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Immunology

Primary structure of an amyloid prealbumin variant in familial polyneuropathy of Jewish origin

(amyloidosis/autosomal dominant inheritance)

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ABSTRACT **The complete amino acid sequence of three related amyloid proteins (M_r 14,000, 10,000, and 5,000) derived from tissues of a Jewish patient who suffered from a variant of familial polyneuropathic amyloidosis was determined. The protein, which contains 127 residues, is identical to a human serum prealbumin subunit. Only one amino acid substitution, glycine for threonine, was detected at position 49, where enzymatic cleavage occurred, yielding M_r 5,000 and 10,000 fragments which represent the amino terminus (residues 1–48) and carboxyl terminus (residues 49–127) of the molecule, respectively. Thus, a prealbumin variant and its fragments constitute the amyloid fibrils in a heredofamilial amyloidosis syndrome of dominant inheritance.**

Amyloidosis comprises a heterogeneous group of diseases of diverse etiology characterized by the extracellular deposition of a number of fibrillar proteins which may be distinguished from each other biochemically and immunohistologically. Fibrils are composed of low molecular weight subunits that appear to originate by proteolysis and polymerization of normal serum proteins. In idiopathic and myeloma-associated amyloidoses, subunits derive from circulating monoclonal immunoglobulin light chains and comprise mainly the variable region (1). In secondary amyloidosis, as well as in familial Mediterranean fever, the subunit is the AA protein (2, 3) which is related to a major normal acute-phase reactant, designated SAA (4, 5). Recent studies have shown that a third form of systemic amyloidosis may occur in association with familial amyloidotic polyneuropathy, a heredofamilial form of amyloidosis (6) that demonstrates autosomal dominant inheritance. Fibril subunit protein with cross-antigenicity to prealbumin has been reported in Portuguese patients affected by this syndrome (7) and limited amino-terminal sequence homology to prealbumin has been shown in two affected American kindreds of Swedish origin (8). In a previous paper we reported a case of familial amyloidotic polyneuropathy in a Jewish patient of Ashkenazi origin and the partial characterization of the amyloid protein (9). This paper presents the amino acid sequence of three related amyloid proteins derived from tissue of the same patient, SKO.

EXPERIMENTAL PROCEDURES

Amyloid fibrils were isolated from thyroid and spleen tissues of patient SKO, as described by Pras *et al.* (10). The yield from 18 g of wet thyroid tissue was 2.1 g of dry fibrils and from 60 g of wet spleen tissue, 1.8 g of dry fibrils. The amyloid fibrils were solubilized in 6 M guanidine·HCl/0.1 M Tris (pH 10.2) containing 0.17 M dithiothreitol or 1% 2-mercaptoethanol and were fractionated on a Sephadex G-75/G-100 (Pharmacia) column equilibrated with 5 M guanidine·HCl in 1 M acetic acid. SKO peaks III, IV, and V (Fig. 1) were further purified by gel filtration on an Ultrogel AcA 54 (LKB) column equilibrated with 5 M guanidine·HCl in 1 M acetic acid.

The purity and molecular weight of each protein fraction were determined in 17% polyacrylamide gels containing 0.1% $NaDodSO_4$ (11). Molecular weight standards included bovine serum albumin (M_r 65,000), ovalbumin (M_r 45,000), chymotrypsinogen A (M_r 25,000), ribonuclease A (M_r 13,700) (Pharmacia), and human protein AA (M_r 8,500).

Amyloid protein SKO was completely reduced in 6 M guanidine·HCl/0.6 M Tris/1 mM EDTA/50 mM dithiothreitol, pH 8.2, for 1 hr at 37°C. Alkylation was performed by making the solution 110 mM in iodo[^{14}C]acetic acid (0.7 Ci/mol; 1 Ci = 3.7 × 10^{10} becquerels; Amersham) (12). Completely reduced and alkylated protein SKO was dissolved at 5–10 mg/ml in 70% formic acid, to which was added a 50-fold excess (wt/wt) of cyanogen bromide (Pierce). The reaction was allowed to proceed at room temperature with constant stirring for 72 hr and was stopped by the addition of 10 vol of distilled water, followed by lyophilization (13).

Because the two resultant cyanogen bromide fragments had M_r of 1,600 and 12,400, respectively, they were purified by exhaustive dialysis by using Spectrapor no. 1 membrane tubing, which has a M_r cutoff of 6,000–8,000.

Protein SKO was digested by trypsin treated with *N*-tosylphenylalanine chloromethyl ketone (Worthington) in 0.2 M NH_4HCO_3 at pH 8.2 for 1 hr at room temperature at an enzyme-to-substrate ratio of 1:100 (wt/wt). Tryptic peptides were isolated by reversed-phase chromatography on a μBondapak C_{18} column (0.78 × 30.0 cm; Waters Associates), with a gradient of 0–66% acetonitrile (Burdick and Jackson, Muskegon, MI) in 0.05% trifluoroacetic acid at pH 2.5. Each peptide was rechromatographed on an alkylphenyl μBondapak column (0.38 × 30.0 cm), with a gradient of 0–60% acetonitrile in 0.05 M ammonium acetate at pH 6.5. Digestion by carboxypeptidase A (Sigma) was done in 0.1 M ammonium bicarbonate buffer at pH 8.0 at 37°C for varying time intervals (14).

Amino acid analyses of peptides and proteins were performed with a Durrum D-500 automatic amino acid analyzer (Durrum, Sunnyvale, CA) after hydrolysis in 0.2 ml of 6 M HCl under reduced pressure for 24 hr at 110°C. Forty microliters of a 1% aqueous solution of phenol was added to prevent degradation of tyrosine. Amino-terminal analyses of peptides were done by the Edman degradation technique with amino acid analysis to identify the cleaved derivatized amino acid (15, 16). Automated sequence analyses were performed by using a Beckman 890C sequencer (Beckman) with the Beckman 0.1 M Quadrol program. For small peptides, Polybrene was added to the cup.

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Identification of phenylthiohydantoin amino acids was done by two methods: (*i*) HPLC with Waters model ALC/GPC-204, equipped with a 0.38 × 30.0 cm μBondapak column and eluted with a methanol/water gradient and (*ii*) automated amino acid analysis with a Durrum D-500 analyzer after hydrolysis in 0.2 ml of 6 M HCl containing 5 μl of 5% $SnCl_2$ under reduced pressure for 4 hr at 150°C (17). Cysteine was detected by measuring radioactivity of phenylthiohydantoin [^{14}C]carboxymethylcysteine in a liquid scintillation counter (Beckman model LS-250).

RESULTS AND DISCUSSION

The gel filtration pattern of amyloid fibrils obtained from the spleen (Fig. 1) yielded five peaks. The void volume, peak I, consisted of heterogeneous mucopolysaccharide material present in all amyloid fibrils (18). Peak II contained P component, a serum α-globulin that constituted 5–10% of all amyloid deposits (19, 20), and peaks III, IV, and V were composed of M_r 14,000, 10,000, and 5,000 proteins, respectively. Table 1 shows the amino acid composition of peaks III, IV, and V from the spleen amyloid fibrils compared with human prealbumin and its fragments, residues 1–48 and 49–127. After rechromatography on an Ultrogel AcA 54 column equilibrated in 5 M guanidine·HCl in 1 M acetic acid, the purity of peaks III, IV, and V was determined in $NaDodSO_4$/17% polyacrylamide gels (Fig. 1). The molecular weight, amino acid composition, and partial sequence of peaks III, IV, and V indicated that (*i*) peak III is the intact prealbumin subunit, (*ii*) peak V comprises the first 48 residues of the amino terminus, and (*iii*) peak IV represents residues 49–127 of the carboxyl terminus. Although antiserum

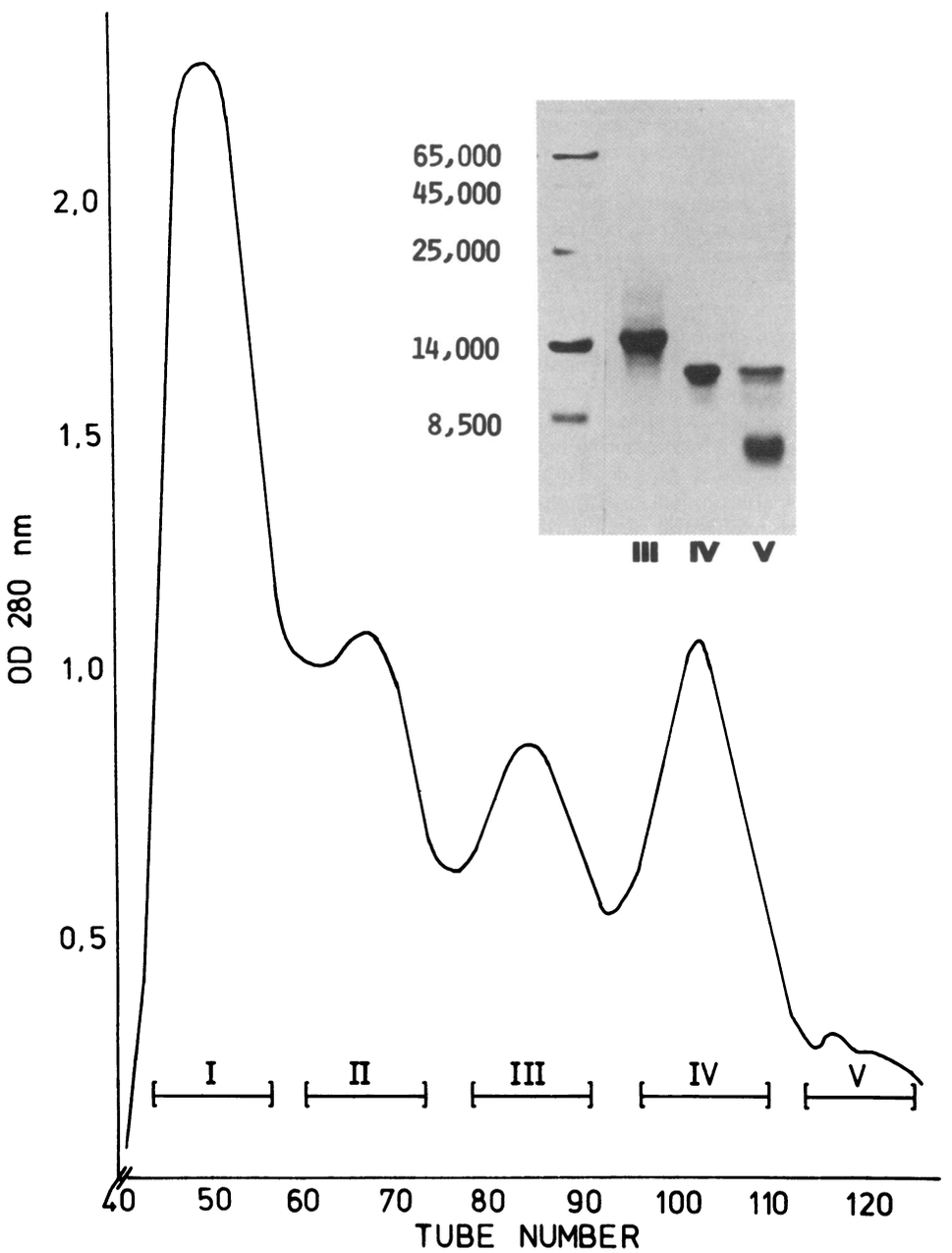

FIG. 1. Gel filtration of amyloid fibrils obtained from spleen of patient SKO on a 2.6 × 100 cm Sephadex G-75/G-100 column equilibrated in 1 M acetic acid/5 M guanidine·HCl. Fractions (3 ml per tube) were collected; 17% polyacrylamide slab gels (containing 0.1% $NaDodSO_4$) of peaks III, IV, and V are shown. Molecular weight markers were: bovine serum albumin, M_r 65,000; ovalbumin, M_r 45,000; chymotrypsinogen A, M_r 25,000; ribonuclease A, M_r 13,700; AA protein, M_r 8,500.

Table 1. Amino acid composition of amyloid proteins SKO III, IV, and V compared with human serum prealbumin

Amino acid	SKO III	Prealbumin	SKO V	Prealbumin residues 1–48	SKO IV	Prealbumin residues 49–127
Cys	0.817	1	1.39	1	0.43	0
Asp	9.112	8	4.78	4	5.281	4
Thr	11.414	12	2.92	3	7.164	9
Ser	10.682	11	3.66	3	8.071	8
Glu	14.377	12	3.01	2	10.088	10
Pro	7.616	8	3.58	4	4.270	4
Gly	8.853	10	3.72	5	6.006	5
Ala	12.526	12	6.48	6	6.772	6
Val	10.635	12	6.23	6	5.028	6
Met	1.606	1	1.01	1	0.345	0
Ile	5.764	5	1.79	1	3.772	4
Leu	8.618	7	2.61	2	5.442	5
Tyr	5.209	5	—		4.001	5
Phe	4.563	5	1.34	2	2.610	3
His	3.271	4	1.13	1	2.921	3
Lys	6.972	8	3.16	4	4.217	4
Arg	4.979	4	1.03	2	2.566	2
Trp	ND	2	ND	1	ND	1

ND, not done.

to peak III (intact molecule) reacted with the amyloid fibrils, prealbumin, and normal human sera, antiserum to peak IV only recognized the amyloid fibrils, suggesting that this may be an antibody to a conformational determinant present in amyloid fibrils or a determinant made accessible by the splitting of residues 1–48. Antibodies to prealbumin reacted with peaks III, IV, and V.

The complete amino acid sequence of peaks III, IV, and V is shown in Fig. 2, and it was deduced as follows: peak III was submitted to automated Edman degradation which gave the sequence of 18 residues from the amino terminus. Automated Edman degradation of intact peak V yielded the sequence of six residues, which were completely homologous to the first six amino acids of peak III. Because the only methionine residue of peak III was identified at position 13, the carboxyl-terminal cyanogen bromide fragment of peak III was subjected to automated sequence analysis and gave the sequence to position 53. Amino-terminal sequence analysis of peak IV aligned with residue 49 of peak III and extended to residue 82. Four peptides were obtained after trypsin digestion of peak IV. The amino acid compositions are shown in Table 2. Each peptide was subjected to automated Edman degradation. The sequence of intact peak

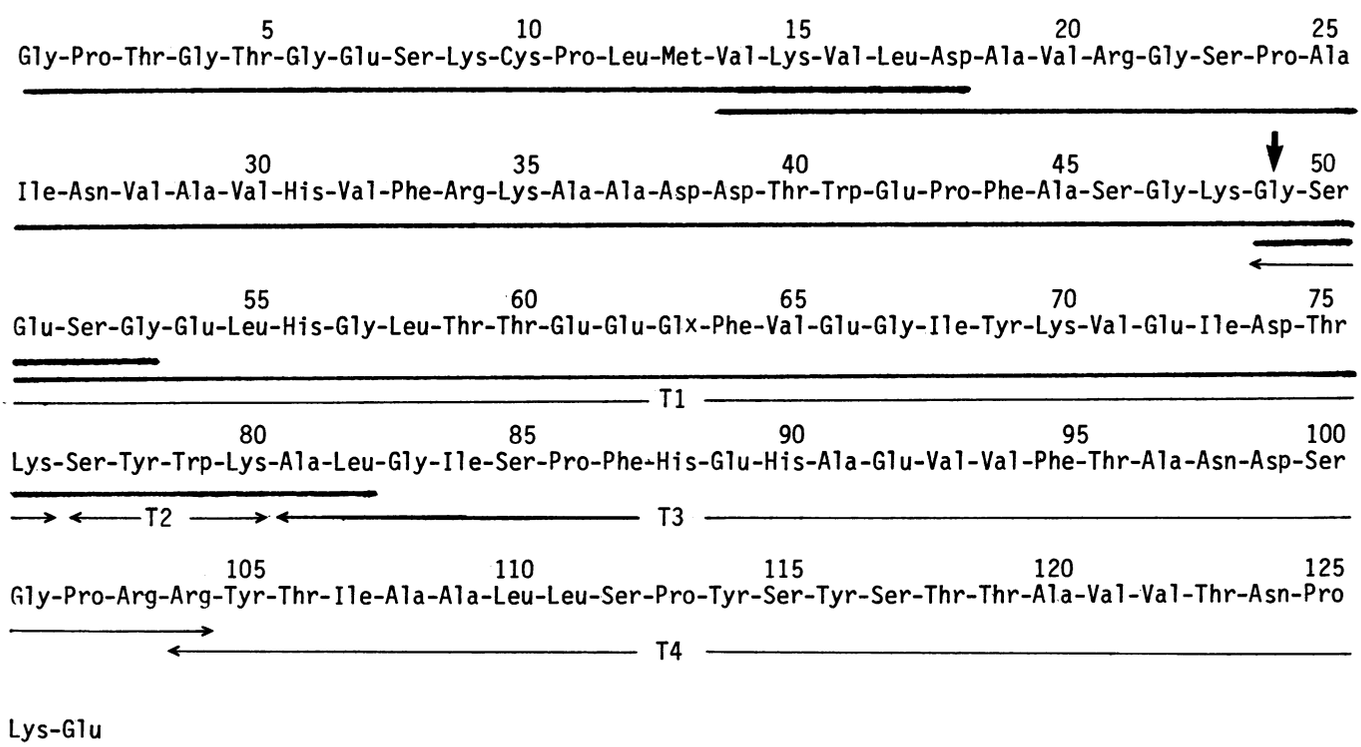

FIG. 2. Sequence of the SKO prealbumin variant. ——, Residues determined by automated sequence analysis; T, tryptic peptides of peak IV; ▼, the position of amino acid substitution.

Table 2. Amino acid composition of the tryptic (T) peptides of peak IV

Amino acid	Tryptic peptides, residues/mole			
	T1	T2	T3	T4
Asp	1.06		2.06	1.10
Thr	3.50		1.00	3.53
Ser	2.01	1.00	1.90	2.88
Glu	6.91		2.19	1.26
Pro			1.80	2.04
Gly	3.24		2.20	
Ala			3.22	3.14
Val	2.04		1.44	1.40
Ile	1.88			
Leu	2.05		1.02	0.93
Tyr	0.97	1.04	1.10	1.99
Phe	1.00			2.83
His	1.00		2.07	
Lys	2.00	1.05	1.92	0.97
Arg			2.41	0.93
Trp		*		
Residues	49–76	77–80	81–104	104–127

* Determined by automated sequence analysis.

IV by automated analysis overlapped tryptic peptides T1, T2, and T3 and established the sequence to arginine 104. Tryptic peptide T4 was assigned as the carboxyl terminus on the basis of a single residue overlap, arginine 104, and the determination by carboxypeptidase digestion of peaks III and IV that lysine and glutamic acid were the carboxyl-terminal residues of both peaks. When this sequence was compared to the sequence of prealbumin (21), only one amino acid substitution, glycine for threonine, was found at position 49 in both peaks III and IV; 10–20% of threonine also was detected at this position in peak III.

The heredofamilial amyloidotic polyneuropathies represent a variety of syndromes that demonstrate autosomal dominant modes of inheritance. Although each syndrome has unique clinical manifestations with regard to age at onset, rate of progression, distribution of organ involvement, and morbidity, every type of heredofamilial amyloidosis exhibits prominent peripheral neuropathy. These hereditary amyloidoses have been customarily classified according to the clinical syndrome and to the country or ethnic group in which they were first described. Human prealbumin is a tetramer composed of four identical subunits and has a M_r of 54,980. Each monomer is composed of 127 amino acids and possesses an extensive β-pleated sheet configuration with only 7.1% α-helix conformation (22). Prealbumin forms a complex with retinol-binding protein, which is a carrier protein for vitamin A and also is involved in the transport of thyroxine (23).

The complete sequence analysis of a protein extracted from amyloid fibrils present in the Jewish variety of familial amyloidotic polyneuropathy revealed it is homologous to the normal plasma protein, prealbumin. Our studies were facilitated by the extraction of three related proteins—peak III, the intact molecule, and peaks IV and V, two fragments resulting from enzymatic cleavage between positions 48 and 49 (Lys, Gly). Because there was only one amino acid substitution, glycine for threonine at position 49, it is conceivable that the SKO prealbumin molecule is the expression of a point mutation and the basis for an abnormal metabolism and amyloid fibril formation.

Alternatively, human prealbumin may be genetically polymorphic and the SKO protein represents an isotype or an allele. Although no additional variations from the prototype sequence of human prealbumin were detected, the 10–20% recovery of threonine at position 49 indicates that normal human prealbumin also was present. Thus, a hereditary defect in the normal metabolism of prealbumin resulting in amyloid fibril formation may be a factor in the disease process of familial amyloidotic polyneuropathy. Because serum from this patient was not available for extensive studies, we cannot answer the questions (*i*) whether two forms of prealbumin, either as a complex or free, were present in the circulation and (*ii*) if present, which form was dominant. Immunochemical analysis of proteins associated with both normals and those with familial amyloidotic polyneuropathy of different ethnic origins will help to answer these questions.

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