AN AMYLOID FIBRIL PROTEIN OF UNKNOWN ORIGIN: PARTIAL AMINO-ACID SEQUENCE ANALYSIS

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SUMMARY: The amino-terminal amino acid sequence of a purified amyloid protein lacking threonine, valine, proline and half-cystine has been determined. The sequence data do not correspond to the sequence of any previously reported immunoglobulin protein.

Chemical analysis of amyloid fibrils has recently led to an understanding of the origin of these proteins. Amino-terminal amino acid sequence analysis of two such fibrils has clearly shown them to have a homogeneous sequence and to be derived from the variable region of kappa light chains (1). One of these fibrils comes from a patient with primary amyloidosis, the other from a patient with a secondary form of the disease (2). Immunochemical analyses of additional fibrils has shown these to be antigenically cross-reactive with either kappa or lambda human Bence Jones proteins, i.e. light chains (3).

During the course of these investigations the protein, Amyloid IV (2,4), of a fibril with the typical tinctorial and ultrastructural features of other amyloid fibrils was found to have a molecular weight of 5,000 and an unusual amino acid composition. This fibril protein totally lacks valine, proline, threonine and half-cystine while containing a total of over 40 amino acids. No stretch of 40 amino acids from any immunoglobulin light chain sequenced to date is missing all these amino acids. This suggests that the Amyloid IV fibril protein might have another origin. Accordingly, the amino-terminal amino acid sequence of this protein was studied in an attempt to elucidate

its derivation.

MATERIALS AND METHODS: The methods and conditions for the purification of Amyloid IV protein by column chromatography have been previously described (2,4). The purified protein gave a single band on sodium dodecyl sulfate-polyacrylamide (10 percent) disc gel electrophoresis at pH 7.1. The amino acid composition and peptide maps of this protein have been previously reported (4).

The sequence of the amino-terminal amino acids of Amyloid IV was determined twice with an automatic amino acid sequencer, Beckman Model 890 according to the method of Edman and Begg (5) using 2 mg and 4 mg of protein. The thiazolinone derivatives obtained from the sequencer were converted to the phenylthiohydantoin-amino acids and identified by liquid-gas chromatography (6). Identification of basic amino acids was achieved by hydrolysis of the phenylthiohydantoin derivatives to the free amino acids which were analyzed using the Beckman Model 121 amino acid analyzer.

RESULTS: The amino-terminal amino acid sequence of Amyloid IV was:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Arg-	Ser-	Phe-	Phe-	Ser-	Phe-	Leu-	Gly-	Glu-	Ala-	Phe-	Asp-	Gly-	Ala-	Arg-

The yield of Arg-1 was approximately 50% of theoretical, a result comparable to that found with other homogeneous proteins. No other amino acid in position 1 or 2 was detected. After position 15 the yield fell off rapidly despite the initial presence of large amounts of protein and no further residues could be identified with certainty.

DISCUSSION: The amino-terminal amino acid sequence of Amyloid IV reported here bears no homology to the published sequences of immunoglobulin kappa and lambda light chains, heavy chain variable regions or to gamma heavy chain constant regions. This is in marked contrast to other amyloid fibril proteins which represent fragments of light chains comprising primarily the variable regions (1).

The origin of this amyloid protein is at present unknown. It could conceivably derive from a hitherto unknown type of light chain, from an as

yet unsequenced heavy chain, i.e. IgA, IgD, etc. or from other immunoglobulin-associated polypeptides such as J chain (7). Alternatively, this protein might not derive at all from immunoglobulins. Amyloid IV was isolated from the tissues of a patient with rheumatoid arthritis. Thus it is possible that certain chronic inflammatory diseases elicit the synthesis of non-antibody proteins capable of becoming amyloid fibrils. The discovery of the origin of this unusual protein will clearly be important in understanding completely the pathogenesis, classification and perhaps treatment of amyloidosis.

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