THE COMPLETE AMINO ACID SEQUENCE OF AN AMYLOID FIBRIL PROTEIN AA[1] OF UNUSUAL SIZE (64 RESIDUES)

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SUMMARY: An amyloid fibril protein AA[1] of unusual size (64 amino acid residues) was characterized by amino acid sequence analysis. The protein was derived from the amyloid-laden liver of a patient with amyloidosis associated with ankylosing spondylitis and comprised 45 per cent of the total amyloid fibril proteins. Apart from the difference in size and with the exception of an aspartic acid-asparagine substitution in position 60 the protein was identical to a 76 amino acid residue protein AA derived from another patient with chronic rheumatic disease and amyloidosis. Protein AA most probably results from proteolytic cleavage at different positions of a larger precursor of unknown origin.

INTRODUCTION: During recent years it has become evident that amyloid fibrils with similar staining properties and identical ultrastructure consist of different protein sub-units. One such type of protein is monoclonal immunoglobulin light chains (1). Another major amyloid component is the protein AA (2,3). The origin of protein AA is not known, but it appears to derive from a closely related protein, SAA, found in serum of patients with amyloidosis, in patients with diseases prone to get amyloidosis and in very small amounts in normal serum (3,4,5). The human amyloid protein AA

1 For nomenclature see: Nomenclature for amyloid proteins and related serum components. In Amyloidosis. Eds. O. Wegelius and A. Pasternack. Academic Press, New York, London. In press.

is unique in its primary structure and has been characterized by complete amino acid sequence by several groups (6,7,8). It consists reportedly of from 45 to 76 amino acids, corresponding to molecular weights varying from 4.500 to 9.145 daltons in different amyloid preparations. Some amino acid substitutions have been reported, particularly in the carboxy-terminal part of the protein AA polypeptide chain (8).

In the present communication we report the complete amino acid sequence of protein AA from amyloid fibrils secondary to a chronic inflammatory condition - ankylosing spondylitis - and compare this protein with other known sequences of protein AA. This is the first report of protein AA consisting of 64 amino acid residues, however, the sequence was otherwise identical to that of a 76 amino acid protein AA from another patient with amyloidosis associated with chronic inflammation, except for a possible aspartic acid-asparagine substitution in position 60.

MATERIALS AND METHODS: Amyloid-laden tissues were obtained from the liver and spleen of a 25 years old male (J.L.), who had severe generalized amyloidosis secondary to ankylosing spondylitis, and died in uremia due to renal amyloidosis. Amyloid fibrils were extracted with water after repeated washings of homogenized tissue with physiological saline (9), degraded with 6M guanidine in 0.55M Tris - HCl containing 0.1M dithiothreitol, and fractionated on a Sephadex G-100 column (92 x 3 cm) equilibrated with 5M guanidine - 1M acetic acid (10). The retarded peak containing protein AA was dialyzed against water, lyophilized and further purified by gel filtration on Sephadex G-25 fine, equilibrated with 10% formic acid as described in detail elsewhere (10). Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis was performed using the procedure of Swank and Munkres (11).

Acid hydrolysis and amino acid analysis were carried out as described by Sletten and Husby (8). A JEOL-JAS-47K Sequence Analyzer was used for the automated Edman degradations with samples of 100 to 250 nanomoles. A tryptic peptide was sequenced by manual Edman degradation (12). The reagents and solvents were obtained from Pierce Chemical Company. The phenylthiohydantoin amino acids (PTH-AA) were identified

as previously described (8). Cyanogen bromide cleavage and purification of the resulting fragments were carried out (8). Tryptic digestion was performed on the C-terminal fragment and the resulting peptides were purified by ion-exchange chromatography and by thin-layer chromatography (8).

RESULTS AND DISCUSSION: The yield of protein AA from the liver of patient J.L. was found to be 45 per cent of the total protein eluted. N-terminal sequence analysis indicated a high purity of the protein, but the amino acid composition (Table 1) revealed that some of the amino acids did not come out as whole residues, which indicated some degree of heterogeneity. SDS polyacrylamide gel electrophoresis showed one major band corresponding to a molecular weight of approximately 8.000 daltons and a minor one corresponding to a molecular weight of approximately 11.000. Both polypeptides reacted antigenically identical with a 76 amino acid residues protein AA (T.H.) described previously (8) when a monospecific antiserum to protein AA(T.H.) was utilized.

Edmand degradation of the protein elucidated the N-terminal sequence up to residue 45, except for some uncertainty for residue 37 and 39 (Fig. 1). Amino acid composition of the cyanogen bromide fragments CB-1 and CB-2 (Table 1) confirmed this sequence data. The amino acid composition of CB-3, based on one residue of histidine (Table 1), showed that the data for aspartic acid, glutamic acid, alanine, and phenylalanine is slightly off of what was found in the sequence, thus indicating some degree of heterogeneity in the C-terminal region. Edman degradation of CB-3 fragment resulted in only one amino acid at each step and the sequence up to residue 62 was elucidated (Fig. 1). The sequence was also verified by isolation of the tryptic peptides

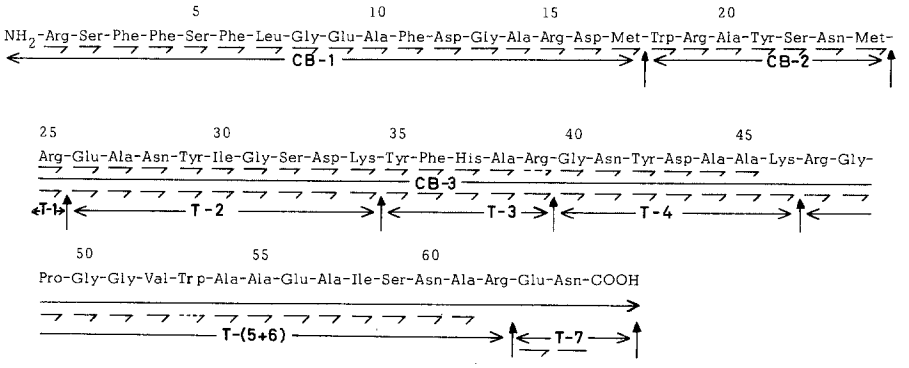

Fig. 1 The amino acid sequence of amyloid protein AA (J.L., liver) from ankylosing spondylitis. The peptides are indicated by double-headed arrows. Amino acid residues identified by Edman degradation (——→) (- - →) and by amino acid analysis on the residual material (——) are so indicated. Abbreviations used are: CB for cyanogen bromide fragments and T for tryptic peptides.

T-1+2, T-2, T-4, and T-5+6 (Fig. 1). Free arginine was also found, which would correspond to T-1 and T-5. In addition, a tryptic peptide, T-7, was isolated and the sequence was determined by manual Edman degradation to be glutaminyl asparagine. As this tryptic peptide did not contain any arginine or lysine, the peptide has to be the C-terminal one. No other peptides were obtained. However, as the SDS polyacrylamide gel electrophoresis revealed two bands there is obviously a heterogeneity in the polypeptide length. Calculations on the molecular weight from the complete amino acid sequence is 7.225 and fits well with that determined by SDS polyacrylamide gel electrophoresis (8.000). The molecular weight of the main protein AA is thus smaller in this case than that reported by Levin et al. (7) and by Sletten and Husby (8), but larger than that reported by Ein et al. (6).

A serum protein, SAA, with a molecular weight of 14.000

TABLE 1

Amino acid composition of human amyloid protein AA and of the fragments obtained from cyanogen bromide cleavage of the protein.

Amino acid	Protein AA	CB-1	CB-2	CB-3	Protein AA from sequence
Aspartic acid	8.48 (8-9)	2.15 (2)	1.03 (1)	5.58 (6)	9
Threonine	0.28	-	-	0.17	
Serine	4.48 (5)	1.60 (2)	0.81 (1)	1.93 (2)	5
Glutamic acid	4.82 (5)	1.16 (1)	-	3.40 (3)	5
Proline	1.19 (1)	-	-	0.93 (1)	1
Glycine	6.83 (7)	2.02 (2)	0.09	4.79 (5)	7
Alanine	10.2 (10-11)	2.26 (2)	1.03 (1)	7.35 (8)	11
Half-cystine	-	-	-	-	
Valine	1.15 (1)	0.08	-	0.97 (1)	1
Methionine	1.94 (2)	0.46(1)[a]	0.65(1)[a]	-	2
Isoleucine	2.23 (2)	0.08		1.88 (2)	2
Leucine	1.41 (1)	0.91 (1)		0.29	1
Tyrosine	3.97 (4)	0.32	0.49 (1)	2.70 (3)	4
Phenylalanine	5.35 (5)	3.31 (4)	0.06	1.47 (1)	5
Histidine	1.18 (1)	-	-	1.00 (1)	1
Lysine	2.14 (2)	-	-	1.80 (2)	2
Arginine	7.05 (7)	1.92 (2)	0.94 (1)	3.97 (4)	7
Tryptophan	1.16 (2)	-	- (1)	(1)	2
Total residues		17	7	40	64

a Determined as homoserine/homoserine lactone.

daltons and which is antigenically related to the amyloid protein AA has recently been studied in our laboratories (4,13). Sequence studies revealed complete identity among the first twenty amino acid residues in the N-terminal part of protein AA and protein SAA[1]. The observation that

1 Anders, R.F., Sletten, K., and Natvig, J.B., manuscript in preparation.

protein AA studied here consisted of 64 amino acid residues, and two others of 76 amino acid residues (7,8) and one of 45 residues (6) is evidence that protein AA is a product from proteolytic digestion of a precursor protein. The fact that two polypeptides with different molecular weights occurred in the amyloid preparation reported here gives further evidence to this suggestion. Whether protein SAA is the precursor for protein AA, or whether both proteins derive from another larger precursor protein remains to be elucidated. The protein AA was also isolated from the spleen of the same patient. SDS polyacrylamide gel electrophoresis revealed two bands corresponding to molecular weights very similar to that found for protein AA isolated from the liver. N-terminal sequence analysis showed, however, that about 50 per cent of the protein lacks arginine in position 1 and starts with residue 2, whereas protein AA from the liver lacks about 10 per cent of arginine.

The amyloid fibril protein AA reported here was derived from a patient with amyloidosis secondary to ankylosing spondylitis, a chronic inflammatory disorder. It was striking that the primary structure of this protein AA was identical, with one exception, to protein AA from a patient with juvenile rheumatoid arthritis studied by us (8). The only difference was residue 60, where asparagine was found instead of aspartic acid, which could be due to deamidation occurring during the isolation procedure. These two patients were both of Norwegian origin. In contrast, two amino acid substitutions were observed when comparing with a protein AA sequence of amyloid associated with familial Mediterranean fever, an inherited disease seen mostly among Jewish and Arabic

people in Mediterranean areas (14). One can speculate whether this variability observed reflects different disease processes or whether it is due to differences in the genetic material coding for the precursor protein.

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