HUMAN AMYLOID PROTEIN: DIVERSITY AND UNIFORMITY

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SUMMARY: The major protein of amyloid fibril concentrates of ten tissues obtained from six patients has been fractionated by sequential gel filtration on Sepharose 4 B and Sephadex G-100 columns using 5 M guanidine-HCl in 1 N acetic acid with removal of over 28% of minor constituents. The major amyloid protein from each individual differs from that of the others in molecular weight, amino acid composition and peptide map profile. The major protein from the liver and spleen of the same individual, however, are identical. A serum component having an immunologic relationship to the patients' major amyloid protein has been detected migrating to the γ-globulin region on immunoelectrophoresis.

Amyloidosis is a pathologic condition in which a fibrillar glycoprotein, amyloid, is deposited extracellularly in various organs of the human body leading to metabolic disturbances in the involved tissue (e.g. heart, liver, kidney). The identifying characteristics of native human amyloid fibrils are their green polarization birefringence after staining with Congo red, constant tryptophan content, fibrillar appearance when examined by electron microscopy and x-ray diffraction crystallographic pattern revealing a "β-pleated sheet" configuration (1). In order to identify and characterize the major protein constituents of amyloid fibrils, a method for fractionation of denatured amyloid fibril concentrates by gel filtration in guanidine-HCl was recently described by us (2). The present study extends the investigation of the isolation of the major protein components of amyloid fibrils to preparations derived from the same tissue source in different patients and different tissue sources in the same patient.

MATERIALS AND METHODS: Ten organs of six individuals dying of amyloidosis were obtained at necropsy and contained amyloid deposits representing from 50 to 90% of the total tissue bulk. The following tissues were obtained: patient 1,

liver (Amyloid III); patient 2, spleen (Amyloid IV); patient 3, liver (Amyloid V), spleen (Amyloid VI) and serum (serum 3); patient 4, liver (Amyloid VII) and spleen (Amyloid VIII); patient 5, spleen (Amyloid IX), liver (Amyloid X) and serum (serum 5); patient 6, spleen (Amyloid XIV) and liver (Amyloid XV). All tissues were processed by differential centrifugation to produce an amyloid fibril concentrate (3,4). Twenty to 30 mg of the lyophilized protein was dissolved in 3 ml of N_2 gassed 6 M guanidine-HCl containing 0.1 M Tris-HCl, pH 8.0 and 19.5 μmoles of dithiothreitol, and agitated under N_2 at 25°C for one to 24 hrs. The resulting solution was cooled to 4°C, diluted to 5 M in guanidine-HCl with 1 ml of 2 M guanidine-HCl in 1 N acetic acid and was applied directly to a 1.5 x 90 cm column of Sepharose 4 B equilibrated with 5 M guanidine-HCl in 1 N acetic acid. Descending chromatography was performed at 25°C with a pressure of 15 cm and a flow rate of 5 ml per hr. (2). Fractions were assayed for protein at 278 mμ with a Beckman DU-2 spectrophotometer. Fractions containing the major protein(s) were pooled, exhaustively dialyzed with distilled water and lyophilized. This protein was dissolved as for Sepharose chromatography and applied to a 1.5 x 90 cm column of Sephadex G-100 equilibrated with 5 M guanidine-HCl in 1 N acetic acid and descending chromatography performed as above. Fractions containing the major protein components were pooled, dialyzed with distilled water and lyophilized (2).

Amino acid analysis and peptide mapping of a tryptic digest of the major amyloid proteins from each amyloid fibril preparation were performed as previously described (2,3). Reduction and alkylation of purified proteins were performed using 3 mg of protein in 3 ml of N_2 gassed 6 M guanidine-HCl in 0.1 M Tris-HCl, pH 8.2, to which 3.3 μmoles of dithiothreitol was added and agitated for 1 hr. at 25°C. Iodoacetamide, 35 μmoles, was added with gentle shaking for 30 min. at 25°C and 42 μmoles of dithiothreitol added, mixed and the preparation dialyzed with distilled water and lyophilized. N-terminal analysis on the purified protein was performed using the dinitrophenylation technique (5). Molecular weights were determined both by the SDS polyacrylamide disc gel

electrophoresis method of Shapiro, et al (6) as modified by Weber and Osborn (7) and by gel filtration on Sephadex G-100 in 5 M guanidine-HCl (8). SDS polyacrylamide disc gels were stained for tryptophan (9). Immunologic analysis of the purified proteins, denatured amyloid fibril concentrates and patients' sera was performed as previously described (2).

RESULTS: Sequential gel filtration of dithiothreitol-reduced protein using 5 M guanidine-HCl in 1 N acetic acid on Sepharose 4 B and Sephadex G-100

TABLE I

AMINO ACID ANALYSIS*, N-TERMINAL GROUP AND MOLECULAR WEIGHT OF MAJOR AMYLOID PROTEINS

Amyloid Protein	IIIa	IIIb	IV	VI	VIIa	VIIb	IX	XIV
Amino Acid								
Aspartic Acid	5.51	5.98	6.71	4.30	9.20	8.73	7.10	6.91
Threonine	5.85	5.24	0	5.30	6.82	7.10	6.18	6.15
Serine	8.18	7.87	3.07	9.51	9.23	8.72	7.98	10.95
Glutamic Acid	7.77	6.85	2.18	7.60	9.99	9.35	7.81	9.02
Proline	5.70	6.11	0	4.85	4.98	6.48	5.60	7.32
Glycine	6.09	6.33	4.47	8.36	6.17	7.47	7.02	11.13
Alanine	6.71	6.55	7.76	6.46	5.62	6.12	4.34	8.26
Half Cystine	1.71	1.29	0	1.80	1.81	1.33	1.50	1.64
Valine	5.44	4.86	0	4.50	5.28	5.50	3.22	6.20
Methionine	.23	.33	1.89	0	.75	.63	.80	0
Isoleucine	2.18	2.79	.82	3.16	4.06	5.29	3.47	4.68
Leucine	6.12	6.27	1.09	6.26	7.14	6.65	4.34	7.79
Tyrosine	2.31	2.09	3.58	3.40	3.50	4.08	3.76	3.68
Phenylalanine	1.60	1.81	4.96	1.85	4.09	5.36	3.30	3.22
Lysine	3.93	3.39	1.09	4.65	4.95	4.97	3.18	2.29
Histidine	.85	.60	.89	1.30	1.15	1.03	0	1.23
Arginine	1.81	2.05	4.80	3.20	3.50	3.65	2.38	1.11
Tryptophan	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
N-terminus	U**	U	U	U	Asx	Asx	Asx	U
Molecular Weight	27,600	13,700	5,000	15,400	31,200	18,300	7,500	14,600

* Micromoles per micromole of tryptophan.

** U: unreactive to fluorodinitrobenzene.

columns succeeded in fractionating one major protein from the amyloid fibril concentrates of Amyloids III, IV, V and VI, IX and X, XIV and XV to afford, after reduction and alkylation, a single tryptophan reactive band in SDS polyacrylamide disc gels. Removal of minor protein constituents as estimated by column chromatography was over 28% in each case. The amyloid fibrils derived from the organs of 2 of the 6 patients had two major tryptophan containing proteins (IIIa and IIIb; VIIa and VIIb, VIIIa and VIIIb) with similar amino acid compositions (Table I), but not completely identical peptide maps (Fig. 1 A and C).

The major protein(s) of the amyloid fibrils obtained from the same tissue source of different patients was found by SDS polyacrylamide disc electrophoresis and gel filtration to have different molecular weights and amino acid analyses (Table I) and revealed striking differences in amino acid composition though all had tryptophan, a high dicarboxylic and short chain amino acid composition and absent hydroxyproline, hydroxylysine and desmosine. In addition the amyloid protein from 5 of the 6 patients was deficient in one or more commonly occuring amino acids. N-terminal analysis revealed 4 of the 6 patients' amyloid protein to be unreactive to fluorodinitrobenzene while the remaining two patients' amyloid had N-terminal aspartic-asparagine (Asx). Peptide maps of the major amyloid protein(s) revealed marked differences from patient to patient (Fig. 1 A and D).

The major protein(s) of the amyloid fibrils obtained from different tissues of the same patient (e.g. IX and X) had identical N-terminal group and amino acid analyses, molecular weights and peptide maps (Fig. 1 A and B).

Immunologic studies revealed an identity between the amyloid protein derived from the liver, spleen and serum of the same patient (Figs. 2 and 3). However, antisera to the amyloid protein derived from the tissues of any one patient failed to form a precipitin line with the tissues or sera of other amyloidotic patients. With the more sensitive radioimmunassay technique a 7 to 10% cross-reactivity between the major amyloid protein from different

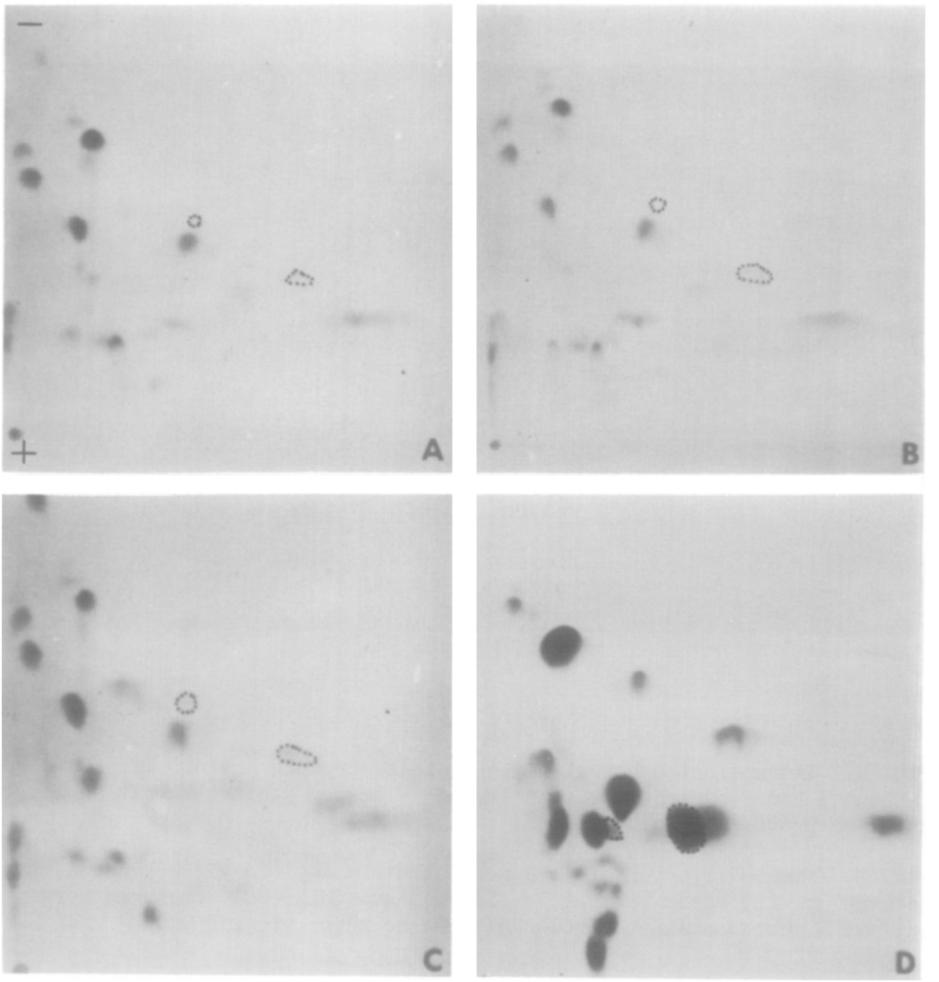

Fig. 1. Peptide maps of amyloid proteins with tryptophan peptides circled: (A) liver protein Amyloid VIIb, (B) spleen protein Amyloid VIIb and (C) liver protein Amyloid VIIa from patient 4; (D) spleen protein Amyloid IV from patient 2.

patients was obtained (unpublished data). A serum component traveling in the γ-globulin region on immunoelectrophoresis (Fig. 2) was shown to cross-react with the major amyloid protein obtained from the spleen of the same patient (Fig. 3). The nature of this serum protein has not as yet been identified.

DISCUSSION: Amyloid fibrils have been shown to have a characteristic crystallographic pattern on x-ray diffraction (1) and a similar (10), but occasionally

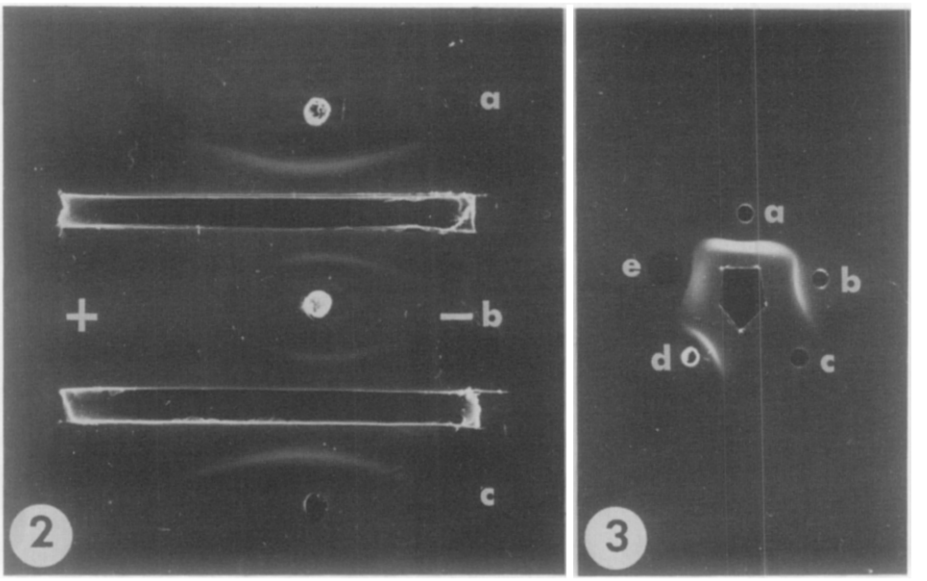

Fig. 2. Immunoelectrophoresis of (a) major protein Amyloid VI (b) serum patient 3 and (c) major protein Amyloid V tested against rabbit antisera to major protein Amyloid VI.

Fig. 3. Immunodiffusion of (a) major protein Amyloid VI; (b and d) serum patient 3; (c) serum patient 5: (e) 6 M guanidine-HCl denatured serum patient 3 tested against rabbit antisera to major protein VI.

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variable appearance (3,4), by high resolution electron microscopy. Recent immunologic evidence indicated both differences and similarities between amyloid fibril proteins obtained from the tissues of different patients (11). The biochemical evidence reported here indicates marked differences in amino acid composition of the major protein(s) obtained from amyloid fibrils of the same tissue of different patients and identity of the major amyloid protein obtained from two different tissues of the same patient. In addition, in those patients for which a serum sample was available, antibodies directed against the major amyloid protein from the tissues of that patient cross-reacted with a component in his serum migrating in the γ-globulin region on immunoelectrophoresis.

In view of the marked chemical similarity of amyloid protein and immunoglobulin protein, e.g. unreactive or Asx N-terminal groups, constant tryptophan content, heterogeneity between individuals and homogeneity within the individual; and the molecular weight ranges of amyloid fibril protein reported here, the possibility that amyloid may be derived from the N-terminal variable segment of the light (12) or heavy chain of immunoglobulin proteins (e.g. the Fd fragment) must be considered and is presently under investigation.

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