THE PARTIAL AMINO ACID SEQUENCE OF THE MAJOR LOW MOLECULAR WEIGHT COMPONENT OF TWO HUMAN AMYLOID FIBRILS*

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Received 16 January 1972

1. Introduction

Amyloid fibrils from several patients with secondary amyloidosis or amyloidosis associated with Familial Mediterrean Fever (FMF), when treated with dilute acid yield a major low molecular component which appears to be similar in all, and unrelated to any known immunoglobulin.

In an effort to separate the protein subunit present in amyloid fibrils from mucopolysaccharides, amyloid fibrils isolated by the method of Pras et al. [1] were extracted with dilute acid. This procedure yielded a low molecular weight substance which migrated as a single band on disc electrophoresis, had a molecular weight of around 6,000 daltons, bound Congo red, and could be made to assume again a fibrillar appearance which however differed from that of typical amyloid fibrills [2]. In the present study we present the partial amino acid sequences of this component from two patients and show them to be identical.

2. Materials and methods

The acid soluble low molecular weight fraction was extracted from amyloid fibrils isolated from the liver of a patient with Hodgkin's disease and the spleen of a patient with FMF by treating the fibrils with 0.02 M HCl for 1 hr at 60°. After centrifugation, the supernatant which contained 40–50% of the dry weight was dialyzed against 0.15 M NaCl and then against distilled water. The material was redissolved in 0.02 M HCl and further purified by vacuum dialysis. The dialysate was then lyophilized prior to use [2].

N-terminal analyses were done by the dansyl chloride method [3] and identified on polyamide thin-layer plates. The partial amino acid sequence was determined in a Beckman Model 890 sequencer by the method of Edman and Begg [4]. Released thiazolinone derivatives were converted to phenythiohydantoin (PTH) derivatives with 1 N HCl at 80° for 10 min and were identified as such or as the silylated derivative by gas chromatography using a Hewlett Packard gas chromatograph [5] and by amino acid analysis after hydrolysis using a Beckman 121 amino acid analyzer.

3. Results

Table 1 lists the amino acid composition of the 2 preparations which appear to be similar but not identical. Both samples are devoid of cysteine, poor in threonine and rich in aspartic acid, phenylalanine, glycine, alanine, and arginine. The amino terminal residue of both was serine although small amounts of arginine were also identified in the first step of the Edman degradation.

Fig. 1 lists the amino acid sequence of the first 33 residues of the Hodgkin's protein, 21 residues of the FMF protein and compares them to the 24 residues of

* Supported by U.S.P.H.S. Grant No. AM 02594, and No. AM 01431 (National Institutes of Health), The Health Research Council of the City of New York, The Arthritis Foundation, Inc. and the National Institute of General Medical Sciences No. 5-T05GM0 1668.

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North-Holland Publishing Company – Amsterdam

Table 1
Amino acid composition of acid soluble fraction from 2 human amyloid fibrils.

Amino acid	Number of residues found*	
	FMF	Hodgkins
Lysine	2.0	2.3
Histidine	1.1	1.8
Arginine	7.0	6.6
Aspartic acid	9.1	7.3
Threonine	0.9	0.2
Serine	4.6	4.0
Glutamic acid	6.3	5.4
Proline	1.0	0.8
Glycine	7.6	6.6
Alanine	11.6	10.0
Half Cystine	---	---
Valine	0.9	1.1
Methionine**	(2)	(2)
Isoleucine	2.0	2.0
Leucine	2.2	1.2
Tyrosine	3.7	3.4
Phenylalanine	4.7	5.0
Tryptophan**	+	

* Mean of 3 analyses after 21 hr hydrolysis.
** Identified from amino acid sequence analysis in fig. 1.

protein A from a patient with tuberculosis recently sequenced by Benditt et al. [6]. It is apparent that all 3 appear to be identical in the regions examined.

4. Discussion

From the data presented in this study it would appear that a protein seemingly unrelated to any known immunoglubulin may constitute up to 50% of the dry weight of amyloid fibrils isolated from 2 subjects with amyloidosis associated with Hodgkin's disease and FMF. This material appears to be identical to protein A found in secondary amyloidosis, one sample of which was recently partially sequenced by Benditt et al. [6, 7]. Thus it seems likely that it may constitute a major component of amyloid substance of diverse origin [2, 6, 7]. This component seems to be present in addition to the immunoglubulin fragments demonstrated by Glenner et al. [8] since examination of the peptide map of one of these proteins (FMF) indicates the presence of peptides characteristic of the acid soluble fraction as well as those which appear to be com-

Source	Residues 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Hodgkin's liver	*Ser-	Phe-	Phe-	**Ser-	Phe-	Leu-	Gly-	Glu-	Ala-	Phe-	Asp-	Gly-	Ala-	Arg-	Asp-	Met-	Trp-	Arg-	Ala-	Tyr-	**Ser-	Asp-	Met-	Arg-	Glu-	Ala-	Asn-	Tyr-	Ileu-	Gly-	**Ser-	Asp	
F.M.F. spleen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	[ ]	—	[ ]	—	—	—											
Tubercu-losis liver (ref. 6)	Arg	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

Fig. 1. Amino acid sequence of acid soluble fractions of amyloid.
* While Ser was the N-terminal of both proteins, small amounts of Arg were identified in the first step. Consequently, numbering has been made to correspond to that of Benditt et al. [6].
** Uncertain; [ ] - not identified.

mon to light chains.

Whether this protein is synthesized and deposited as such, or represents a fragment of a larger protein remains to be determined. The heterogeneity and presence of arginine at the amino terminus raise the possibility that it is the product of proteolytic digestion of a larger protein.

The presence of this protein in 3 subjects, a similar one which has not yet been fully characterized in several others [2, 7], taken together with the ability to bind Congo red and to assume a fibrillar appearance, suggest an important role in the genesis of amyloid. If the same protein can be identified also in primary and myeloma associated amyloid it seems possible that it may represent a basic defect common to all amyloid.

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