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The complete amino acid sequence of a prototype immunoglobulin-λ light-chain-type amyloid-fibril protein AR

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The amino acid sequence of an amyloid-fibril protein of immunoglobulin light-chain type (AL) was elucidated. The sequence determination involved digesting the protein with trypsin, thermolysin and pepsin. The protein was found to consist of 154 amino acid residues and is thus missing about half of the constant region of a light chain. A certain heterogeneity in the length of the polypeptide was observed in the *C*-terminal region. The amino acid sequence from CDR (complementary-determining region) 1 and FR (framework region) 3 indicated an oligoclonal origin of the protein. By comparing the primary structure of protein AR with other λ- and even κ-chains, it was revealed that protein AR had an insertion of two residues of aspartic acid, namely residues 68 and 69, which has not been reported previously in light chains. The overall sequence homology in the variable region showed that protein AR is more similar to VλII and VλV than to the other subgroups [Kabat, Wu & Bilofsky (1979) *Variable Regions of Immunoglobulin Chains*, Medical Computer Systems, Bolt, Beranek and Newman, Cambridge, MA].

Tissue deposition of amyloid is a pathological process often resulting in functional disturbances and/or growth of various organs (Glenner *et al.*, 1973; Husby, 1975). The amyloid fibril is an essential component of the amyloid substance. The chemical composition of the amyloid fibrils differs among individual patients, and this difference is clearly related to the various clinical types of amyloidosis. Immunoglobulin light chains or fragments thereof comprise a major component of the fibrils in cases of primary and myeloma-associated amyloidosis [for reviews, see Glenner *et al.* (1973) and Sletten *et al.* (1974)]. The amyloid protein AA plays a similar role in secondary amyloidosis (Benditt *et al.*, 1971; Glenner *et al.*, 1973; Sletten & Husby, 1974).

In a previous paper we described the characterization of an immunoglobulin-λ light-chain amyloid-fibril protein AR, isolated from the spleen of a patient (AR) with primary amyloidosis (Husby *et al.*, 1974; Sletten *et al.*, 1974). This protein was antigenically unique and structurally different from other known immunoglobulin-λ light chains. For these reasons the protein AR was proposed as the prototype of a new λ-subgroup (Sletten *et al.*, 1974).

In the present paper we report the complete amino acid sequence of the amyloid protein AR.

Abbreviation used: dansyl, 5-dimethylaminonaphthalene-1-sulphonyl. In immunoglobulin light chains, FR1, FR2, FR3 and FR4 comprise residues 1–23, 35–49, 57–88 and 98–107, and CDR1, CDR2 and CDR3 comprise residues 24–34, 50–56 and 89–97F respectively. FR, framework region; CDR, complementary-determining region.

Materials and methods

Preparation of protein AR

Amyloid fibrils were obtained from the spleen of a patient AR with a malignant course of primary amyloidosis. The patient died only 4–5 months after the diagnosis was established. The purification of amyloid fibrils has been described in detail previously (Sletten *et al.*, 1974). Briefly, the amyloid fibrils were extracted with water after repeated washings of homogenized amyloid-laden spleen tissue with saline (0.9% NaCl) to remove all soluble proteins (Pras *et al.*, 1968). Protein AR was prepared by gel filtration of amyloid fibrils treated with 0.55 M-Tris/HCl buffer, pH 8.5, containing 6 M-guanidine and 0.1 M-dithiothreitol. The fractions containing protein AR were dialysed against water, freeze-dried, dissolved in 10% (v/v) formic acid and gel-filtered on Sephadex C-25 (Fine grade) equilibrated with 10% (v/v) formic acid.

Amino acid analysis

Protein samples were hydrolysed for 24, 42 and 72 h with 6 M-HCl in evacuated tubes at 108–110°C. Samples for determination of tryptophan were

hydrolysed for 30h with 6M-HCl containing 2½% (v/v) thioglycollic acid (Matsubara & Sasaki, 1969). Half-cystine was determined as cysteic acid after performic acid oxidation and as carboxymethylcysteine after carboxymethylation (Crestfield *et al.*, 1963) and as *S*-methylcysteine after *S*-methylation (Eyem *et al.*, 1976). Peptides were routinely hydrolysed for 24h in 6M-HCl containing 0.05% thioglycollic acid. Amino acid analysis was performed on a BIO CAL BC-200 instrument, which was connected to an AUTOLAB AA computing integrator system.

Citraconylation

The citraconylation of the protein as well as the deblocking of the citraconylated peptides were essentially performed as described by Jaton (1974).

Proteolytic digestions

Tryptic digestion of *S*-methylated citraconylated protein (4μmol) was performed in 4ml of 0.2M-NH_4HCO_3, pH8.3, with an enzyme/substrate ratio 1:50 for 3h at 37°C. The reaction was stopped by freeze-drying.

Carboxymethylated protein (1.6μmol) was digested with thermolysin in 5ml of 0.1M-NH_4HCO_3, pH8.0, with an enzyme/substrate ratio of 1:200 for 1h at 37°C. The digestion was stopped by adding 50% formic acid dropwise until the pH fell to 3. Digestion with carboxypeptidases A and B obtained from Koch–Light and Worthington was performed in 0.1M-NH_4HCO_3, pH7.9, at 38°C, with an enzyme/substrate ratio of 1:50. Portions were removed after 3, 13, 55 and 130min of digestion and the reaction was terminated by adding 5% (v/v) formic acid followed by freeze-drying before amino acid analysis. Peptic digestion of *S*-methylated protein was performed in pyridine/acetate, pH3.0, at 24°C for 8h, at an enzyme/substrate ratio of 1:35. The digest was stopped by freeze-drying.

Dilute acid cleavage

A peptide obtained from the digestion with thermolysin was cleaved in 0.03M-HCl for 15h at 108–110°C.

Purification of peptides

Trypsin-digested *S*-methylated and citraconylated protein was gel-filtered on a column (1.8cm × 108cm) filled with Sephadex G-50 (Fine grade) and equilibrated with 0.5M-$NaHCO_3$. The effluent was monitored at 280nm by using an LKB 8300 A Uvicord II spectrophotometer, and fractions (5.5ml) were collected. The flow rate was 10.9ml/h. The fractions were freeze-dried and then desalted on a column with Sephadex G-10 before deblocking was performed. Fractions were further purified by t.l.c. on precoated plates obtained from Merck and Machery–Nagel and Co. The silica thickness was 0.2mm and 0.25mm respectively. The following solvent systems were used: (1) chloroform/methanol/25% (v/v) NH_3 (2:2:1, by vol.); (2) butan-2-ol/acetic acid/water (3:1:1, by vol.); and (3) propan-2-ol/formic acid/water (40:2:10, by vol.). Separation of peptides obtained from the digestion with thermolysin was achieved by using Aminex A-5 resin (Sletten & Husby, 1974). The ion-exchange-chromatography fractions were further fractionated, either by gel filtration on a column (1cm × 140cm) of Sephadex G-25 with 10% (v/v) formic acid as eluent or by t.l.c. with solvent systems 1, 2 and 3. The peptides eluted from the columns were tested by t.l.c. and detected by spraying with ninhydrin and by chlorbenzidine.

Peptide 'maps' were obtained by t.l.c. in the first direction by using solvent system 2, and electrophoresis in pyridine/acetate, pH3.6, at 1000V for 60min in the second direction.

Initially, a small amount of performic acid-oxidized protein was digested with trypsin and the peptides were purified by ion-exchange chromatography on Aminex A-5 resin (Sletten & Husby, 1974) and by t.l.c.

Peptic peptides were purified by gel filtration on a column (1.5cm × 217cm) of Sephadex G-50 (Fine grade), with 10% (v/v) formic acid as effluent. Fractions were further purified by t.l.c.

N-Terminal analysis

Dansylation of peptides and separation of the dansyl-amino acids were performed as described by Gray (1972). Edman degradation was performed automatically on a JEOL JAS-47K sequence analyser by using the protein program for both small and large peptides. The program included double coupling in 0.1M-Quadrol buffer at the first cycle, otherwise single coupling and double cleavage were used. To prevent losses of peptides from the spinning cup, 1mg of Polybrene was added and taken through a cycle before the peptide was applied (Tarr *et al.*, 1978; Hunkapillar & Hood, 1978). Samples in the range of 50–300nmol were used. The thiazolinone amino acids were converted into their phenylthiohydantoin derivatives by incubation in 0.2ml of 1M-HCl at 80°C for 10min. The amino acid phenylthiohydantoin derivatives were analysed by gas chromatography in a JEOL JGC-20K gas chromatograph equipped with a glass column with 10% SE on Gas Chrom Q (Supelco) and by t.l.c. on Merck DC-Alufolien Kieselgel 60 F 254 in the following solvent systems: (A) chloroform/ethanol/methanol (441:9:50, by vol.); (B) chloroform/ethanol (49:1, v/v). For identification of arginine and histidine phenylthiohydantoin derivatives, and for supplementary identification of other amino acid residues, a portion of the sample was hydro-

lysed in 6M-HCl at 150°C for 18h (Van Orden & Carpenter, 1964) and analysed on the amino acid analyser.

Peptide nomenclature

The following prefixes were used to denote the origin of the various peptides: T, tryptic digest of performic acid-oxidized protein; Tc, tryptic digest of citraconylated *S*-methylated protein; Th, thermolytic digest of *S*-carboxymethylated protein; P, peptic digest of *S*-methylated protein: Da, dilute-acid cleavage of peptides. Peptides are numbered in order of their position in the final sequence, except for the peptic and the dilute-acid-cleaved peptides.

Results

The protein was isolated from the spleen of patient AR, a 57-year-old male with symptoms of primary amyloidosis. The yield of the protein from the amyloid fibrils was about 65%. From the gel-filtration studies a mol.wt. of about 16000 was estimated (Husby *et al.*, 1974). *N*-Terminal analysis revealed only one amino acid derivative at each position among the first 21 residues. The amino acid sequence of the first 46 amino acid residues and the yield obtained at the different positions is shown in Fig. 1. Automatic degradations of the protein revealed trace amounts of glutamic acid in position 24, asparagine in position 26 and serine in position 27, in addition to glycine in these positions. At this stage glycine was ruled out, since this amino acid may appear as background in later degradation steps.

C-Terminal analysis with carboxypeptidase A and B showed that the main polypeptide has a *C*-terminal sequence of Ala-Val. The amino acid composition of the protein was determined from several batches, and the mean values are shown in Table 1.

Tryptic digest of citraconylated protein AR

The trypsin-digested material was gel-filtered on a column (1.8cm × 108cm) filled with Sephadex G-50 (Fine grade), 0.5M-$NaHCO_3$ being used as eluent. The elution pattern obtained is shown in Fig. 2. Fractions were collected as indicated and freeze-dried before desalting on a column of Sephadex G-10. Desalted fractions were freeze-dried, deblocked and gel-filtered on a column of Sephadex G-25. Peptides Tc-1 and Tc-2 were separated from each other by t.l.c. on DC-Alufolien Kieselgel 60 F254 by using solvent system 2. In this system, Tc-1 stayed at the origin whereas Tc-2 moved. Tc-3 was characterized in the form in which it was obtained after the deblocking procedure. The amino acid composition of the tryptic fragments Tc-1, Tc-2 and Tc-3 obtained after acid hydrolysis is shown in Table 2. Except for the residue numbers of aspartic acid, glutamic acid and alanine, there is good agreement with the composition of Tc-1 and the results obtained from the *N*-terminal analysis of the protein (residues 1–40). Fragment Tc-1 contains the only residue of methionine in the protein. Four steps of Edman degradation of Tc-1 confirmed the above results and showed that it was slightly contaminated. The amino acid composition of Tc-2 revealed a peptide consisting of about 22 amino acid residues, including two residues of arginine (Table 2). Fourteen steps of Edman degradation showed that the amino acid sequence of the first six residues was the same as that found in positions 41–46 of the protein and thus gave the sequence up to position 54 (Fig. 1). The analysis further revealed that Tc-2 was contaminated with Tc-1 to the extent of about 20%.

The amino acid composition of Tc-3 indicated that this peptide was the *C*-terminal one of the protein, since the peptide contained no arginine. The data from gel filtration, as well as from amino acid analysis, indicated a mol.wt. of about 9000. *N*-Terminal analysis gave phenylalanine and alanine in a ratio of about 3:2 (Fig. 1). However, further degradation made it clear that the contaminant originated from the main peptide being digested by trypsin at the lysine residue in position 115 in the constant region of the λ-chain. In this way it was possible to establish the amino acid sequence of residues 63–85 and verify the sequence in the

Table 1. *Amino acid composition of amyloid protein AR*
The values are averages for nine different analyses; hydrolysis was for 24, 42 and 72h.

	Composition (residues/molecule)	
Amino acid	Found	From the sequence
Aspartic acid	14.0	15
Threonine	11.0	13
Serine	17.9	21
Glutamic acid	13.5	13
Proline	10.8	11
Glycine	12.5	13
Alanine	9.04	11
Half-cystine*	2.22	3
Valine	10.1	13
Methionine	1.09	1
Isoleucine	4.23	5
Leucine	7.29	8
Tyrosine	5.54	6
Phenylalanine	6.49	7
Histidine	2.94	3
Lysine	4.98	6
Arginine	3.74	3
Tryptophan	1.4	2
Total residues		154

* Half-cystine was calculated as cysteic acid after performic acid oxidation, as *S*-methylcysteine after *S*-methylation and as carboxymethylcysteine after carboxymethylation.

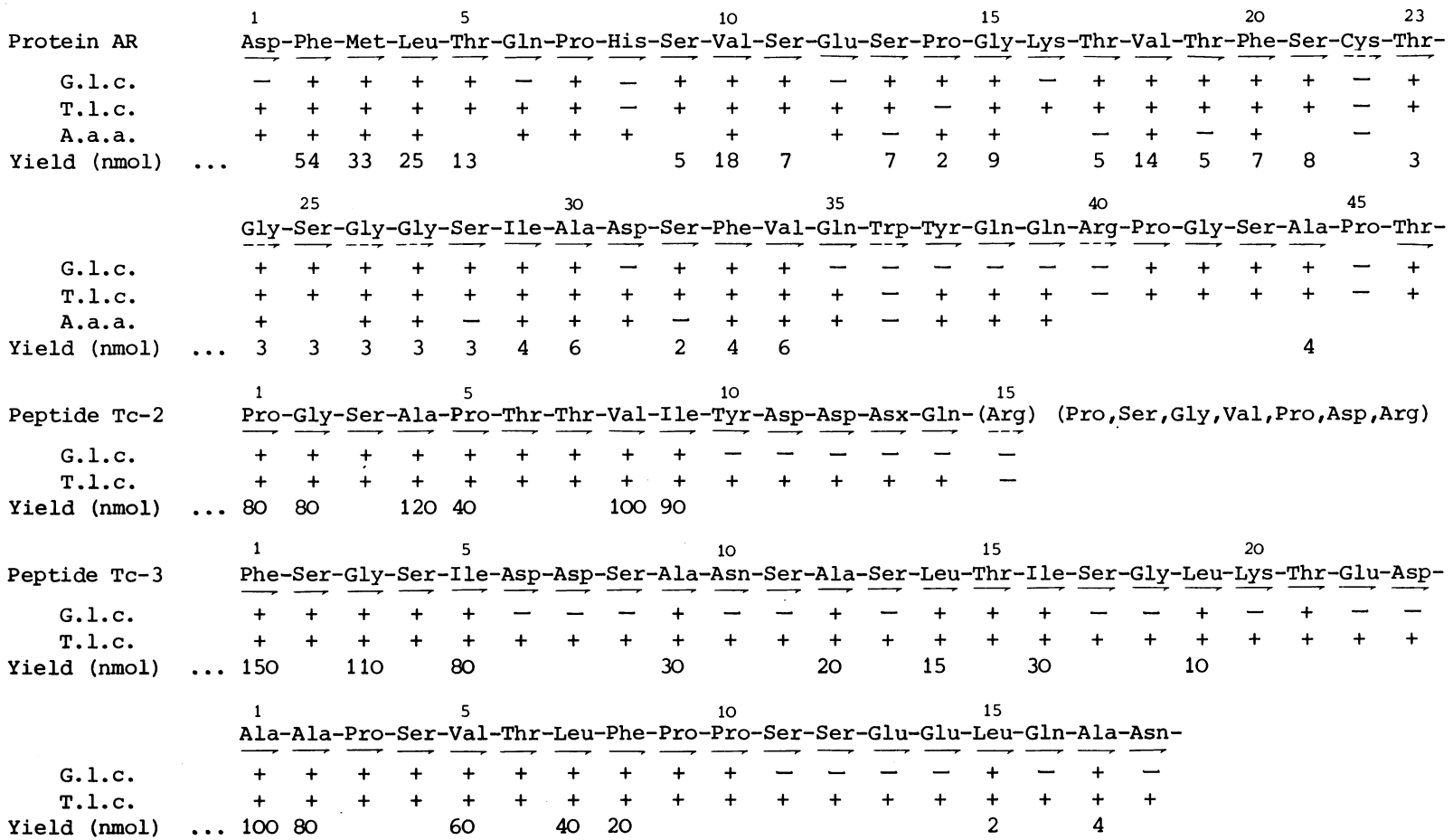

Fig. 1. *N-Terminal analysis of protein AR and of tryptic peptides obtained from the citraconylated protein*

Identification methods used for analyses of the amino acid phenylthiohydantoin derivatives released at each degradation step were g.l.c., t.l.c. and amino acid analysis (A.a.a.) of the 6 M-HCl hydrolysate of the amino acid derivative. Yields were calculated by comparing peak heights of samples with those of relevant standard derivatives by g.l.c.

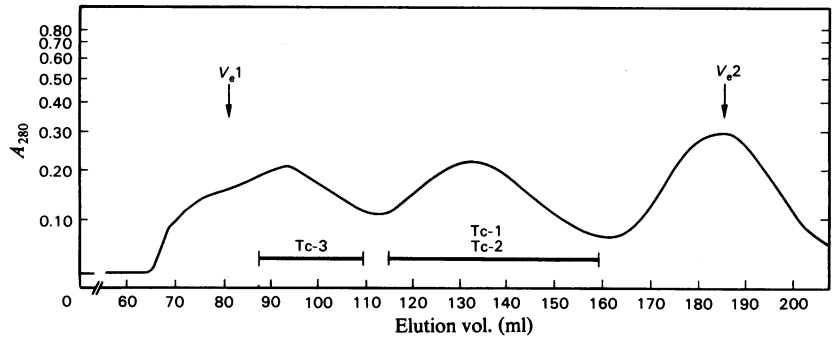

Fig. 2. *Elution profile of a tryptic digest of citraconylated protein AR*
A portion (65 mg) of digested material was applied to a column (1.8 cm × 108 cm) of Sephadex G-50 (Fine grade) and eluted with 0.5 M-$NaHCO_3$ at 10.9 ml/h. Fractions (5.5 ml) were collected. For testing the column, chymotrypsinogen A (V_e1) and glycyltryptophan (V_e2) were used.

Table 2. *Amino acid composition of peptides obtained from trypsin-digested citraconylated protein AR*
Residues in parentheses are obtained from sequence studies.

Amino acid Peptide ...	Composition (residues/peptide molecule) Tc-1	Tc-2	Tc-3
Asp	2.98 (2)	3.93 (4)	9.46 (9)
Thr	3.52 (4)	2.05 (2)	6.74 (7)
Ser	7.45 (7)	2.46 (2)	10.9 (12)
Glu	3.89 (5)	1.75 (1)	9.18 (7)
Pro	2.2 (2)	3.3 (4)	5.5 (5)
Gly	4.47 (4)	2.38 (2)	7.18 (7)
Ala	1.73 (1)	1.19 (1)	6.64 (9)
½-Cys*	0.65 (1)		0.7 (2)
Val	3.00 (3)	1.78 (2)	5.44 (8)
Met	0.77 (1)		
Ile	1.19 (1)	0.72 (1)	2.64 (3)
Leu	0.89 (1)		6.18 (7)
Tyr	0.81 (1)	0.77 (1)	3.80 (4)
Phe	1.98 (3)	0.28	3.98 (4)
His	0.43 (1)		2.02 (2)
Lys	1.19 (1)	0.22	3.96 (5)
Arg	0.98 (1)	1.85 (2)	
Trp	(1)		(1)
Residue nos.	1–40	41–62	63–154

* Half-cysteine was measured as *S*-methylcysteine.

constant region from position 116 to 133. The reason why the lysine residue in position 115 was incompletely citraconylated, or that trypsin cleaved at this position, is unclear. From a tryptic digestion of performic acid-oxidized protein, peptides T-1 (residues 1–16) and T-6 (residues 83–107) were characterized by amino acid composition and *N*-terminal analysis and peptides T-4 (residues 56–62), T-5 (residues 63–82), T-7 (residues 108–115) and T-9I (residues 135–148) by amino acid composition (Table 3 and Fig. 3).

Peptides obtained after digestion with thermolysin

The thermolysin-digested material was chromatographed on Aminex A-5 resin and fractions were further fractionated on Sephadex G-25 (Fine grade) and by t.l.c. Table 4 shows the amino acid composition of 24 purified peptides, obtained after acid hydrolysis of ninhydrin-stained material. The yield of the *N*-terminal residue is about 20% lower than the other amino acid residues. Peptides obtained from the thermolysin digest were analysed by

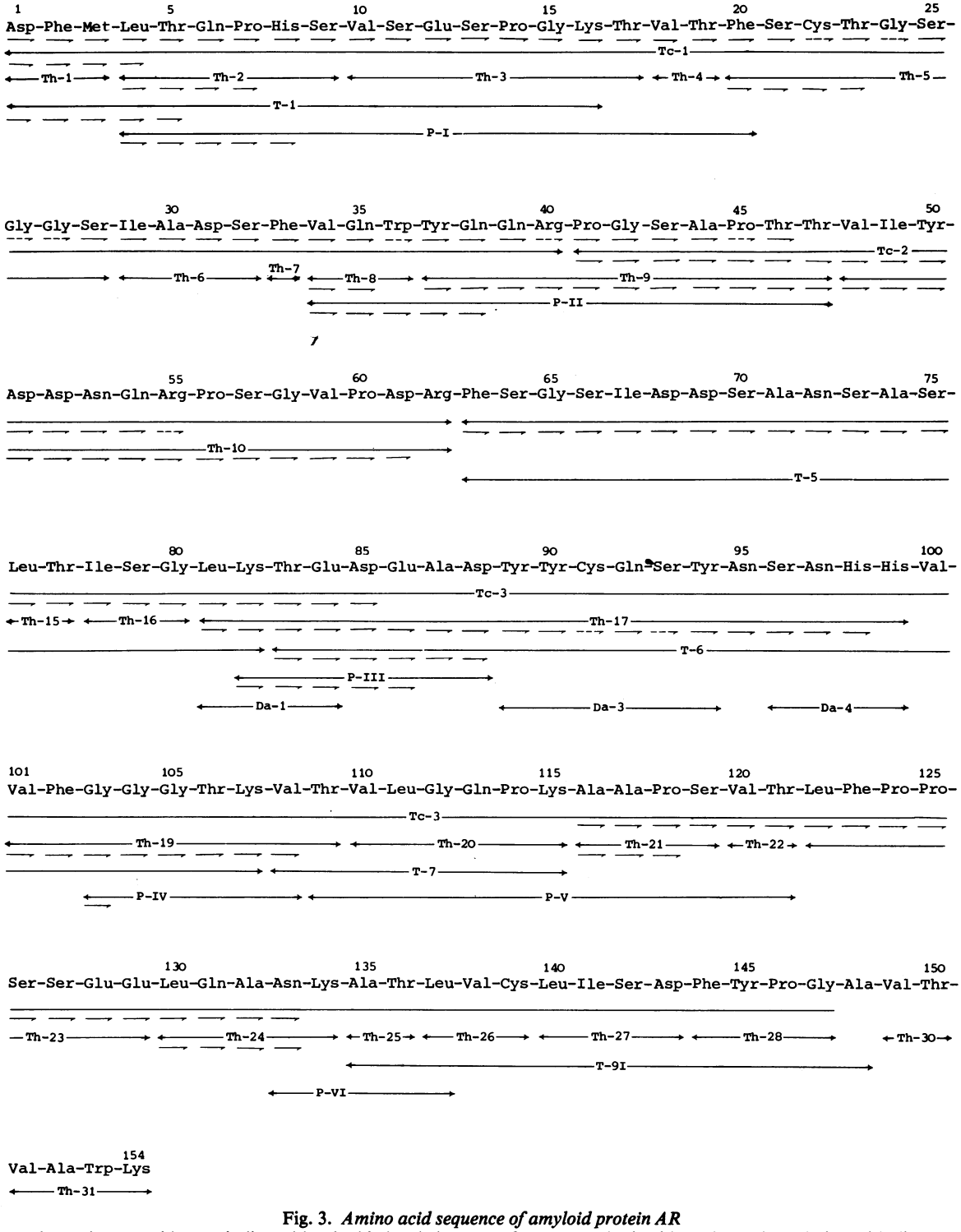

Fig. 3. *Amino acid sequence of amyloid protein AR*

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 pointing to the right. Broken half-arrows indicate amino acid phenylthiohydantoin derivatives not identified directly by Edman degradation. Tc and T are tryptic peptides. Th, P and Da are thermolysin, peptic and dilute-acid-cleaved peptides respectively.

Table 3. *Amino acid composition of tryptic peptides obtained from oxidized protein AR*
Data for peptides T-1 and T-5 are from ninhydrin-stained material.

Amino acid	Peptide ...	Composition (residues/molecule) T-1	T-4	T-5	T-6	T-7	T-9I
Asp		1.01 (1)	0.11	3.02 (3)	4.00 (4)		1.01 (1)
Thr		0.95 (1)		1.00 (1)	1.89 (2)	0.94 (1)	1.11 (1)
Ser		2.86 (3)	2.21 (2)	5.46 (6)	2.31 (2)		0.96 (1)
Glu		2.27 (2)	0.91 (1)	0.30	3.36 (3)	1.16 (1)	0.42
Pro		1.6 (2)	0.9 (1)			0.93 (1)	1.0 (1)
Gly		1.04 (1)	1.02 (1)	1.82 (2)	3.43 (3)	1.05 (1)	1.10 (1)
Ala		—		2.19 (2)	1.07 (1)		1.52 (1–2)
½-Cys*		—			0.99 (1)		0.70 (1)
Val		1.14 (1)	1.00 (1)	0.20	1.50 (2)	1.96 (2)	1.17 (1–2)
Met		1.02 (1)					
Ile		—		2.06 (2)			0.76 (1)
Leu		0.97 (1)		1.91 (2)		1.01 (1)	1.59 (2)
Tyr		—			2.50 (3)		0.22 (1)
Phe		0.96 (1)		0.98 (1)	1.12 (1)		0.84 (1)
His		1.01 (1)			1.84 (2)		
Lys		0.8 (1)	0.81 (1)	0.7 (1)	0.83 (1)	0.96 (1)	
Arg							
Trp							
Residue nos.		1–16	56–62	63–82	83–107	108–115	135–148

* Half-cystine was measured as cysteic acid.

the automatic sequence analyser, with the protein program and the addition of Polybrene. The results are summarized in Table 5. No thermolytic peptides from the region of residues 63–75 could be isolated. From peptide 'mapping', 35 ninhydrin-positive spots could be seen. Peptide Th-17 was further degraded by cleavage with dilute acid, and the resulting peptides purified by t.l.c. in solvent system 2. The amino acid composition of the peptides is shown in Table 6. The fraction containing peptide Da-2 was not purified.

Peptides obtained after peptic digestion

Peptic digestion was performed in order to get more information about the region from positions 60 to 75. The digested material was gel-filtered on a column (1.5 cm × 217 cm) of Sephadex G-50 (Fine grade), followed by t.l.c. Six peptides, P-1 (residues 4–10), P-II (residues 34–47), P-III (residues 82–88), P-IV (residues 103–108), P-V (residues 109–121) and P-VI (residues 133–137) were purified and characterized (Table 7 and Fig. 3).

Deduction of the sequence

The sequence data are summarized in Fig. 3.

Residues 1–46. *N*-Terminal analysis of the protein elucidated the amino acid sequence to residue 46 (Fig. 3), except for residues 22, 24, 26, 27, 36, 40 and 45. Peptides Th-1, Th-2, Th-3, Th-4, Th-5, Th-6, Th-8 and Th-9 obtained from the thermolysin digest of the protein completed and confirmed the data (Fig. 3). Additional information was also obtained from tryptic peptides T-1, Tc-1, Tc-2, P-I and P-II.

Residues 47–62. Automatic Edman degradation of Tc-2 resulted in the amino acid sequence of residues 41–54. The sequence of this region was confirmed by peptides Th-9 and Th-10 (Fig. 3).

The amino acid composition of Tc-2 is also in agreement with the residues elucidated by Edman degradation (Table 2 and Fig. 3). Trace amounts of a tryptic heptapeptide containing two residues of serine and one residue each of glycine, valine, proline, glutamic acid and lysine was isolated. No other structural studies could be performed, but this peptide could possibly derive from the same region as T-4, namely residues 56–62.

Residues 63–82. Automatic Edman degradation of Tc-3 revealed the amino acid sequence of two peptides. The elucidation could be done because the yield of the two peptides was different and because the peptide with the lowest yield originated from the constant region of λ-chain. From the main peptide, the amino acid sequence of residues 63–85 was established. The covalent structure of this region was also verified by peptides Th-15, Th-16, Th-17 and T-5 (Fig. 3).

Residues 83–107. Automatic Edman degradation of peptide Th-17 elucidated the amino acid sequence

Table 4. *Amino acid composition of peptides obtained from thermolysin-digested carboxymethylated protein AR*
The analyses of the different peptides were made on ninhydrin-stained fractions, except fraction Th-17. The content of tryptophan was determined from A_{280}.

Amino acid	Peptide ...	Composition (residues/molecule)								
		Th-1	Th-2	Th-3	Th-4	Th-5	Th-6	Th-8	Th-9	Th-10
Asp		0.72 (1)	0.20	0.15			1.01 (1)		0.16	4.04 (4)
Thr			0.90 (1)	0.92 (1)	1.00 (1)	0.98 (1)			1.85 (2)	
Ser			1.02 (1)	1.88 (2)		2.96 (3)	0.93 (1)		1.05 (1)	0.95 (1)
Glu			1.11 (1)	1.08 (1)				1.00 (1)	2.15 (2)	1.12 (1)
Pro			1.07 (1)	1.03 (1)					2.0 (2)	2.0 (2)
Gly		0.29	0.16	0.97 (1)	0.11	3.15 (3)	0.11		1.05 (1)	0.95 (1)
Ala							0.99 (1)		0.96 (1)	
½-Cys*						0.99 (1)				
Val				0.88 (1)	0.78 (1)			0.97 (1)		1.28 (2)
Met		0.91 (1)								
Ile							0.79 (1)			0.34 (1)
Leu			0.74 (1)							
Tyr									0.86 (1)	0.63 (1)
Phe		1.09 (1)				0.75 (1)				
His			0.92 (1)							
Lys				0.80 (1)						
Arg									0.89 (1)	1.96 (2)
Trp								(1)		
Residue nos.		1–3	4–9	10–17	18–19	20–28	29–32	34–36	37–47	48–62

	Peptide ...	Th-15	Th-16	Th-17	Th-17 I	Th-19	Th-20	Th-21	Th-22	Th-23
Asp				4.25 (4)	2.05 (2)					
Thr		1.00 (1)	0.20	0.95 (1)		1.98 (2)			1.00 (1)	0.38
Ser			0.91 (1)	1.90 (2)	0.92 (1)			0.96 (1)		1.64 (2)
Glu				3.21 (3)		0.12	1.01 (1)			2.01 (2)
Pro							1.00 (1)	1.0 (1)		2.1 (2)
Gly			1.09 (1)	0.20		3.04 (3)	0.99 (1)	0.20	0.11	
Ala				0.98 (1)				1.61 (2)		
½-Cys*				0.90 (1)						
Val			0.15			1.73 (2)	0.77 (1)		0.78 (1)	0.33
Met										
Ile			0.74 (1)							
Leu		0.4 (1)		0.99 (1)			0.81 (1)			0.84 (1)
Tyr				2.90 (3)	0.52 (1)					
Phe						0.99 (1)				0.93 (1)
His				1.84 (2)	1.95 (2)					
Lys				1.02 (1)		0.64 (1)	0.86 (1)			
Arg										
Trp										
Residue nos.		76–77	78–80	81–99	94–99	101–109	110–115	116–119	120–121	122–129

	Peptide ...	Th-24	Th-25	Th-26	Th-27	Th-28	Th-30	Th-31
Asp		1.30 (1)			1.05 (1)			
Thr		0.19	1.00 (1)				1.00 (1)	
Ser			0.16		0.91 (1)			
Glu		1.00 (1)						
Pro						1.0 (1)		
Gly		0.13	0.41			1.10 (1)	0.11	
Ala		1.00 (1)	0.63 (1)					1.00 (1)
½-Cys*				0.88 (1)				
Val				1.12 (1)			0.78 (1)	0.95 (1)
Met								
Ile					0.95 (1)			
Leu		0.84 (1)		0.73 (1)	0.63 (1)			
Tyr						0.77 (1)		
Phe						0.60 (1)		
His								
Lys		0.74 (1)						0.73 (1)
Arg								
Trp								(1)
Residue nos.		130–134	135–136	137–139	140–143	144–147	149–150	151–154

* Half-cystine was measured as carboxymethylcysteine.

Table 5. *Structural studies of peptides obtained from thermolysin-digested protein AR*

→ , Edman degradation with direct positive identification of the phenylthiohydantoin derivative; --→ , the residue was indirectly identified by Edman degradation; residues in parentheses were deduced from amino acid composition, from the specificity of thermolysin and from the already established sequence.

Peptide	Position	Amino acid sequence
Th-1	1–3	(Asp-Phe-Met)
Th-2	4–9	Leu-Thr-Gln-Pro(His-Ser)
Th-3	10–17	(Val-Ser-Glu-Ser-Pro-Gly-Lys-Thr)
Th-4	18–19	(Val-Thr)
Th-5	20–28	Phe-Ser-Cys-Thr-Gly-Ser-Gly-Gly-Ser
Th-6	29–32	(Ile-Ala-Asp-Ser)
Th-7	33	Phe
Th-8	34–36	Val-Gln-Trp
Th-9	37–47	Tyr-Gln-Gln-Arg-Pro-Gly-Ser-Ala-Pro-Thr-Thr
Th-10	48–62	Val-Ile-Tyr-Asp-Asp-Asn-Gln-Arg-Pro-Ser-Gly-Val-Pro-Asp-Arg
Th-15	76–77	Leu-Thr
Th-16	78–80	(Ile-Ser-Gly)
Th-17	81–99	Leu-Lys-Thr-Glu-Asp-Glu-Ala-Asp-Tyr-Tyr-Cys-Gln-Ser-Tyr-Asn-Ser-Asn-His-His
Th-17 I	94–99	Tyr-Asn-Ser-Asn-His-His
Th-18	100	Val
Th-19	101–109	Val-Phe-Gly-Gly-Gly-Thr-Lys-Val-Thr
Th-20	110–115	(Val-Leu-Gly-Gln-Pro-Lys)
Th-21	116–119	Ala-Ala-Pro-Ser
Th-22	120–121	(Val-Thr)
Th-23	122–129	(Leu-Phe-Pro-Pro-Ser-Ser-Glu-Glu)
Th-24	130–134	Leu-Gln-Ala-Asn-Lys
Th-25	135–136	(Ala-Thr)
Th-26	137–139	(Leu-Val-Cys)
Th-27	140–143	(Leu-Ile-Ser-Asp)
Th-28	144–147	(Phe-Tyr-Pro-Gly)
Th-29	148	Ala
Th-30	149–150	(Val-Thr)
Th-31	151–154	(Val-Ala-Trp-Lys)

Table 6. *Amino acid composition of peptides obtained after diute-acid cleavage of peptide Th-17*

Amino acid	Peptide ...	Composition (residues/molecule) Da-1	Da-3	Da-4
Asp				1.02 (1)
Thr		1.00 (1)		
Ser		0.15	0.92 (1)	0.87 (1)
Glu		1.00 (1)	1.25 (1)	0.26
Ala				
½-Cys*			1.0 (1)	
Leu		0.90 (1)		
Tyr			2.85 (3)	
His				1.98 (2)
Lys		0.91 (1)		
Residue nos.		81–84	89–94	96–99

* ½-Cys was measured as carboxymethylcysteine.

of residues 81–93. Th-17 was also cleaved by dilute acid, and the amino acid composition was determined for peptides Da-1, Da-3 and Da-4 (Table 6). Another peptide, Th-17 I, obviously a subfragment of Th-17, was also isolated from the digest. Five steps of Edman degradation of this hexapeptide completed the sequence of residues 81–99. Eight steps of Edman degradation of peptide Th-19 were

Table 7. *Amino acid composition of peptides obtained from a peptic digest of protein AR*
Tryptophan composition was determined from A_{280}.

Amino acid	Peptide ...	Composition (residues/molecule) P-I	P-II	P-III	P-IV	P-V	P-VI
Asp		0.46		1.99 (2)	0.22	0.34	1.00 (1)
Thr		2.70 (3)	2.00 (2)	0.91 (1)	1.07 (1)	1.80 (2)	1.07 (1)
Ser		2.86 (3)	1.21 (1)		0.18	1.10 (1)	
Glu		2.03 (2)	3.04 (3)	2.15 (2)	0.22	1.20 (1)	
Pro		2.2 (2)	2.4 (2)			2.0 (2)	
Gly		1.46 (1)	1.00 (1)		2.93 (3)	1.35 (1)	
Ala		0.15	1.00 (1)	0.95 (1)		1.74 (2)	1.09 (1)
½-Cys							
Val		2.16 (2)	0.94 (1)		0.96 (1)	2.00 (2)	
Met							
Ile			0.35				
Leu		0.92 (1)				0.96 (1)	0.96 (1)
Tyr		0.25	0.70 (1)			0.42	
Phe		1.28 (1)				0.20	
His		1.05 (1)				0.86 (1)	
Lys		1.00 (1)		0.88 (1)	1.05 (1)		0.89 (1)
Arg			1.05 (1)				
Trp			(1)				
Residue nos.		4–20	34–47	82–88	103–108	109–121	133–137

performed, and as this peptide contained a small amount of a peptide starting with residue 100 (valine), the amino acid sequence up to residue 109 could be established. The amino acid composition of tryptic peptide T-6 and peptic peptides P-III and P-IV confirmed the data for residues 83–107 (Tables 3 and 7).

Residues 108–154. Peptide Th-19 overlapped peptides T-6 and T-7, which, together with Th-20, established the amino acid sequence to residue 116. The amino acid sequence of residues 116–133 was elucidated from Tc-3 and verified by the peptides Th-21, Th-22, Th-23, Th-24, P-V and P-VI (Fig. 3). Finally, peptides Th-25, Th-26, Th-27, Th-28, Th-30, Th-31 and T-9I confirmed the covalent structure of the constant region to residue 154 (Fig. 3). The results obtained from *C*-terminal analysis showed that the main polypeptide had a *C*-terminal sequence of Ala-Val, which would correspond to residues 148–149.

Discussion

The amyloid protein AR described here is, to our knowledge, the first amyloid-fibril protein of immunoglobulin light-chain type (AL) to be characterized by complete amino acid sequencing. The protein was shown to consist of 154 amino acid residues, corresponding to a mol.wt. of approx. 16000, which is in good agreement with the molecular weight previously estimated by gel-filtration studies (Husby *et al.*, 1974). Thus the polypeptide AR represents an incomplete protein comprising about 75% of the entire immunoglobulin-λ light chain. It comprises a whole variable region and has the typical constant-λ region sequence. *C*-Terminal analysis of the protein as well as of isolated peptides from the *C*-terminal end revealed a definite heterogeneity with respect to the length of the polypeptide. This observation is noteworthy in view of our recent finding of a similar heterogeneity in the *C*-terminus of another, non-related, amyloid-fibril protein of the AA type (Møyner *et al.*, 1980). The genesis of amyloid fibrils of both the AL and the AA type may be due to a defective enzymic degradation of intact immunoglobulin light chains and protein SAA respectively (Glenner *et al.*, 1973; Møyner *et al.*, 1980).

The primary structure of amyloid protein AR has been determined by sequence analysis of the *N*-terminus of four tryptic fragments obtained after citraconylation of the protein and by characterization of peptides obtained from digestion of the protein with thermolysin, trypsin, pepsin and dilute acid. No overlapping peptide could be found for residues 63 and 64, but as the amino acid sequence has identified the protein as an incomplete λ-chain, residue 64 was positioned by homology with other λ-chains. The amino acid sequence of protein AR presented in Fig. 3 has been corrected from that published previously (Sletten *et al.*, 1974) in positions 24, 26, 27 and 40. The *N*-terminal analysis revealed, in addition to glycine in positions 24, 26 and 27, glutamic acid, asparagine and serine

Table 8. *Amino acid-sequence homologies between the V-region of protein AR and that of other λ-chains*
The sequence data of the different λ-chains are as listed by Kabat *et al.*, (1979).

Subgroup	...	VλI			VλII				VλIII				VλIV	VλV	
Segment	Protein ...	HA	NEW	VOR	NEI	TRO	BOH	VIL	HIL	X	BAU	DEL	SH	BO	MCG
FR1		13	13	12	13	14	14	12	12	12	11	10	9	13	12
CDR1		2	3	3	3	4	3	3	0	3	2	4	1	3	3
FR2		10	8	9	10	9	10	8	11	11	10	9	11	10	9
CDR2		4	5	3	4	4	4	3	5	5	4	4	4	3	4
FR3		24	21	24	24	24	25	27	20	22	21	21	23	26	26
CDR3		1	2	0	4	3	3	5	1	3	2	1	3	4	3
FR4		8	11	11	9	10	9	10	10	8	10	10	10	9	10
	Sum	62	63	62	67	68	68	68	59	64	60	59	61	68	67

respectively. However, the amount of glycine was taken as background. Peptide Th-5 revealed three residues of glycine, and the covalent structure of this region could be established. A microheterogeneity in this region can, however, not be excluded. Some microheterogeneities were also apparent in the region of residues 56–62. A tryptic heptapeptide with an amino acid composition very similar to that found for the same region in human VλI VOR (Engelhard & Hilschmann, 1975) was isolated in trace amounts. These results from the hypervariable regions show that the amyloid-fibril proteins of the immunoglobulin type may not be entirely homogeneous (Glenner *et al.*, 1976).

The primary structure of protein AR revealed that this λ-chain has a characteristic insertion of two residues of aspartic acid in positions 68 and 69. This has not been observed in any other λ- or κ-light chain (Kabat *et al.*, 1979). The significance of this is, however, unclear.

Three λ-light-chain proteins with a *N*-terminal amino acid sequence very similar to that of protein AR have been reported [JAM (Skinner *et al.*, 1975), YAM (Wang *et al.*, 1976) and RS (Natvig *et al.*, 1980)]. Protein JAM has only been characterized up to residue 16, protein YAM up to residue 22 and protein RS up to residue 34. It will be of great interest to see whether this observed homology will be consistent for the entire V-region. One of these λ-chains, protein YAM, was isolated from a monoclonal immunoglobulin A(λ) protein obtained from a patient with multiple myeloma, and it is not known if this patient had amyloidosis. It is, however, noteworthy that the three remaining proteins, AR, JAM and RS, were all derived from patients with primary amyloidosis. The amyloid protein AR was originally established as a prototype of a new λ-subgroup, namely subgroup VλV, but later this subgroup has been assigned to protein DEL (W. C. Barker, personal communication). Recently, Kabat *et al.* (1979) have assigned the three λ-light-chain proteins (AR, JAM and YAM) to a subgroup called VλVI. In that paper, Kabat *et al.* (1979) have considered the variable regions of light and heavy chains to be made up of four framework regions separated by three complementary-determining regions. In Table 8, the sequence homologies between the FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4 of protein AR and that of λ-chains from five different subgroups are shown. In total the most extensive sequence homology is seen with the proteins belonging to the VλII and VλV subgroups. If only the total complementary-determining regions are being compared, the three proteins NEI, TRO and VIL, subgrouped in VλII, are those with the highest similarities to protein AR. However, if only the total framework regions are being compared, the highest homology is seen with both proteins BOH (VλII) and BO (VλV). On the basis of these data the assignment of a subgroup is rather unclear, and in that case we would suggest that protein AR is assigned to subgroup VλVI. This would also be in agreement with that already established on the partial sequence (Kabat *et al.*, 1979).

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