

A VARIANT OF PREALBUMIN FROM AMYLOID FIBRILS IN  
FAMILIAL POLYNEUROPATHY OF JEWISH ORIGIN\*

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Amyloidosis constitutes a heterogeneous group of diseases characterized by the deposition of proteinaceous material composed mainly of fibrils having a  $\beta$ -pleated sheet configuration (1). The nature of some of these deposits has been clarified in recent years, and it has been shown that under appropriate conditions a number of proteins can form fibrils that exhibit the histologic and physicochemical properties of amyloid. In the form associated with plasma cell proliferation, the fibrillar protein is an intact immunoglobulin light chain and/or its amino terminal variable-region fragment (2), whereas the deposits in the acquired "secondary amyloidosis" and in familial Mediterranean fever consist of the AA protein, which is a degradation product of an acute phase reactant, serum AA protein (3, 4). The nature of the fibrillar protein in certain hereditary forms of amyloidosis that demonstrate autosomal-dominant inheritance is only now being defined. In the case of familial amyloidotic polyneuropathy of Portuguese origin, it was shown that the fibrillar protein was antigenically related to prealbumin (5), and in two Swedish relatives, preliminary biochemical analyses support this suggestion (6, 7).

The purpose of this paper is to report on the purification and primary structure of proteins isolated from amyloid fibrils from a patient of Jewish origin suffering from familial amyloidotic polyneuropathy with systemic involvement.

#### Materials and Methods

*Source of Tissue Purification of Amyloid Fibrils and Subunits.* The patient (S.K.O.), a 28-year-old Ashkenazi Jew, suffered from progressive neuropathy and blindness because of vitreous deposits of amyloid. His father had died at the age of 29 with an identical disease (B. Fishel et al., manuscript in preparation). Tissues were obtained from the patient at an autopsy after he committed suicide. Extraction of amyloid fibrils was performed from the thyroid and spleen, as described by Pras et al. (8). The yield from the thyroid was 2.1 g of dry fibrils from 18 g of wet tissue and from the spleen, 1.8 g of dry fibrils from 60 g of wet tissue. Amyloid fibrils were dissolved in 6 M guanidine HCl, pH 10.2, in the presence of 0.17 M dithiothreitol or 1%  $\beta$ -mercaptoethanol and then fractionated on Sephadex G-100 columns equilibrated with 5 M guanidine HCl in 1 M acetic acid. Purity of protein fractions and molecular weight were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (9). Antibodies were made in rabbits by repeated injections of purified peak IV (Fig. 1) in complete Freund's adjuvant.

*Amino Acid Analysis and Amino Acid Sequence.* Amino acid analysis was performed on a Durrum model D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, Calif.). Samples were hydrolyzed in 0.2 ml 6 N HCl under vacuum for 24 h at 110°C. 40  $\mu$ l of a 10% aqueous solution of phenol was added to prevent degradation of tyrosine. Automated amino acid

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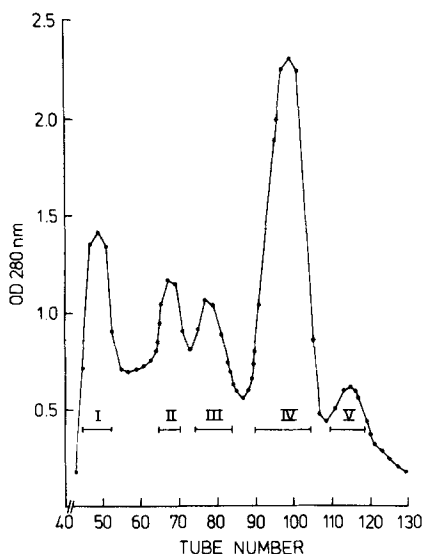


FIG. 1. Fractionation of S.K.O. amyloid fibrils from thyroid on a  $2.6 \times 100$ -cm Sephadex G-100 column equilibrated in 1 M acetic acid/5 M guanidine. Fractions (2.9 ml/tube) were collected.

sequencing was performed on a Beckman 890C sequencer (Beckman Instruments, Inc., Fullerton, Calif.). Phenylthiohydantoin amino acids were identified by high pressure liquid chromatography (HPLC) performed on a Waters HPLC model ALC/GPC-204 (Waters Instruments, Inc., Rochester, Minn.) prepacked with a C18/ $\mu$ -Bondapak column (Waters Instruments, Inc.) and eluted with a methanol water gradient and by amino acid analysis after back hydrolysis of the phenylthiohydantoin amino acids (10).

### Results

Fig. 1 shows the pattern obtained when the amyloid from the thyroid was fractionated on Sephadex G-100. Peak I (void volume) contained mucopolysaccharide material, which is found in all types of amyloid fibrils (11). Peak II consisted of P component (1) as indicated by antigenic analysis and molecular weight determination on SDS gels. Peaks III, IV, and V contained 14,000-, 9,000-, and 5,000-mol wt proteins, respectively. Peaks III and IV were rechromatographed on a G-75 Sephadex column and peak V on Ultragel AC54 (LKB Instruments Inc., Rockville, Md.) in 5M Guan/1 M HAC. Their purity was determined on SDS gels (Fig. 2), and their amino acid compositions are shown in Table I. Fractionation of amyloid fibrils obtained from spleen yielded a similar pattern to that in Fig. 1, although peaks IV and V were less prominent.

The partial amino acid sequences of peaks III, IV, and V are shown in Fig. 3 and compared to the sequence of serum prealbumin (12). Based on their amino acid composition, molecular weight, and partial sequence, it would appear that peak III represents the intact prealbumin subunit, peak IV consists of the carboxy terminus starting at residue 49, and peak V contains the first 48 residues. Although the homology is striking, the sequence shows a substitution of glycine for threonine at position 49. The heterogeneity at positions 52, 59, and 62 (Leu, Glu, and Val, respectively), in both thyroid and spleen, probably resulted from a second cleavage between positions 51 and 52, which gave rise to a minor contaminant.

A sample of vitreous fluid was obtained at surgery and was extracted once with

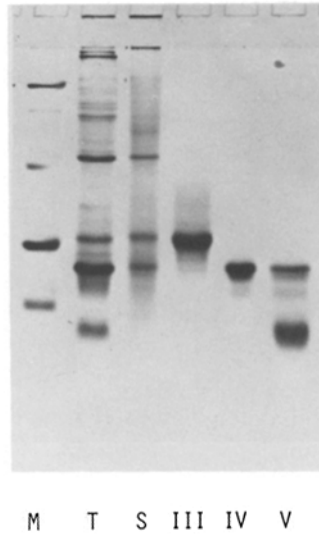


FIG. 2. SDS-PAGE 17% slab gel of impure amyloid fibrils extracted from thyroid (T) and spleen (S). Peaks III, IV, and V are purified components from Fig. 1. M, markers; bovine serum albumin, 67,000 mol wt; ovalbumin, 45,000 mol wt; chymotrypsinogen A, 25,000 mol wt; ribonuclease A, 13,700 mol wt; and AA protein, 8,000 mol wt.

TABLE 1  
*Amino Acid Composition\* of Peaks III, IV, and V from Fig. 1, Compared with Human Prealbumin and with Fragments 1-48 and 49-127*

	S.K.O. III	Prealbumin	S.K.O. IV	Prealbumin (49-127)	S.K.O. V	Prealbumin (1-48)
Cys‡	0.9	(1)§			1.0	(1)
Asp	9.2	(8)	4.8	(4)	4.4	(4)
Thr	10.4	(12)	7.7	(9)	2.6	(3)
Ser	11.2	(11)	7.7	(8)	3.6	(3)
Glu	14.4	(12)	10.0	(10)	2.7	(2)
Pro	7.4	(8)	4.5	(4)	3.8	(4)
Gly	9.5	(10)	5.8	(5)	3.7	(5)
Ala	12.7	(12)	7.0	(6)	6.0	(6)
Val	10.5	(12)	5.8	(6)	5.6	(6)
Met	1.1	(1)			1.0	(1)
Ile	5.7	(5)	3.8	(4)	1.6	(1)
Leu	8.7	(7)	5.3	(5)	2.5	(2)
Tyr	5.5	(5)	4.6	(5)		
Phe	4.6	(5)	3.2	(3)	1.1	(2)
His	3.9	(4)	2.8	(3)	1.0	(1)
Lys	7.4	(8)	4.0	(4)	3.0	(4)
Arg	4.0	(4)	1.9	(2)	2.0	(2)
Trp	ND	(2)	ND	(1)	ND	(1)

\* Mean value of four runs (not corrected).

‡ Determined as cysteic acid.

§ In parentheses are expected residues based on the published amino acid sequence of the intact prealbumin subunit and its amino and carboxy terminal fragments (12).

|| Not done.

distilled water and subjected to PAGE, amino acid, and N terminal analysis. A single band with an ~15,000 mol wt was obtained with an amino acid analysis similar to prealbumin. The amino terminus was glycine.

Antiserum to peak IV (which is a weak antiserum) reacted against peaks III and IV and the patient's serum. It did not react with normal human sera, isolated immuno-

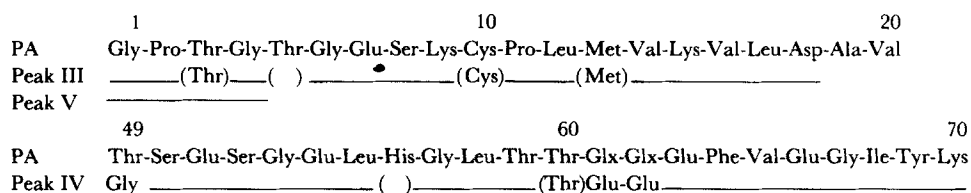


FIG. 3. The amino terminal sequence of fragments Peaks III, IV, and V from Fig. 1 compared with that of prealbumin (PA [12]). Empty parentheses, no amino acid detected; amino acid in parentheses, tentative.

globulin, prealbumin, and serum AA proteins. On the other hand, antibodies to human prealbumin reacted with peaks III and IV.

### Discussion

The neuropathic heredofamilial amyloidoses represent a variety of syndromes that generally demonstrate autosomal-dominant modes of inheritance (13). Although amyloid deposits can be detected in several tissues, such as liver, spleen, thyroid, gastrointestinal tract, and heart, in many of the heredofamilial types there is prominent involvement of the peripheral nerves, and they have been classified according to the clinical syndrome and to the country or ethnic group in which they were first described. Recently, Costa et al. (5) presented evidence that the amyloid deposits in Portuguese familial amyloid polyneuropathy shared common antigenic determinants with human prealbumin. This appears to be true also in amyloid obtained from two Swedish patients (6, 7), in whom limited amino acid sequence studies supported this possibility. Human prealbumin is a protein (54,980 mol wt) that possesses a  $\beta$ -structure and is composed of four identical subunits. Each subunit has a 13,745 mol wt and contains 127 amino acids. Prealbumin forms a complex with retinol-binding protein, which is a carrier protein for vitamin A and is also involved in the transport of thyroxine (12).

The amino acid sequence of amyloid fibrils extracted from organs of a patient (S.K.O.) of Jewish origin indicates that it is also a prealbumin-related protein. The S.K.O. proteins are particularly informative because the amyloid fibrils were composed of the intact prealbumin molecule (Fig. 1, peak III) and two fragments (peaks IV and V) resulting from enzymatic cleavage between positions 48 and 49 (Lys-Gly). Furthermore, at position 49 there appears to be an amino acid substitution because Thr was found in normal human prealbumin (12) and Gly in S.K.O. amyloid. Based on the x-ray diffraction studies of Blake et al. (14) and the tendency of Gly to act as a helix breaker (15), it seems possible that the molecule is more susceptible to digestion at this site. It is conceivable, therefore, that in the S.K.O. family a mutation has occurred, leading to the production of an unusual prealbumin that is abnormally degraded, bound, and/or precipitated.

Alternatively, prealbumin may be genetically polymorphic, and S.K.O. may represent an as yet undiscovered isotype or an allelic form. It is possible that additional variations from the prototype sequence exist. Studies are in progress to answer this question and to detect other polymorphisms.

### Summary

Amyloid fibrils were isolated from spleen and thyroid obtained at autopsy from one patient (S.K.O.) of Jewish origin with familial amyloidotic polyneuropathy. Gel filtration on Sephadex G100 after solubilization in 5 M guanidine HCl yielded three

major components with 14,000, 9,000, and 5,000 mol wt, respectively. The two larger components shared antigenic determinants with human prealbumin. Amino acid analysis and amino terminal sequence studies revealed the 14,000-mol wt protein to be an intact prealbumin subunit. The 9,000-mol wt fragment obtained in highest yield encompassed the region from position 49-127 and the 5,000 mol wt fraction encompassed the amino terminal of prealbumin (position 1-48). An amino acid substitution (Gly/Thr) was detected at position 49, where enzymatic cleavage occurred. Thus, several prealbumin-derived fragments, predominantly the carboxyl end, constitute the amyloid fibrils in a hereditary amyloidosis syndrome of dominant inheritance.

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