High homology is present in the primary structures between murine senile amyloid protein (AS_{SAM}) and human apolipoprotein A-II

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The primary structure of a murine senile amyloid protein (AS_{SAM}) was determined. The protein consists of a single polypeptide chain of 78 amino acid residues. The amino-terminus is blocked with pyrrolidonecarboxylic acid. The sequence differs from that of the known murine amyloid A protein and is highly homologous to human apolipoprotein (apo) A-II. The result indicates that the putative precursor of the senile amyloid protein is apo A-II in mice.

Primary structure *Murine senile amyloid protein* *Apolipoprotein A-II*

1. INTRODUCTION

Amyloidosis is characterized by extracellular deposition of fibrillar amyloid protein. In human, the systemic amyloidoses have been classified as immunoglobulin (primary), reactive (secondary) and heredofamilial [1]. They have been well characterized biochemically but their pathogenesis is not clearly understood.

The mouse has been considered an appropriate animal for studying a wide variety of pathological processes. Therefore the murine model has been used to elucidate amyloidogenesis. In mice, secondary amyloidosis has been produced experimentally by parenteral administration of a variety of inflammatory agents. It has been shown that murine amyloid A protein (AA) is highly homologous to human AA and is derived from one isotype of serum AA protein (SAA), which is apo SAA_2 protein [2]. On the other hand, though spontaneous amyloidosis has been reported to occur in various strains of mice, this type of amyloidosis is not well characterized biochemically.

A murine model of accelerated senescence, consisting of SAM-P (accelerated senescence prone mice, P/1, P/2, P/3 and P/4 series) and SAM-R (accelerated senescence resistance mice, R/1, R/2 and R/3 series), which have normal ageing characteristics, was developed in our laboratory [3]. In these senescence accelerated mice, spontaneous age-associated amyloidosis is one of the most characteristic pathological findings [4]. A unique amyloid fibril protein termed 'AS_{SAM}' has been isolated from SAM-P/1. This protein has been shown to differ from the amyloid proteins from any other spontaneous or secondary murine amyloidosis in their biochemical and immunochemical properties [5,6].

Here we report the primary structure of AS_{SAM} and find high homology between AS_{SAM} and human apo A-II.

Abbreviations: CNBr, cyanogen bromide; SAM, senescence accelerated mouse; HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; PTH, phenylthiohydantoin

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2. MATERIALS AND METHODS

2.1. *Purification of $AS_{SAM}-1$ and $AS_{SAM}-2$*

AS_{SAM} was isolated from the water soluble fraction from the livers of SAM-P/1 mice and purified by gel chromatography through Sephadex G-100 as described [5]. Analysis of this material on DEAE-cellulose (DE52) chromatography with a linear gradient elution from 0.01 to 0.1 M Tris-HCl, pH 8.2, in 6 M urea gave two peaks, designated $AS_{SAM}-1$ (major component) and $AS_{SAM}-2$ (minor component). The proteins in each peak were further purified by reverse-phase HPLC on a Baker bond wide pore Butyl column (0.46 × 25 cm, C4, 330 Å). The column was equilibrated with 0.1% trifluoroacetic acid (TFA) and elution was performed by a linear gradient (0–60%) of 2-propanol/acetonitrile (7:3, v/v) in 0.1% TFA for 1 h at a flow rate of 1 ml/min.

2.2. *Removal and identification of amino-terminal pyrrolidone-carboxylic acid (PCA)*

Removal of the amino-terminal PCA from the protein was performed using calf liver pyroglutamyl peptidase (L-pyroglutamyl-peptide hydrolase, EC 3.4.19.3, Sigma) by the method of Podell and Abraham [7]. The PCA released was identified by reverse-phase HPLC according to the method described [8]. Authentic PCA, Ac-Glu and Ac-Gln were used for comparison.

2.3. *Peptide fragmentation and separation on reverse-phase HPLC*

$AS_{SAM}-1$ and $AS_{SAM}-2$ were separately digested with *Achromobacter lyticus* protease I (AP) at 37°C for 6 h in 10 mM Tris, pH 9.5 (enzyme/substrate = 1:100, mol/mol). Methionyl bonds in $AS_{SAM}-1,-2$ were cleaved with a 100-fold molar excess of CNBr to methionine residues in 70% formic acid at 37°C for 18 h. Digestion of $AS_{SAM}-1,-2$ with *Staphylococcus aureus* V8 protease (SP) was performed in 1.5% NH_4HCO_3 at 37°C for 18 h (enzyme/substrate = 1:100, mol/mol). Peptides were fractionated on a reverse-phase C-4 column as described above.

2.4. *Amino acid and sequence analyses*

Amino acid analysis was carried out with a Hitachi 835S amino acid analyzer. Proteins were hydrolyzed for 24 or 72 h in 6 N HCl containing 0.2% phenol at 110°C in vacuo. Peptides were hydrolyzed by the same method for 24 h. Automated Edman degradation was performed with a 470A protein sequencer (Applied Biosystem) using the standard program for sequencing [9]. PTH-amino acids were identified by reverse-phase HPLC as reported [10].

3. RESULTS AND DISCUSSION

$AS_{SAM}-1$ and $AS_{SAM}-2$ each exhibited a single band on polyacrylamide gel electrophoresis containing 8 M urea, and $AS_{SAM}-2$ has a slightly greater mobility than $AS_{SAM}-1$ (not shown).

Table 1 shows the amino acid composition of $AS_{SAM}-1$ and $AS_{SAM}-2$. Both proteins gave the same amino acid compositions and are rich in glutamic acid/glutamine and lack half-cystine and tryptophan. The values are compatible, within ex-

Table 1

Amino acid composition of $AS_{SAM}-1$ and $AS_{SAM}-2$

Amino acid	Residues/mol	
	$AS_{SAM}-1$	$AS_{SAM}-2$
Asp	5.0(5)[a]	5.2(5)
Thr[b]	5.9(6)	5.8(6)
Ser[b]	7.6(8)	7.6(8)
Glu	15.3(15)	15.2(15)
Pro	2.8(3)	2.5(3)
Gly	3.4(3)	3.2(3)
Ala	8.0(8)	8.4(8)
½Cys	0 (0)	0 (0)
Val[c]	2.9(3)	3.3(3)
Met	2.5(3)	2.6(3)
Ile[c]	1.0(1)	1.2(1)
Leu	6.9(7)	7.4(7)
Tyr	2.9(3)	2.9(3)
Phe	4.9(5)	4.7(5)
Lys	5.9(6)	5.5(6)
His	1.1(1)	1.0(1)
Trp	0 (0)	0 (0)
Arg	1.2(1)	1.4(1)
Total	(78)	(78)

[a] Numbers in parentheses are based on the amino acid sequence (fig.1)

[b] Values are those extrapolated to zero hour

[c] Values are those of 72 h hydrolysis

perimental error, with those calculated from the established amino acid sequence as shown later. When Edman degradation was performed on the intact $AS_{SAM}-1$, no PTH-amino acids were found and a blocked amino-terminus was suspected. The protein was then incubated with calf liver pyroglutamyl peptidase. As a result, the amino group of the second residue (Ala) from the N-terminus was exposed and the pyroglutamic acid released was identified by reverse-phase HPLC [8]. By subsequent automated Edman degradation with a gas-phase sequencer, the sequence of the first 27 amino-terminal residues of the protein from Ala[2] was determined.

To determine the complete amino acid sequence of $AS_{SAM}-1$, the protein was individually subjected to digestion with AP, SP, and chemical cleavage with CNBr. Nine peptides (AP1–AP8 and AP6-7) were isolated by digestion with AP the yield being 25–88%. Except for a peptide blocked at the amino-terminus (AP1), six peptides (AP2–AP6 and AP8) were completely and two (AP6-7 and AP7) partially sequenced. In this case, AP cleaved all lysyl bonds in the protein and partially the Arg[54]-Ser[55] bond (about 50%). To align the sequenced AP peptides and elucidate the ambiguous sequences in AP6-7 and AP7, $AS_{SAM}-1$ was fragmented with SP and by CNBr. Seven peptides (SP1–SP7) were obtained by the V8 protease digestion and four (CB1–CB4) by CNBr cleavage. The amino acid sequences of seven critical peptides (SP2–SP5, SP7, CB2 and CB4) were determined and the complete amino acid sequence of $AS_{SAM}-1$ was thus established as shown in fig.1. The protein consists of a single polypeptide chain of 78 amino acid residues with an M_r of 8721. The amino acid sequence of $AS_{SAM}-1$ differs from those of the known amyloid proteins [2,11–14]. Though heterogeneity of the amino-terminus has been often reported in the amyloid protein, $AS_{SAM}-1$ sequenced in the present experiment gave PCA as a sole N-terminus. The amino acid sequence of $AS_{SAM}-2$ was also determined by the same procedure (not shown). It is surprising that, in spite of the different electrophoretic mobility of $AS_{SAM}-1$ and $AS_{SAM}-2$, their amino acid sequences are identical. While we have no definite explanation for this reason, it seems likely that native $AS_{SAM}-2$ may undergo some minor but distinct modification of the tertiary structure, its electrophoretic behavior being affected in the absence of SDS.

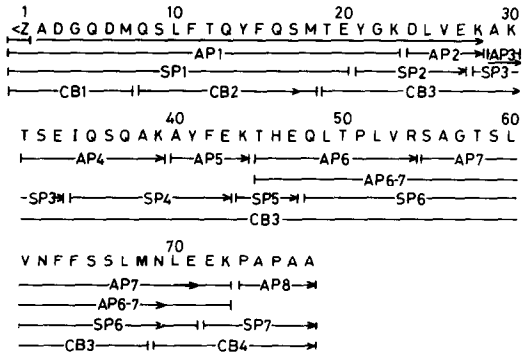

Fig.1. Amino acid sequence of $AS_{SAM}-1$. Residues identified by Edman degradation are shown by arrows. The solid line denotes the portions of the peptides whose amino acid compositions were determined but sequences were not analyzed. AP, SP and CB represent peptides obtained *Achromobacter lyticus* protease I, *Staphylococcus aureus* V8 protease digestion and cyanogen bromide degradation, respectively.

It should be noted that high homology is present between the primary structures of $AS_{SAM}-1$ or $AS_{SAM}-2$ and human apo A-II (fig.2). The two structures are identical in 43 of 78 residues (55%). It has been shown that apo A-II is a major constituent of HDL and that a putative precursor of AS_{SAM}, termed apo SAS_{SAM} (serum AS_{SAM}-related antigenic substance), is associated with circulating HDL [16]. The results obtained in this study suggest that apo SAS_{SAM} is the murine apo A-II. Although the metabolism and the biosynthesis of human apo A-II were examined [17,18], little is known about its function. Therefore we believe that the elucidation of this amyloidogenesis should aid in determining the function of human apo A-II.

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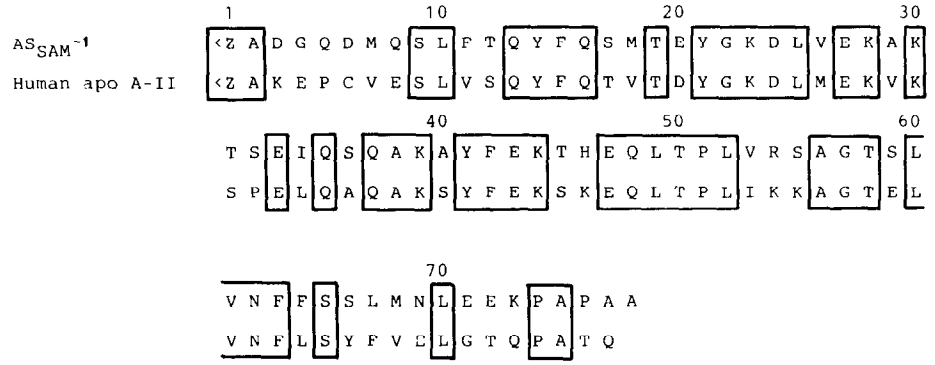

Fig.2. Comparison of the amino acid sequence between $AS_{SAM}-1$ and human apo A-II(15). Identical residues are boxed.

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