Complete Amino Acid Sequence Determinations Demonstrate Identity of the Urinary Bence Jones Protein (BJP-DIA) and the Amyloid Fibril Protein (AL-DIA) in a Case of AL-Amyloidosis

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ABSTRACT: The complete primary structures of both the main amyloid fibril protein component (AL-DIA) and the soluble Bence Jones protein (BJP-DIA) obtained from the same patient with AL-amyloidosis are reported for the first time. The amino acid sequences were determined by automated Edman degradation following proteolytic digestion of the isolated proteins and HPLC separation of the resulting fragments and by amino-terminal sequencing after treatment with pyroglutamate aminopeptidase. Sequencing data were confirmed by amino acid analysis and plasma desorption mass spectrometry (PDMS). Molecular weights of the complete proteins were determined by laser desorption mass spectrometry. The amyloid fibril preparation contained a complete monoclonal λ immunoglobulin light chain (subgroup 1.2) as well as different-sized fragments thereof which were identified by immunoblotting and amino-terminal sequencing following immobilization of electrophoretically-separated proteins on poly(vinylidene difluoride) (PVDF) membranes. The soluble urinary Bence Jones protein (BJP-DIA) was a dimer of monoclonal L-chains with a primary structure identical to that of the amyloid L-chain (AL-DIA) and thus represented the amyloid precursor protein.

AL-amyloidosis is characterized by the extracellular deposition of monoclonal immunoglobulin light chains, amino-terminal fragments thereof, or both (Glenner, 1980).

Soluble Bence Jones proteins were identified as the amyloid precursor molecules in three patients with AL-amyloidosis. The amino-terminal amino acid sequences of the amyloid fibril proteins were shown to be identical to those of the corresponding Bence Jones proteins in 26 out of 27 compared residues in two cases with κ AL-amyloidosis (Terry et al., 1973; Eulitz & Linke, 1982) and in 32 out of 34 residues in a case with λ AL-amyloidosis (Solomon et al., 1986). One residue in each of the two κ fibril proteins, one residue in the λ fibril protein, and one amino acid in the λ Bence Jones protein could not be identified.

A detailed analysis of the primary structures of soluble κ light chain fragments isolated from the serum, the urine, and the liver and of the insoluble amyloid fibril proteins from patient MAL suggested the existence of two clones of plasma cells, one clone producing amyloidogenic and the other producing non-amyloidogenic monoclonal κ light chains (Rodilla Sala et al., 1991). Interestingly, the first 27 amino-terminal residues of the two κ light chains isolated from patient MAL were identical with the exception of position 24, which is exactly the position that was not identified in the κ fibril proteins studied by Terry et al. (1973) and Eulitz and Linke (1982).

Point mutations in the genes coding for amyloid precursors or fibril proteins varying from their normal counterparts by single amino acid substitutions, respectively, have been identified in different hereditary forms of amyloidosis. These include familial Alzheimer's disease (Goate et al., 1991), hereditary cerebral hemorrhage with amyloidosis of Dutch (Levy et al., 1990) and of Icelandic (Ghiso et al., 1986) origin, familial amyloidosis (Finnish type) (Maury et al., 1990; Ghiso et al., 1990), familial amyloid polyneuropathy of Japanese (Tawara et al., 1983), Swedish (Dwulet & Benson, 1983), Portuguese (Saraiva et al., 1984), and Iowan (Nichols et al., 1988) origin, and familial amyloid cardiomyopathy (Danish type) (Nordlie et al., 1988).

These findings are in support of the concept that specific features of the primary structure of the amyloid precursors are crucial in the process of amyloid fibril formation and that even a single amino acid substitution might be responsible for amyloidogenicity.

Considering these observations, we decided to determine the complete primary structures including the constant regions of the Bence Jones protein as well as that of the main amyloid fibril protein component from a patient with λ AL-amyloidosis in order to determine whether the finding of amyloidogenic and non-amyloidogenic monoclonal light chains in one patient (Rodilla Sala et al., 1991) was a unique observation.

Materials and Methods

Fibril Preparation. The source of material was the liver (autopsy material) obtained from a 70-year-old female who had suffered from plasmocytosis and amyloidosis. Amyloid fibrils were isolated by water extraction according to Pras et al. (1968) with minor modifications, from the tissue which had been stored at −20 °C. Briefly, after removal of soluble proteins by repeated washings with 150 mM NaCl, 10 mM Tris-HCl, pH 7.3, containing 0.1 mM PMSF,[1] amyloid fibrils

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[1] Abbreviations: AL-DIA, amyloid light chain DIA; BJP, Bence Jones protein; Da, dalton; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FPLC, fast protein liquid chromatography; HPLC, high-performance liquid chromatography; PDMS, plasma desorption mass spectrometry; PMSF, phenylmethanesulfonyl fluoride; PTH, phenylthiohydantoin; PVDF, poly(vinylidene difluoride); RP-HPLC, reversed-phase high-performance liquid chromatography; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SV8, *Staphylococcus aureus* V8; TBS, tris-buffered saline; TFA, trifluoroacetic acid.

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were extracted with bidistilled water containing 0.1 mM PMSF. Fibril-containing supernatants were pooled, dialyzed against bidistilled water, and lyophilized.

Isolation of AL-DIA Light Chain. A total of 60 mg of lyophilized fibrils was solubilized in 4 mL of 6 M guanidine hydrochloride, 0.1 M Tris-HCl, pH 8.3, containing 1 mM EDTA. A total of 40 mg of DTT was added, and after stirring for 6 h at 37 °C, thiol groups were carboxymethylated with 96.5 mg of iodoacetic acid neutralized with NaOH. The reaction was stopped after 30 min at 37 °C by the addition of 130 μL of β-mercaptoethanol. Insoluble material was removed by centrifugation, and the sample was subsequently subjected to gel filtration on Sephacryl S-200 Superfine in the presence of 6 M guanidine hydrochloride, 0.1 M Tris-HCl, pH 8.5. Fractions were checked for purity by SDS–PAGE, pooled, and lyophilized after dialysis against bidistilled water.

Purification of Urinary Bence Jones Protein BJP-DIA. Proteins were precipitated from whole urine by 37% saturation with solid ammonium sulfate. Precipitates were redissolved in bidistilled water and dialyzed against water.

Lyophilized dialysate was dissolved in 10 mM Tris-HCl, pH 8.0. Insoluble material was removed by centrifugation, and the sample was fractionated by anion-exchange chromatography on a self-made column of Whatman DE 52 (10 × 70 mm) (Whatman, Maidstone, England) on FPLC using a linear gradient of 0–500 mM NaCl.

Fractions were checked for purity by SDS–PAGE, pooled, dialyzed against bidistilled water, and lyophilized.

Reduction and Carboxymethylation of Isolated Bence Jones Protein. A total of 180 μg BJP dimer was reduced for 6 h at 37 °C with 0.5 mg of DTT in 1 mL of 6 M guanidine hydrochloride, 0.1 M Tris-HCl, pH 8.3. A total of 5.6 mg of iodoacetic acid neutralized with NaOH was added, and the reaction was stopped after 30 min at 37 °C by the addition of 50 μL of β-mercaptoethanol.

Proteolytic Digestions. (a) Tryptic digestion was performed in 0.05 M NH_4HCO_3, pH 7.8, for 4 h at an enzyme to substrate ratio of 1:50 (trypsin, HPLC grade; Boehringer Mannheim, Germany).

(b) Cleavage with SV8-Protease: 40 μg of SV8 protease (endoproteinase Glu-C from *Staphylococcus aureus* V8; Boehringer Mannheim, Germany) was added to 2 mg of purified AL-DIA L-chain in 0.05 M NH_4HCO_3, pH 7.8, and the solution was stirred at 37 °C. The same amount of enzyme was added after 12 h, and the mixture was incubated at 37 °C for another 12 h.

(c) Endoproteinase Asp-N: Selected tryptic and SV8 peptides were specifically cleaved with endoproteinase Asp-N (Boehringer Mannheim, Germany) in 50 mM sodium phosphate buffer, pH 8.0, for 18 h at 37 °C at an enzyme to substrate ratio of 1:40.

(d) Chymotryptic peptides were obtained by digestion with approximately 0.1 μg of α-chymotrypsin/nmol of peptide (Worthington, Freehold, NJ) in 50 mM NH_4HCO_3, pH 7.8, at 37 °C for 30 min.

Peptides obtained by proteolytic digestion were separated by *RP-HPLC* using Synchropak RPP, Shandon ODS Hypersil, Spherisorb ODS II, and Vydac C18 columns equilibrated in 0.1% TFA or 0.025 M ammonium acetate with a gradient of CH_3CN.

Deblocking of the N-termini of the reduced and carboxymethylated amyloid L-chain and the reduced and carboxymethylated Bence Jones L-chain was performed with pyroglutamate aminopeptidase (Sigma, Deisenhofen, Germany) as described by Podell and Abraham (1978).

Sequence Determination. The amino acid sequences of the purified peptides were determined on an automated gas-phase sequencer with online PTH detection (Models 470 A and 120 A; Applied Biosystems, Weiterstadt, Germany). Calculated molecular masses of the peptides were confirmed by plasma desorption mass spectrometry using a Bio Ion mass spectrometer (Applied Biosystems, Weiterstadt, Germany).

Amino acid compositions of the tryptic peptides of the amyloid L-chain were determined by the method of Moore and Stein (1963), using a Durrum D500 automated amino acid analyzer (Dionex, Palo Alto, CA), following gas-phase hydrolysis (60 min at 150 °C with 6 M HCl containing 1% phenol). Hydrolytic losses were corrected by multiplication of the values with the following correction factors: 1.05 (Thr, Tyr) and 1.11 (Ser). Additionally, the value for Glu was corrected by multiplication with the correction factor 0.9.

Gel electrophoresis on homogeneous and gradient gels was performed according to Laemmli (1970) and Laemmli and Favre (1973). Electrophoretically-separated proteins were transferred to PVDF membranes (Millipore, Eschborn, Germany) by semidry blotting (Hirano, 1989) at 2.3 mA/cm^2 for 20 min using a Sartoblot II apparatus (Sartorius Göttingen, Germany). The blotting sandwich was assembled as follows (from anode to cathode): One sheet of Whatmann 3MM filter immersed in buffer A [300 mM Tris/20% (v/v) methanol], one sheet of Whatmann 3MM soaked with buffer B [25 mM Tris/20% (v/v) methanol], one or two sheets of PVDF membrane equilibrated in buffer B, SDS gel equilibrated in buffer C [25 mM Tris–borate, pH 9/0.1% (w/v) SDS/20% (v/v) methanol], and two sheets of Whatmann 3MM immersed in buffer C.

Electroblotted proteins were reversibly stained with Ponceau S [essentially according to Salinowich and Montelaro (1986)], marked, and immunostained with rabbit antiserum to human Ig/L-chain, type λ (Behringwerke, Marburg, Germany) 1:100 in TBS in combination with peroxidase-conjugated swine anti-rabbit Ig antibodies (Dako GmbH, Hamburg, Germany) 1:500 in TBS/0.5% skim milk. Alternatively, electroblotted proteins were visualized with Coomassie brilliant blue (Burnette, 1981) and subjected to amino-terminal sequencing (Kratzin et al., 1989).

Laser desorption mass spectrometry was performed on a reflector-type time of flight mass spectrometer equipped with a Q-switched quadrupled Nd–Yag Laser (pulse length 10 ns, wavelength 266 nm) as described elsewhere (Karas & Hillenkamp, 1988; Karas et al., 1989).

Results

SDS–PAGE of amyloid fibrils obtained by water extraction demonstrated the presence of a major protein component with an apparent molecular mass of approximately 26–28 kDa (depending on the type of gel) and smaller proteins with apparent molecular weights between 18 and 22 kDa, all of which gave positive reactions with anti-λ antiserum on immunoblots (Figure 1). The complete primary structure of the most prominent protein on SDS–PAGE was determined by automated Edman degradation of peptides obtained by proteolytic digestion and purified by RP-HPLC (Figure 2) and by amino-terminal sequencing of the electroblotted protein after removal of amino-terminal pyrrolidonecarboxylic acid. The complete amino acid sequence of the amyloid L-chain AL-DIA is shown in Figure 3. The proteolytic fragments were further characterized by mass spectrometry. Tables I and II summarize calculated molecular weights and data obtained by PDMS of peptide fragments obtained from digestion with

Table I: Molecular Weights of the Tryptic Peptides of the Amyloid L-chain AL-DIA

peptide	sequence	PDMS[a]	M_r(calcd)[b]
T1	ZSVLTQPPSASGTPGQR	1694.6	1693.8
T1a	TQPPSASGTPGQR	1283.9	1283.4
T2	VTISCSGSSSNIGSNVVTWYQQLPGTAPK	3041.6	3040.3
T3	LLIYTNNQRPSGVPGR	1784.4	1785.0
T4	FSGSK	525.2	524.6
T5	SGTSASLAVSGLQSEDEADYYCATWDDSVNGWVFGGGTK	4088.7	4090.3
T6	LTVLGQPK	855.6	855.0
T7	AAPSVTLFPPSSEELQANK	1987.0	1986.2
T8	ATLVCLISDFYPGAVTVAWK	2213.2	2212.6
T9	ADSSPVK	703.3	702.8
T9 + T10	ADSSPVKAGVETTTPSK	1675.3	1674.8
T10	AGVETTTPSK	991.1	990.1
T11 + T12	QSNNKYAASSYLSLTPEQWK	2317.3	2315.5
T12	YAASSYLSLTPEQWK	1744.1	1743.9
T13	SHK	370.6	370.4
T14	SYSCQVTHEGSTVEK	1712.9	1712.8
T15	TVAPTECS	865.0	864.9
T15a	TVAPTE	616.7	616.7

[a] PDMS = molecular weights measured by PDMS. [b] M_r(calcd) = molecular weight calculated according to the sequence.

Table II: Molecular Weights of the SV-8 Peptides of the Amyloid L-chain AL-DIA

peptide	sequence	PDMS[a]	M_r(calcd)[b]
	(a) SV-8 Peptides		
S1	ZSVLTQPPSASGTPGQRVTISCSGSSSNIGSNVVTWYQQLPGTAPKL-LIYTNNQRPSGVPGRFSGSKSGTSASLAVSGLQSEDE	8560[c]	8609.4
S2	ADYYCATWDDSVNGWVFGGGTKLTVLGQPKAAPSVTLFPPSSEE	4719.8	4721.2
S3	LQANKATLVCLISD	1546.1	1546.8
S4	FYPGAVTVAWKAD	1424.6	1424.6
S5	SSPVAGVE	873.1	872.9
S6	TTTPSKQSNNKYAASSYLSLTPE	2488.0	2488.7
S7	QWKSHKSYSCQVTHE	1906.9	1906.0
S8	GSTVE	491.8	491.5
S9	KTVAPTE	744.8	744.8
	(b) α-Chymotrypsin Cleavage of S1		
S1Ch2	TQPPSASGTPGQRVTISCSGSSSNIGSNVVTW	3221.4	3221.5
S1Ch3	YQQLPGTAPKLLIY	1605.7	1604.9
S1Ch4	TNNQRPSGVPGRF	1430.5	1429.6
S1Ch5	SGSKSGTSASLSAVSGLQSEDE	1998.1	1997.1

[a] PDMS = molecular weight measured by PDMS. [b] M_r(calcd) = molecular weight calculated according to the sequence. [c] Accuracy of measurement is poor due to low signal to noise ratio.

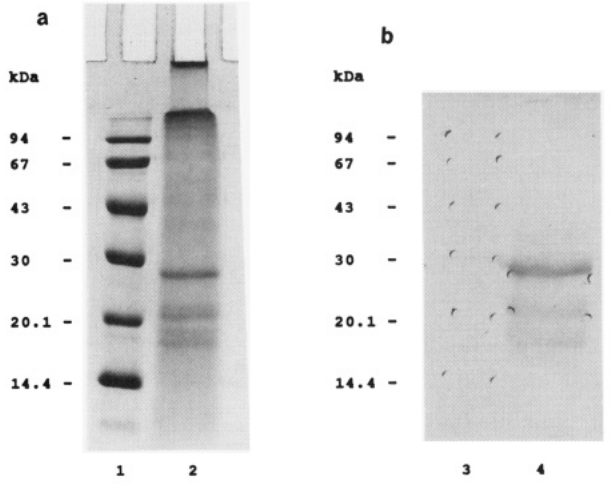

FIGURE 1: SDS-PAGE and immunoblot analyses of water-extracted amyloid fibrils. (a) 15% SDS–polyacrylamide gel stained with Coomassie brilliant blue: lane 1 shows molecular mass markers (Pharmacia LMW-marker), and lane 2 shows approximately 15 μg of water-extracted, lyophilized amyloid fibrils [the sample buffer contained 5% (v/v) β-mercaptoethanol]. (b) Immunostaining of electroblotted proteins using rabbit antiserum to human Ig/L-chain, type λ, in combination with a peroxidase-conjugated second antibody. Punches indicate positions of proteins visible after reversible staining with Ponceau S prior to immunostaining. Lane 3 shows molecular mass markers (Pharmacia LMW-marker), and lane 4 shows approximately 15 μg of water-extracted amyloid fibrils.

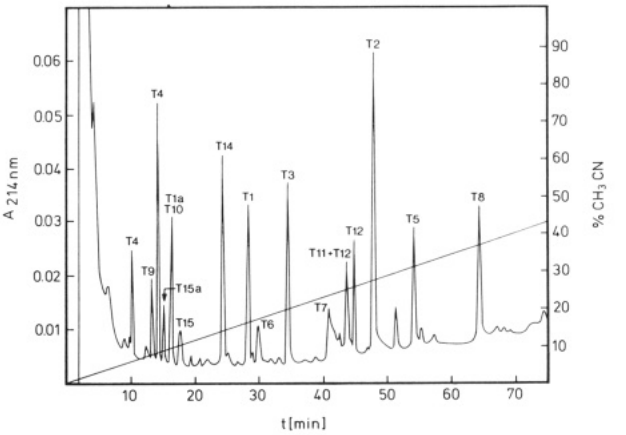

FIGURE 2: Primary separation of peptides generated by digestion of the amyloid L-chain AL-DIA with trypsin. The digest (~4 nmol) was separated on a Vydac C18 column (218TP54) using a TFA/CH_3CN gradient at a flow rate of 1.5 mL/min. Peptides are designated by the prefix T as in Figure 3. The tryptic peptide T13 was purified by rechromatography of material eluting at 0% CH_3CN on a Synchropak RPP column (chromatograms not shown).

trypsin and SV-8 protease. Amino acid compositions of the tryptic peptides are given in Tables III–V. Characterized by subgroup-specific residues Ala 11, Gly 13, Thr 14, Gln 53, Val 58, Ser 72, Ala 74, Ser 76, Ser 80, Glu 81, Ala 89, and Asp 93, the amyloid L-chain AL-DIA belongs to λ light chain

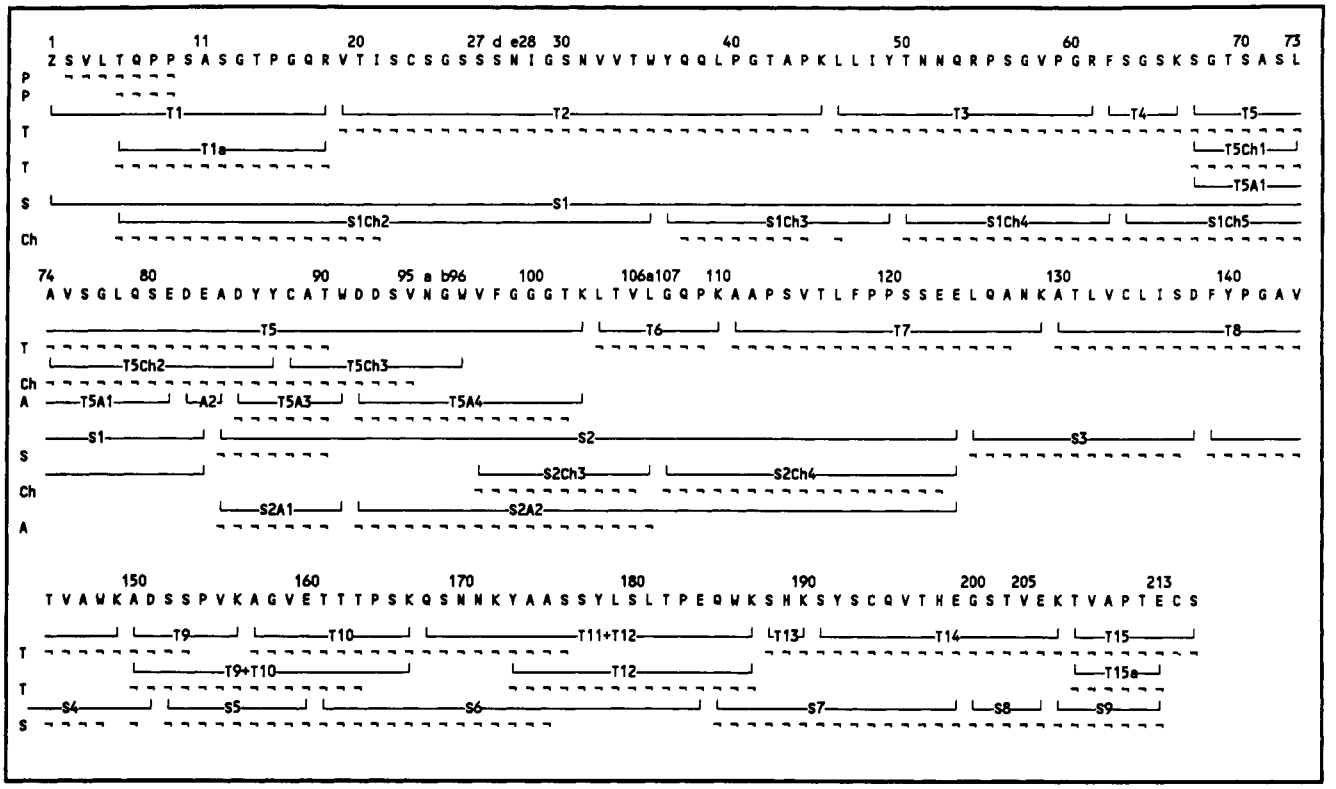

FIGURE 3: Amino acid sequence of amyloid protein AL-DIA. Z indicates pyroglutamate. P indicates peptides sequenced after deblocking of the N-terminus with pyroglutamate aminopeptidase. T indicates peptides isolated after tryptic digestion. Ch indicates peptides obtained after cleavage of selected peptides with α-chymotrypsin. A indicates peptides isolated after cleavage of selected peptides with endoproteinase Asp-N. – indicates amino acids identified as PTH-amino acids after gas-phase sequencing. Amino acid positions were numbered according to Kabat et al. (1987).

Table III: Amino Acid Compositions of the Tryptic Peptides T1–T7 of the Amyloid L-chain AL-DIA[a]

	T1		T2[b]		T3		T4		T5[c]		T6		T7	
	A	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*
CM-C			0.86	1										
D			2.18	2	1.96	2							1.11	1
T	1.93	2	2.89	3	1.03	1					0.91	1	0.91	1
S	2.80	3	5.81	6	1.19	1	1.94	2					2.78	3
E	2.95	3	2.01	2	1.18	1					0.98	1	3.00	3
P	3.02	3	2.01	2	2.00	2					1.00	1	3.17	3
G	2.03	2	3.29	3	2.11	2	0.97	1			1.06	1		
A	1.10	1	1.06	1									3.05	3
V	1.08	1	2.58	3	0.98	1					0.97	1	0.99	1
M														
I			2.0	2	0.82	1								
L	1.05	1	0.96	1	1.91	2					2.08	2	2.01	2
Y			1.17	1	0.88	1								
F							1.01	1					0.98	1
H														
K			1.11	1			1.08	1			0.98	1	1.07	1
R	1.03	1			1.94	2								
W	nd		nd	1	nd		nd		nd		nd		nd	
total	16.99	17	27.97	29	16.0	16	5.0	5			7.98	8	19.07	19

[a] *A* was calculated as the number of amino acid residues per molecule; *Z* is the number of residues in the sequence; CM-C is (carboxymethyl)-cysteine; nd stands for not determined. [b] Determined after gas-phase hydrolysis for 120 min. [c] The amino acid composition of T5 was confirmed by amino acid analysis of peptides obtained after cleavage of T5 with endoproteinase Asp-N and chymotrypsin (Table V).

subgroup 1.2 according to Kametani et al. (1983).

In addition to the complete light chain, bearing pyroglutamic acid at the amino terminus, fragments starting at position 5 of the variable part and molecules lacking two carboxy-terminal amino acids were identified. The absence of an N-glycosylation site (N-X-T/S) (Sox & Hood, 1970) in the amino acid sequence of AL-DIA, as well as the excellent correlation of data obtained by PDMS with the molecular weights calculated from the amino acid sequence, indicates the absence of carbohydrate moieties.

The fibril subunit proteins with apparent molecular masses approximately between 18 and 22 kDa were identified as fragments of the AL-DIA light chain by immunostaining with anti-λ antiserum and by amino-terminal sequence determination following electroblotting on PVDF. Direct sequencing of protein bands with apparent molecular masses of approximately 18 and 21 kDa excised from PVDF membranes after electroblotting and visualization with Coomassie brilliant blue revealed the amino-terminal sequences TQPP and TQPPSAS for the 18- and the 21-kDa proteins, respectively, corresponding

Table IV: Amino Acid Compositions of the Tryptic Peptides T8–T15 of the Amyloid L-chain AL-DIA[a]

	T8		T9 + T10		T10		T11 + T12		T13		T14		T15	
	A	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*
CM-C	0.73	1									0.83	1	0.70	1
D	1.01	1	1.18	1			2.25	2						
T	1.87	2	2.76	3	2.87	3	1.03	1			1.91	2	1.95	2
S	1.01	1	2.83	3	1.01	1	3.92	4	0.90	1	2.92	3	1.01	1
E			1.05	1	1.02	1	2.99	3			3.03	3	1.14	1
P	1.01	1	2.02	2	1.05	1	1.23	1					1.18	1
G	1.11	1	1.30	1	1.05	1					1.10	1		
A	3.09	3	2.11	2	1.02	1	2.03	2					1.07	1
V	3.10	3	1.88	2	0.93	1					2.04	2	0.94	1
M														
I	0.95	1												
L	2.19	2					2.03	2						
Y	0.92	1					1.76	2			0.99	1		
F	1.00	1												
H									0.98	1	1.08	1		
K	1.00	1	1.87	2	1.05	1	1.99	2	1.11	1	1.09	1		
R														
W	nd	1	nd		nd		nd	1	nd		nd		nd	
total	18.99	20	17	17	10	10	19.22	20	2.99	3	14.99	15	7.99	8

[a] *A* was calculated as the number of amino acid residues per molecule; *Z* is the number of residues in the sequence; CM-C is (carboxymethyl)-cysteine; nd stands for not determined.

Table V: Amino Acid Compositions of Peptides Obtained by Cleavage of the Tryptic Peptide T5 with Chymotrypsin and Endoproteinase Asp-N (Amyloid L-chain AL-DIA)[a]

	T5Ch2		T5A1		T5A3		T5A4	
	A	*Z*	*A*	*Z*	*A*	*Z*	*A*	*Z*
CM-C					0.93	1		
D	1.90	2			1.26	1	3.34	3
T			0.92	1	0.89	1	1.10	1
S	1.96	2	5.05	5			1.36	1
E	2.99	3	1.99	2			0.41	0
P								
G	1.17	1	2.13	2			3.89	4
A	2.12	2	2.03	2	1.02	1		
V	1.07	1	0.85	1			1.73	2
M								
I								
L	1.10	1	1.98	2				
Y	1.67	2			1.88	2		
F							0.78	1
H								
K							0.89	1
R								
W	nd		nd		nd	1	nd	1
total	13.98	14	14.93	15	5.98	7	13.37	14

[a] *A* was calculated as the number of amino acid residues per molecule; *Z* is the number of residues in the sequence; CM-C is (carboxymethyl)-cysteine; nd stands for not determined.

to the variable part of the AL-DIA light chain starting at position 5.

The complete primary structure of the Bence Jones protein BJP-DIA purified from the urine of the same patient by ion-exchange chromatography was determined by gas-phase sequencing of the tryptic peptides of the reduced and carboxymethylated protein and by amino-terminal sequencing following treatment with pyroglutamate aminopeptidase. The amino acid sequence of the Bence Jones protein BJP-DIA (Figure 4) was found to be identical to that of the amyloid fibril subunit AL-DIA. Sequencing data were confirmed by PDMS (Table VI).

The molecular weights of the BJP and the AL-light chain (both in reduced and carboxymethylated form) were determined by laser desorption mass spectrometry. The measured mass of 22850 ± 70 Da for the BJP (Figure 5a) is in agreement with the value of 22910 calculated from the sequence. The mass of 22740 ± 50 Da obtained for the AL-light chain (Figure 5b) presumably reflects the finding of heterogeneous amino and carboxy termini.

Discussion

The immunoglobulin light chain origin of amyloid fibrils in AL-amyloidosis was first demonstrated by Glenner et al. (1971a). Since then complete light chains, amino-terminal fragments, or both have been identified in AL-amyloid deposits (Glenner 1980). The λ to κ ratio of approximately 2:1, that is, the reverse of that seen in multiple myeloma (Levo et al., 1976; Kyle, 1982; Kyle & Gertz, 1990), the observation that proteolytic digestion of some but not all Bence Jones proteins leads to fibril formation in vitro (Glenner et al., 1971b), and the overrepresentation of λ VI subgroup (Solomon et al., 1982) supported the hypothesis that distinct structural features are responsible for "amyloidogenicity" of the light chains found in amyloid deposits.

In a search for amyloidogenic sequences or amino acid residues, a number of fibril proteins were sequenced (Sletten et al., 1981, 1983; Dwulet et al., 1985, 1986; Eulitz & Linke,

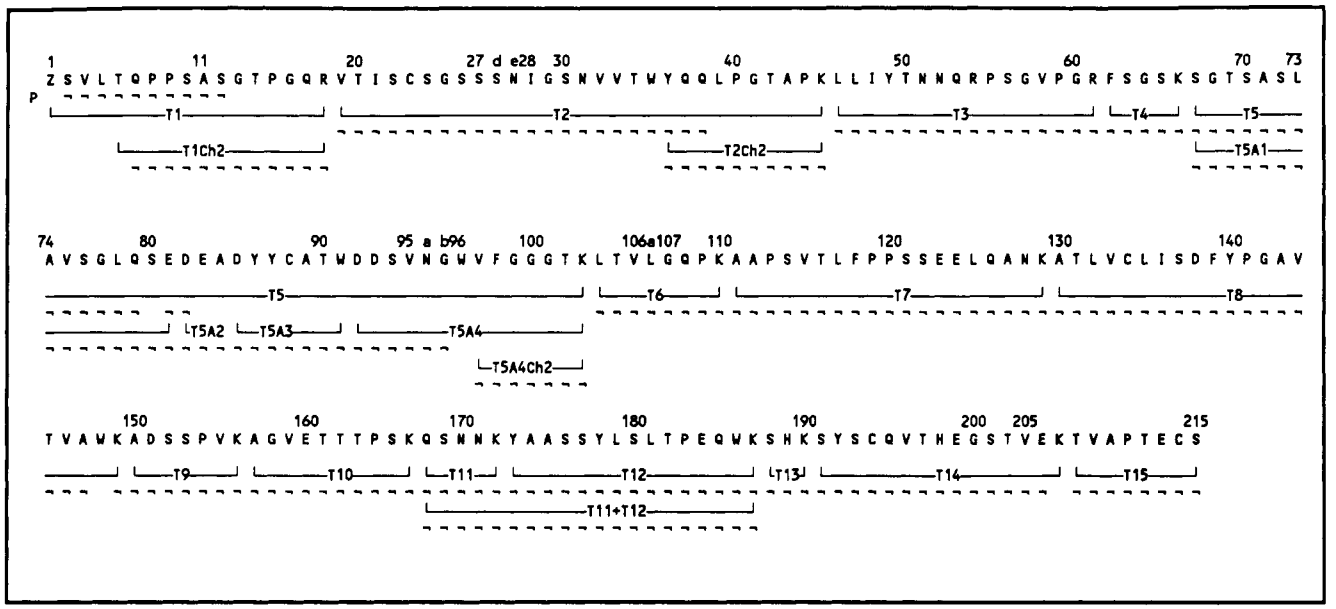

FIGURE 4: Amino acid sequence of BJP-DIA. Z indicates pyroglutamate. P indicates residues sequenced after deblocking of the N-terminus with pyroglutamate aminopeptidase. T indicates peptides isolated after tryptic digestion. Ch indicates peptides isolated after cleavage of selected peptides with α-chymotrypsin. A indicates peptides isolated after cleavage of selected peptides with endoproteinase Asp-N. ¬ indicates amino acids identified as PTH-amino acids after gas-phase sequencing. Amino acid positions were numbered according to Kabat et al. (1987).

Table VI: Molecular Weights of the Tryptic Peptides of the Bence Jones Protein BJP-DIA

peptide	sequence	PDMS[a]	M_r(calcd)[b]
T1	ZSVLTQPPSASGTPGQR	1694.3	1693.8
T2	VTISCSGSSSNIGSNVVTWYQQLPGTAPK	3041.2	3040.3
T3	LLIYTNNQRPSGVPGR	1785.7	1785.0
T4	FSGSK	525.2	524.6
T5	SGTSASLAVSGLQSEDEADYYCATWDDSVNGWVFGGGTK	4089.7	4090.3
T6	LTVLGQPK	855.4	855.0
T7	AAPSVTLFPPSSEELQANK	1986.4	1986.2
T8	ATLVCLISDFYPGAVTVAWK	2216.2[c]	2212.6
T9	ADSSPVK	704.4	702.8
T10	AGVETTTPSK	990.9	990.1
T11	QSNNK	591.1	589.6
T11 + T12	QSNNKYAASSYLSLTPEQWK	2316.2	2315.5
T12	YAASSYLSLTPEQWK	1744.2	1743.9
T13	SHK	370.9	370.4
T14	SYSCQVTHEGSTVEK	1713.0	1712.8
T15	TVAPTECS	864.9	864.9

[a] PDMS = molecular weight measured by PDMS. [b] M_r(calcd) = molecular weight calculated according to the sequence. [c] Accuracy of measurement is poor due to low signal to noise ratio.

1985; Gertz et al., 1985; Toft et al., 1985; Tveteraas et al., 1985; Holm et al., 1986; Fykse et al., 1988; Benson et al., 1989; Ferri et al., 1989; Liepnieks et al., 1990). In addition, the sequences of amyloidogenic Bence Jones proteins were also determined (Frangione et al., 1983; Tonoike et al., 1985; Isobi et al., 1986; Solomon et al., 1982; Eulitz et al., 1987), on the basis of the demonstration of the identity of Bence Jones protein and fibril protein by Terry et al. (1973), Eulitz and Linke (1982), and Solomon et al. (1986). However, no common primary structural feature responsible for amyloidogenicity has been identified so far.

In fact, the comparisons of 27 amino-terminal residues of κ BJP and fibril protein TEW (Terry et al., 1973) and MEV (Eulitz & Linke, 1982) and 34 residues in λ Bence Jones protein and fibril protein GIO (Solomon et al., 1986) were insufficient proof of identity, since only 26 out of 27 amino acids in the κ light chains and 32 out of 34 residues in the λ light chain were shown to be identical. In AL-amyloidosis patient MAL, two monoclonal κ light chains were detected, differing in 21 amino acids in total but being identical in 26 of the 27 amino-terminal amino acids (Rodilla Sala et al., 1991)!

Our determinations of the complete primary structures of both the soluble Bence Jones protein and the fibril subunit from the same patient were motivated by two observations: first, the finding that single amino acid substitutions may possibly be responsible for some hereditary forms of amyloidosis (Goate et al., 1991; Levy et al., 1990; Ghiso et al., 1986, 1990; Maury et al., 1990; Tawara et al., 1983; Dwulet & Benson, 1983; Saraiva et al., 1984; Nichols et al., 1988; Nordlie et al., 1988); and second, the possibility of more than one monoclonal protein being present in the same patient, due to biclonality or the existence of subclones arisen from a common precursor clone or production of two different paraproteins by the same clone (van Camp et al., 1978; Kyle et al., 1981).

This is the first time that the soluble Bence Jones protein and the amyloid fibril protein in a case of AL-amyloidosis have been shown to be identical by determination of the complete amino acid sequences. With regard to the primary structure and to posttranslational modifications, no differences between the BJP and the AL-amyloidosis light chain were detected by gas-phase sequencing and mass spectrometry.

In addition to the complete λ light chain, fragments lacking 4 amino-terminal and/or 2 carboxy-terminal residues and

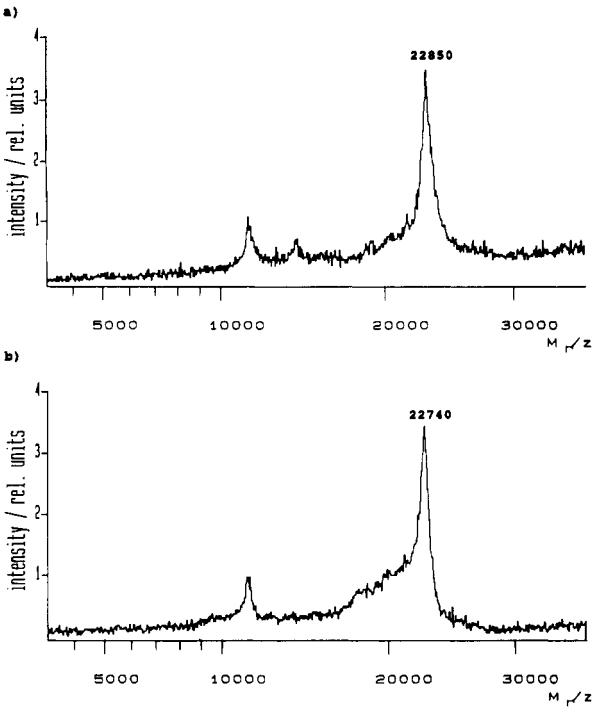

FIGURE 5: Laser desorption/ionization mass spectra of the uncleaved proteins. (a) Mass spectrum of the reduced and carboxymethylated Bence Jones protein BJP-DIA. Measured molecular mass: 22 850 ± 70 Da. (b) Mass spectrum of the reduced and carboxymethylated amyloid light chain AL-DIA. Measured molecular mass: 22 740 ± 50 Da.

smaller fragments starting at position 5 of the variable part with apparent molecular masses between approximately 18 and 22 kDa were identified in the amyloid fibril preparation.

It remains unresolved if proteolytic fragments are essential for amyloid formation or if the intact light chain is sufficient for fibrillogenesis.

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Mutations Affecting the Activity of the Shiga-like Toxin I A-Chain†

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ABSTRACT: Like ricin, *Escherichia coli* Shiga-like toxin I (SLT-I) inactivates eukaryotic ribosomes by catalytically depurinating adenosine 4324 in 28S rRNA. Although the primary structure of the enzymatic portion of the molecule (Slt-IA) is known to contain regions of significant homology to the ricin A chain (RTA), and although certain residues have been implicated in catalysis, the crystal structure of Slt-IA has not been solved nor has the geometry of its active site been well defined. In order to derive a more complete understanding of the nature of the Slt-IA active site, we placed the *slt*-IA gene under control of an inducible promoter in *Saccharomyces cerevisiae*. Induction of the cloned element was lethal to the host. This lethality was the basis for selection of an attenuated mutant of Slt-IA changed at tyrosine 77, a locus not previously linked to the active site. As well, it permitted evaluation of the toxicity of a number of mutant Slt-IA cassettes that we constructed in vitro. Putative active-site residues implicated in this fashion and in other studies were mapped to an energy-minimized computer model of Slt-IA that had been generated on the basis of the known crystal structure of RTA. A cleft was identified on one face of the protein in which all implicated residues clustered, irrespective of their distances from one another in the primary structure of the molecule. Many of the chemical features anticipated in the active site of an RNA N-glycosidase are indeed present on the amino acid side chains occupying the cleft.

Certain strains of *Escherichia coli* produce Shiga-like toxins (SLTs),[1] potent enterotoxins that have been linked to outbreaks of hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Dickie et al., 1989; Griffin et al., 1988; Karmali et al., 1985). So named because of their similarity to the classic Shiga toxin (ST) of *Shigella dysenteriae*, the SLTs have been divided into two general groups: SLT-I, which is virtually identical to ST both structurally and immunologically, and SLT-II and its variants, which at the amino acid level are about 60% conserved relative to ST but are not immunologically cross-reactive (O'Brien & Holmes, 1987). All are single-site RNA N-glycosidases that depurinate a specific adenosine of 28S eukaryotic rRNA (A_{4324} in rat ribosomes) and thereby irreversibly inhibit protein synthesis (Endo et al., 1988), a phenomenon that results in the death of the target cell.

SLT-I has a molecular mass of ~70 kDa and is composed of a single A subunit (Slt-IA; 32.2 kDa) and multiple copies

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[1] Abbreviations: SLT-I, Shiga-like toxin I holotoxin; Slt-IA, Shiga-like toxin IA subunit; *slt*-IA, Shiga-like toxin IA gene; ST-A, Shiga toxin A subunit; RTA, ricin A chain; PCR, polymerase chain reaction; CFU, colony-forming units; CRM, cross-reactive material.

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