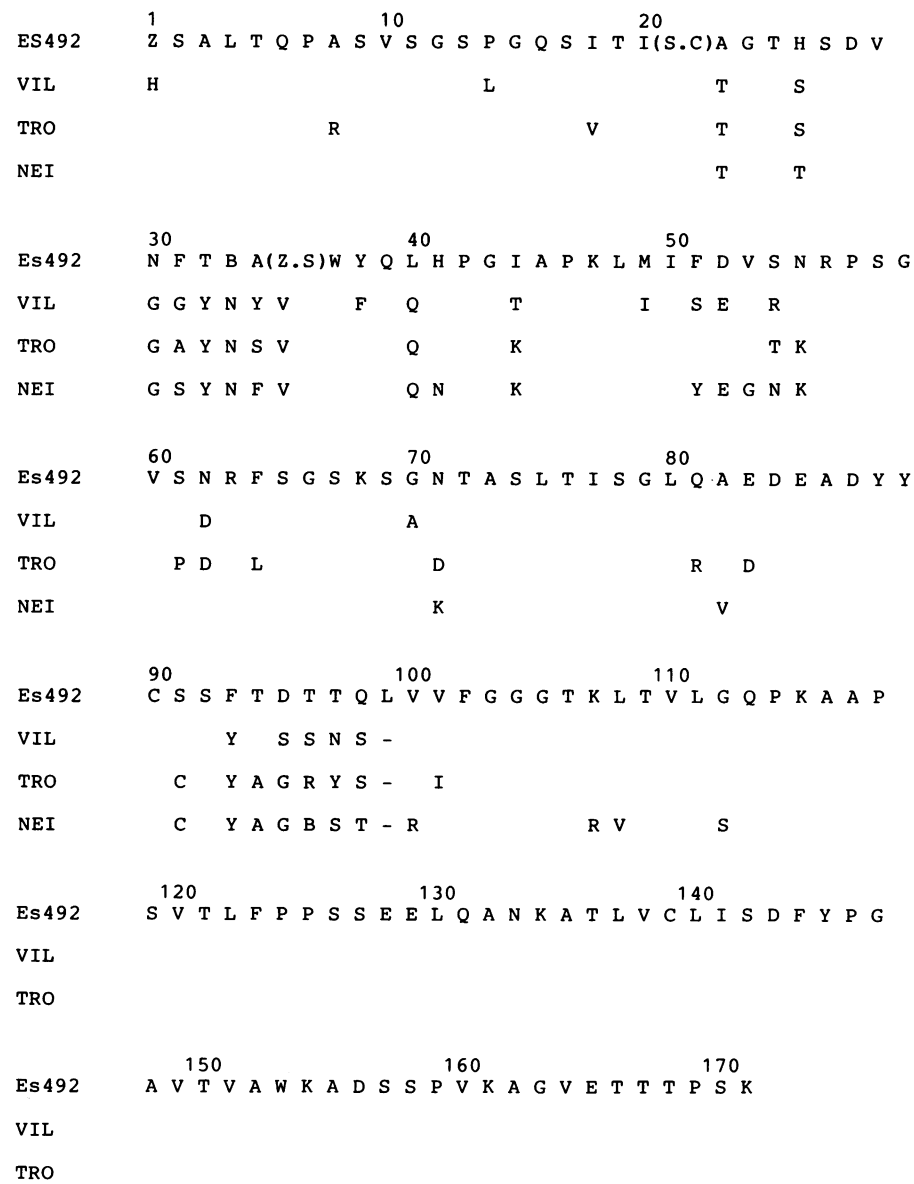

Fig. 2. Comparison of the sequence of the AL protein Es492 with that of other λ-chains of subgroup II

The sequence data are as listed by Kabat *et al.* (1983).

Table 5. Carbohydrate composition of AL protein Es492 and of fragments derived from the protein

The values are averages for three analyses.

	Composition (residues/molecule)		
	AL protein	Peptide CB-1	Peptide T-5
Fucose	—	—	0.3
Mannose	1.6	—	2.0
Galactose	1.3	—	1.2
N-Acetylglucosamine	2.0	1.0	3.0
N-Acetylneuraminic acid	0.6	1.0	3.6
Glucose	1.9	15.0*	—

* Most probably a contaminant from the Sephadex gel filtration.

residues in the following positions have not been observed in other VλII subgroup chains: His-26, Phe-31, Thr-32, Leu-40, Ile-44, Phe-93, Thr-97, Gln-98 and Leu-99 (Kabat *et al.*, 1983). The significance of these residues for the formation of amyloid material is unclear, but most of these amino acid residues have predominant β-pleated sheet potential (Muckle & Goldsmith, 1980).

Another characteristic feature of this AL protein was the content of carbohydrate, which has not been detected in other AL proteins. Of all the VλII light chains characterized, only three contained carbohydrate (Savvidou *et al.*, 1981). The light chains had only one glycosylated site per chain, either around position 25 or around position 94 (Garver & Hilschmann, 1972; Garver *et al.*, 1975; Kiefer *et al.*, 1980). The AL protein presented here was found to contain two glycosylated sites per chain. A thermolytic peptide, Th-7, containing residues 23–30, was by amino acid analyses shown to contain

glucosamine. A CNBr-cleavage fragment, CB-1, containing residues 1–49, was analysed for total carbohydrate composition and found to contain only one residue each of *N*-acetylglucosamine and *N*-acetylneuraminic acid. We have no explanation for this peculiar carbohydrate composition. However, residues 30, 31 and 32 form a part of an appropriate acceptor sequence for *N*-glycosylation.

Evidence for the second glycosylation site was obtained from the tryptic peptide T-5, containing residues 69–107, and which contained 2 residues of mannose, 1.2 residues of galactose, 3 residues of *N*-acetylglucosamine and 4 residues of *N*-acetylneuraminic acid. Digesting this peptide with *S. aureus* V8 proteinase resulted in a peptide P-2, which after acid hydrolysis and amino acid analysis revealed 2.5 residues of glucosamine. A possible glycosylation site could be position 95, where an acceptor sequence for *N*-glycosylation occurs (positions 95, 96 and 97). This glycosylation site would be in the same region as that found in a Bence Jones protein of VλII by Garver & Hilschmann (1972).

What role the carbohydrate plays in the variable region of immunoglobulin light chains is unknown. With the results here reported, questions can be raised whether the carbohydrate plays a part in the antibody combining site or be of importance for the formation of amyloid fibrils from immunogobulin light chains.

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