P-COMPONENT OF AMYLOID: AMINO-TERMINAL SEQUENCE

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SUMMARY: A homogeneous preparation of P-component has been isolated from human splenic amyloid. Twenty-three residues of the amino-terminal sequence of this unique protein have been determined. The sequence is not that of an immunoglobulin and does not correspond to the sequence of any previously reported protein.

P-component (plasma component, pentagonal unit) is a protein known to be imtimately associated with the amyloid fibril and antigenically identical to a constituent of human plasma. It has recently been isolated from amyloid-rich tissues and purified by a combination of gel filtration and ion exchange chromatography (1). It has a characteristic pentagonal ultrastructure, immunologic reactivity and chemical properties which, as previously presented (2,3), have shown it to be distinct from the well-defined amyloid fibril in all respects

In order to determine its precise identity, amino acid sequencing was undertaken.

MATERIALS AND METHODS: The amyloid-laden spleen of a patient (AC 71-173) with primary amyloidosis was used as the source of P-component. Twenty grams of this tissue were repeatedly homogenized in normal saline according to the amyloid isolation procedure of Pras (4). Then, to isolate P-component, the supernatant saline solutions were dialyzed against water, lyophilized and purified in the following manner. Two hundred mg. of lyophilized material were dissolved in 15 ml of 0.1M Tris buffer, pH 8.0, and chromatographed on a column of Sephadex G-200 (superfine), eluted with the same buffer. The peak

Table 1

Amino Acid Analysis of P-component
(residues/1000 residues)

Tryptophan	3.7
Lysine	55.4
Histidine	21.2
Arginine	43.2
Cysteic Acid	36.3
Aspartic Acid	86.2
Threonine	42.6
Serine	74.1
Glutamic Acid	107.2
Proline	55.8
Glycine	79.6
Alanine	50.6
Cystine (half)	8.0
Valine	82.5
Methionine	3.1
Isoleucine	53.0
Leucine	94.6
Tyrosine	54.1
Phenylalanine	49.0
	1000.2

containing P-component (determined by its precipitin reaction with anti-P-component serum) was dissolved in 0.01M phosphate buffer, pH 8.0, and applied to a DEAE cellulose column in the same buffer. This was eluted in a stepwise fashion with a series of phosphate buffers of increasing molarity and decreasing pH to a final buffer of 0.15M phosphate, pH 4.5. P-component, which was eluted with the final buffer, was demonstrated by electron microscopy to consist solely of pentagonal units and was further tested for homogeneity by 7% polyacrylamide disc gel electrophoresis (Canalco 12) in Tris glycine buffer, pH 8.5 (5). Amino-acid analysis (JEOL-5AH) was performed after hydrolysis in p-toluene sulfonic acid (6).

Purified P-component (6.6 mg) was used in sequence analysis on a Beckman Sequencer, Model 890 C following the method of Edman and Begg (7) using the fast protein-quadrol program #072172C. The phenylthiohydantoin (PTH)-amino acids were identified either on a G 65 Beckman gas chromatograph according to Pisano and Bronzert (8) and/or by hydrolysis of the PTH amino acid in 6N HCl under N_2 for 24 hours at 135°C followed by amino acid analysis.

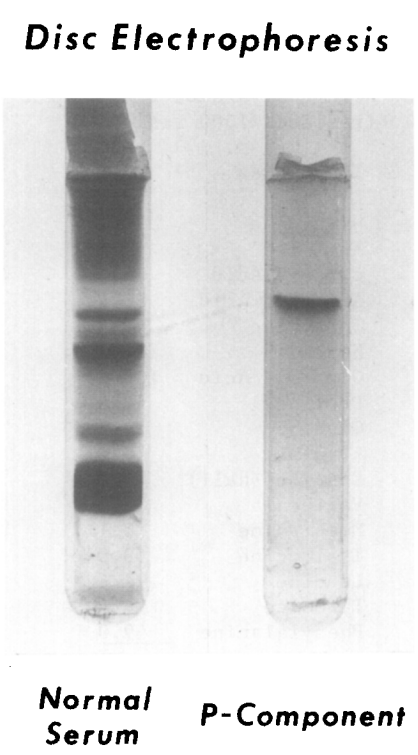

Fig. 1. Polyacrylamide (7%) disc gel electrophoresis in Tris glycine buffer, pH 8.5 of normal serum (left) and P-component (right)

RESULTS: P-component was a single band on polyacrylamide disc gel electrophoresis (Fig. 1). The amino-acid composition is shown in Table 1.

The amino-terminal amino acid sequence analyses was:

1	2	3	4	5	6	7	8	9	10	11	12
His -	Ala -	Asp -	Leu -	( ) -	Thr -	Lys -	Val -	Phe -	Val -	Phe -	( )

13	14	15	16	17	18	19	20	21	22	23
( ) -	Ser -	Glu -	Val -	Val -	( ) -	Ser -	Val -	Val -	Ser -	Ile

Identification of each residue was made by gas chromatography of the PTH amino acid (Table 2). In instances where the residue could not be determined by this method, amino acid analysis was performed after hydrolysis of the PTH residue to the free amino acid.

DISCUSSION: The amyloid fibril, a fibrous protein with characteristic staining pattern, ultrastructure and chemical composition is known to be the major consti-

Table 2

Sequential Degradation of P-component

Step No.	Deduced Residue[a]	GC 65[b]	n moles	AAA[c]
1	HIS	-	-	HIS
2	ALA	ALA	45.9	N.D.
3	ASP	ASP	25.7	ASP
4	LEU	LEU/ILE	43.6	LEU
5	X	-	-	-
6	THR	THR	44.2	N.D.
7	LYS	-	-	LYS
8	VAL	VAL	58.0	VAL
9	PHE	PHE	35.0	PHE
10	VAL	VAL	54.3	N.D.
11	PHE	PHE	27.8	N.D.
12	X	-	-	-
13	X	-	-	-
14	SER	SER	30.2	N.D.
15	GLU	GLU	11.8	GLU
16	VAL	VAL	14.3	N.D.
17	VAL	VAL	14.9	VAL
18	X	-	-	-
19	SER	SER	8.2	N.D.
20	VAL	VAL	17.2	N.D.
21	VAL	VAL	17.6	VAL
22	SER	SER	14.7	N.D.
23	ILE	LEU/ILE	12.7	ILE

a X means residue indeterminant by GC and AAA
b Gas chromatography with SP400 supporting resin
c Amino acid analysis for qualitative identification only
- Residue could not be determined
N.D. not determined

tuent of amyloid deposits (3). In addition to the fibril, a globular protein sharing antigenic identity with a normal plasma component (and therefore named the P-component) has been recognized as a minor constituent of all amyloid tissues studied to date (2,3). Electron microscopic study has shown it to be a pentagonally structured unit which may aggregate laterally to form short rods It has been shown that P-component of amyloid derived from different species (guinea pig and human) differ with respect to antigenic specificity but share chemical and ultrastructural properties (1).

The present study is the first report of the partial amino acid structure of P-component isolated from human primary amyloid by Sephadex G-200 gel filtration and DEAE ion-exchange chromatography. The protein is homogeneous by disc electrophoresis and the amino acid composition shows a minimal amount of tryptophan, cystine, and methionine and large amounts of acidic amino acids (glutamic and aspartic acid). The N-terminal amino acid is histidine and the sequence of the first 23 amino acids is clearly distinct from the amyloid fibril protein derivative of primary amyloids which to date have had amino acid sequences homologous with the variable portion of immunoglobulin light chains. It is also distinct from the protein derivative of secondary amyloids, almost all of which have had a unique amino-terminal sequence.

The present P-component sequence was compared by computer search (courtesy of Dr. Winona C. Barker, National Biomedical Research Foundation, Washington, D.C.) to the 518 sequences published in The Atlas of Protein Sequence and Structure, ed. Margaret O. Dayhoff, and 151 additional sequences in Supplement I of the Atlas and has proven to be distinct from all human protein sequences published to date.

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