The single proline-glutamine substitution at position 5 enhances the potency of amyloid fibril formation of murine apo A-II

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The primary structure of murine apolipoprotein A-II (apo A-II) has been determined. Apo A-II consists of a single polypeptide chain of 78 amino acid residues, of which the amino-terminus is pyrrolidone carboxylic acid. Except for residues 5 and 38, the amino acid sequence is identical to that of murine senile amyloid protein (AS_{SAM}), which has a common antigenicity with apo A-II. Substitution of glutamine (AS_{SAM}) for proline (apo A-II) at position 5 is distinct and may possibly be related to murine senile amyloidogenesis.

Primary structure *Apolipoprotein A-II* *Murine senile amyloid protein* *Proline-glutamine substitution*

1. INTRODUCTION

Apolipoproteins are important structural constituents of lipoprotein particles and are involved in lipoprotein synthesis, secretion, processing and catabolism [1]. Apolipoprotein (apo) A-II is the second most abundant protein constituent of human and murine high density lipoproteins [2]. Although both apo A-II and apo A-I may be potential predictors of cardiovascular disease [3,4], the physiological role of apo A-II in lipoprotein metabolism has not been clearly defined.

Human apo A-II is a dimeric protein composed of two identical peptide chains of 77 amino acid residues. The peptide chains have pyrrolidone carboxylic acid (PCA) at the amino-termini and are linked by a disulfide bridge at Cys 6 [5]. In mice and other species, apo A-II is a monomer [6].

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Abbreviations: CNBr, cyanogen bromide; SAM, senescence accelerated mouse; HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; AP, *Achromobacter* protease I

The mouse is a most useful animal model for studying normal and pathological processes and genetic variations in lipoprotein metabolism. To date, the amino acid sequence of only a few murine apolipoproteins have been determined. We recently determined the primary structure of murine senile amyloid fibril protein (AS_{SAM}) from the murine model for accelerated senescence [7] and found that apo SAS_{SAM}, which is a serum precursor of AS_{SAM}, is murine apo A-II [7,8].

Now we report the purification of apo A-II from murine serum and its determined primary structure. Sequence comparisons revealed one complete and one partial substitution of amino acid residues between apo A-II and AS_{SAM}.

2. MATERIALS AND METHODS

2.1. *Purification of apo A-II*

Murine serum apoproteins in HDL fractions (apo HDL) were obtained from 3-month-old male ICR mice and apo HDL were fractionated on Sephadex G-200, as described [9]. The proteins at the third peak were chromatographed on DEAE-

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cellulose (DE-52) with a linear gradient from 0.01 to 0.1 M Tris-HCl in 6 M urea at pH 8.2. Two proteins (apo A-II-1 and apo A-II-2) in separate peaks reacted with anti-AS_{SAM} serum and each protein was further purified by reverse-phase HPLC on a Baker bond wide pore butyl column (0.46 × 25 cm, C4, 330 Å). Elution was performed with a linear gradient from 0 to 60%, 2-propanol/acetonitrile (7:3, v/v) in 0.1% trifluoroacetric acid (TFA) for 1 h at a flow rate of 1 ml per min.

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

The amino-terminal PCA was liberated from apo A-II-1 and apo A-II-2 using calf liver pyroglutamyl peptidase (L-pyroglutamyl-peptide hydrolase, EC 3.4.19.34, Sigma) and identified as described [7].

2.3. *Peptide fragmentation and separation*

Fragmentation of apo A-II-1 or apo A-II-2 with AP, *Staphylococcus aureus* V8 protease (SP) and CNBr were performed as reported [7]. Peptides were fractionated on a reverse-phase C-4 as described above.

2.4. *Amino acid and sequence analysis*

Amino acid analysis, automated Edman degradation and identifiction of PTH amino acids by reverse-phase HPLC were carried out as described [7].

3. RESULTS AND DISCUSSION

Apo A-II-1, the major component, and apo A-II-2, the minor one, were eluted from a DEAE-cellulose column at positions corresponding to apo SAS_{SAM}-1 and apo SAS_{SAM}-2, respectively. These components have a common antigenicity with AS_{SAM} in serum [8]. On 8 M urea polyacrylamide gel electrophoresis, each of the purified proteins gave a single band and apo A-II-2 had a slightly greater mobility than apo A-II-1 (not shown).

In table 1, the amino acid composition of apo A-II-1 is presented together with that of AS_{SAM}-1. Both proteins possessed the same composition, except for glutamic acid, proline, alanine and valine. Apo A-II-1 had no PTH-amino acid at the first cycle of Edman degradation. The second residue from the N-terminus was exposed when the apo A-II-1 was incubated with calf liver pyroglutamyl peptidase. Subsequently, the first 30 amino acids were determined by automated Edman degradation. In the N-terminal 30 residues, the sole difference between apo A-II-1 and AS_{SAM}-1 was observed at residue 5: glutamine in AS_{SAM}-1 was replaced by proline in apo A-II-1. Pyroglutamic acid liberated from apo A-II was identified by reverse-phase HPLC and amino acid analysis as described [7] (not shown).

Apo A-II-1 was digested with AP and the resulting AP peptides were separated by reverse-phase HPLC (fig.1). The amino acid composition of each peptide was determined and then sequenced. Comparison of the peptide map of apo A-II-1 with that of AS_{SAM}-1 showed critical differences in a few peaks. First, peptide AP1 was

Table 1.
Amino acid composition of apo A-II-1 and $AS_{SAM}-1$

Amino acid	Apo A-II-1 (residues/mol)	$AS_{SAM}-1$[a]
Asp	5.4 (5)[b]	5
Thr[c]	5.7 (6)	6
Ser[c]	7.7 (8)	8
Glu	14.0 (14)	15
Pro	3.7 (4)	3
Gly	3.3 (3)	3
Ala	7.3 (7/8)[d]	8
½Cys	0 (0)	0
Val[e]	3.6 (4/3)[d]	3
Met	2.5 (3)	3
Ile[e]	1.0 (1)	1
Leu	7.2 (7)	7
Tyr	3.0 (3)	3
Phe	4.9 (5)	5
Lys	5.6 (6)	6
His	1.0 (1)	1
Trp	0 (0)	0
Arg	1.1 (1)	1
Total	(78)	(78)

[a] Data from [7]
[b] Numbers in parentheses are based on amino acid sequence (fig.2)
[c] Values are those extrapolated to 0 h
[d] Due to microheterogeneity at position 38. The ratio of Ala to Val at this position is 1:2 based on these numerical values
[e] Values for 72 h hydrolyzate

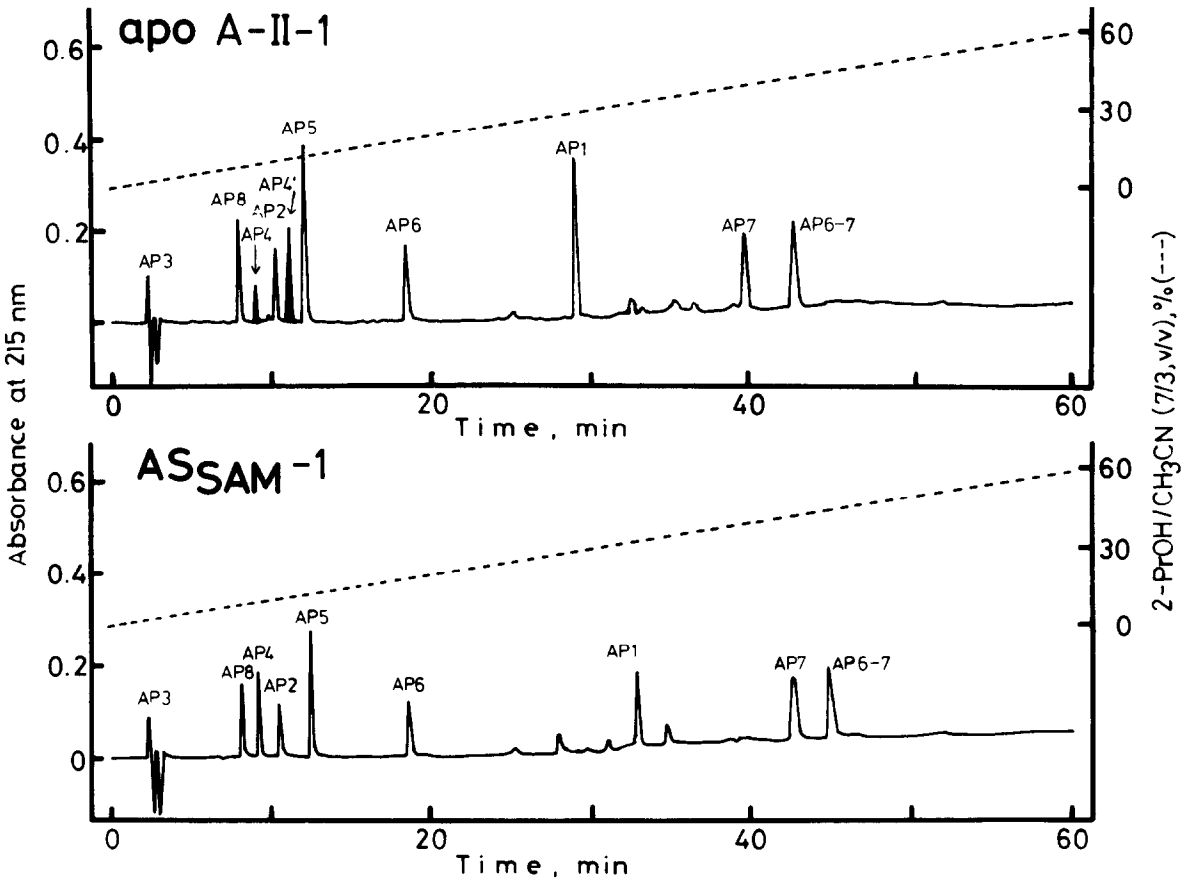

Fig.1. The elution profile obtained by digestion of apo A-II-1 and $AS_{SAM}-1$ with *Achromobacter* protease I.

eluted at a different position, as expected from the finding that apo A-II-1 contains Pro instead of Gln 5 in AS_{SAM}-1. One proline and five glutamic acids were consistently determined by amino acid analysis of peptide AP 1. Secondly, AP4′ was found only in the case of apo A-II-1. The amino acid composition was the same as that of AP4, except for alanine and valine. The molar ratio of AP4 and AP4′ was 1:2. Sequence analysis revealed that AP4′ differs from AP4 only at the residue penultimate to the C-terminal lysine. The residue is valine in the former peptide and alanine in the latter. Thirdly, the elution of AP7 and AP6-7 of apo A-II-1 was more rapid than that of AS_{SAM}-1. However, the same sequences were determined for their counterparts of AS_{SAM}-1. While we have no exact explanation for the difference in the elution behavior of those peptides, the oxidation of methionine during peptide isolation has to be considered. CNBr cleavage and V8 protease digestion were also performed for apo A-II-1 and several peptides thus formed were used to confirm the complete sequence.

The complete amino acid sequence of murine apo A-II-1 is shown in fig.2. Murine apo A-II-1 consists of a single polypeptide chain of 78 amino acid residues with a molecular mass of 8721 Da. The amino-terminus is PCA, that is, the same as human apo A-II protein. Sequence homology is high between murine and human apo A-II. 55% of the amino acids in the two proteins are identical and 34% of the remaining residues represents conservative substitutions [5]. Like the case of AS_{SAM}, the amino acid sequences of two components of apo A-II (apo A-II-1 and -2) are identical in spite of the different electrophoretic mobility. At present we cannot clarify what kind of modification occurs in native apo A-II-2, so further studies are

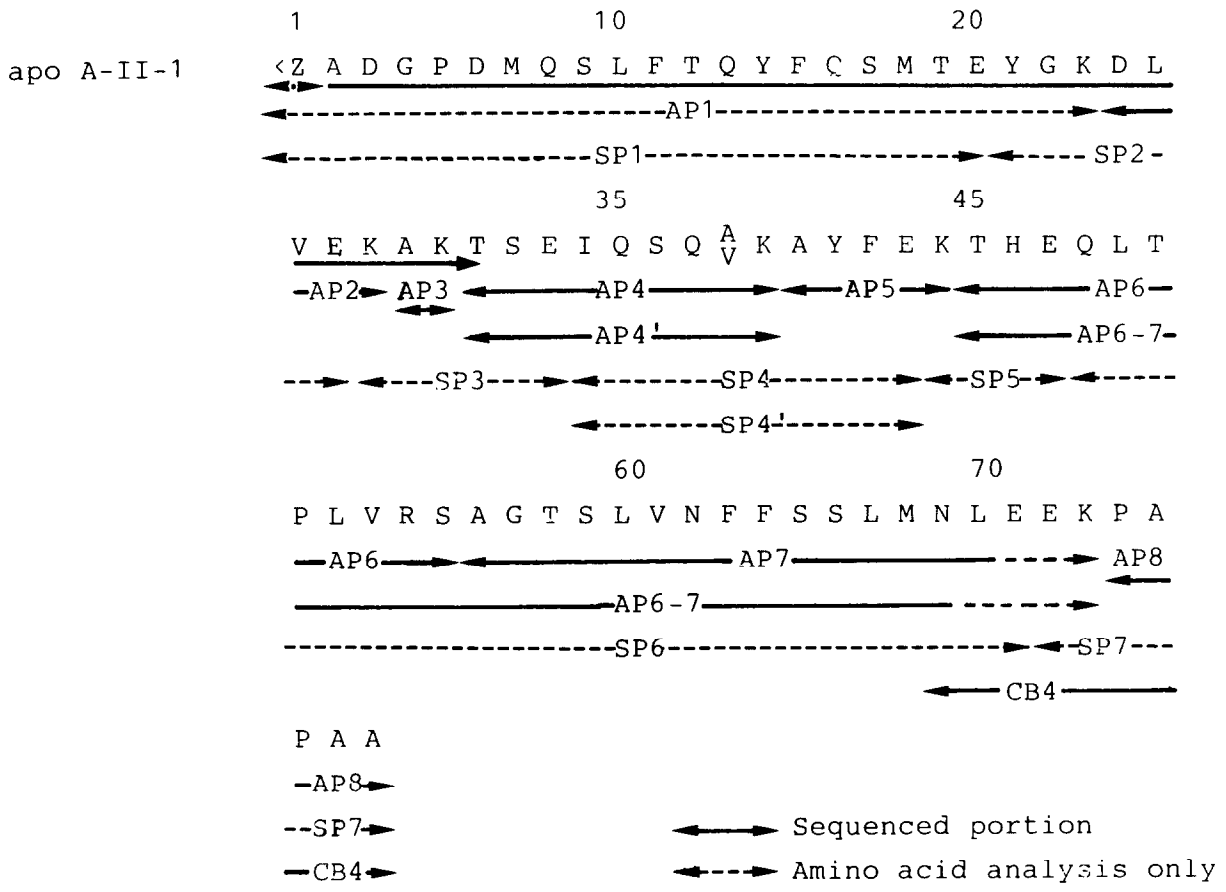

Fig.2. Complete amino acid sequence of apo A-II-1 showing the positions of peptides for which sequence data (solid lines) or composition data (broken lines) were obtained. The arrow (→) represents direct sequencing of the N^{α}-deblocked protein. AP, SP, and CB represent peptides obtained *Achromobacter* protease I, *Staphylococcus aureus* V8 protease digestion and CNBr degradation, respectively.

necessary to elucidate this modification.

It is remarkable that, at position 5, glutamine in AS_{SAM}-1 is substituted for proline in apo A-II-1, since the former amino acid is dinstinct from the latter, in terms of conformational potency (fig.3). The extensive deposition of AS_{SAM} protein which occurred extracellularly with advancing age in the senescence accelerated prone mice (SAM-P) [10] may relate to this particular amino acid substitution. Although a number of mutants of human apolipoproteins has been identified [1,11], a mutation in the apo-II molecule has apparently not been reported. The Pro→Gln substitution described here seems to be the first report of a mutation in apo A-II. In the case of human familial amyloid polyneuropathy (FAP), several types of amino acid substitutions in serum prealbumin molecules seem to closely correlate with amyloid fibril formation [12–14]. Murine apo A-II obtained from random bred ICR mice does have a sequence heterogeneity at position 38, but replacement of valine for alanine at this position is unlikely to produce a significant change in the structure of the protein or have an important effect on apo A-II metabolism.

The mouse has been a species rarely used for lipoprotein research. Our finding of a remarkable variation in apo A-II molecules in this species should enhance the usefulness of the mouse as a model system for examination of the function of apo A-II. The elucidation of this mutation should aid in determining the pathogenesis of amyloidosis.

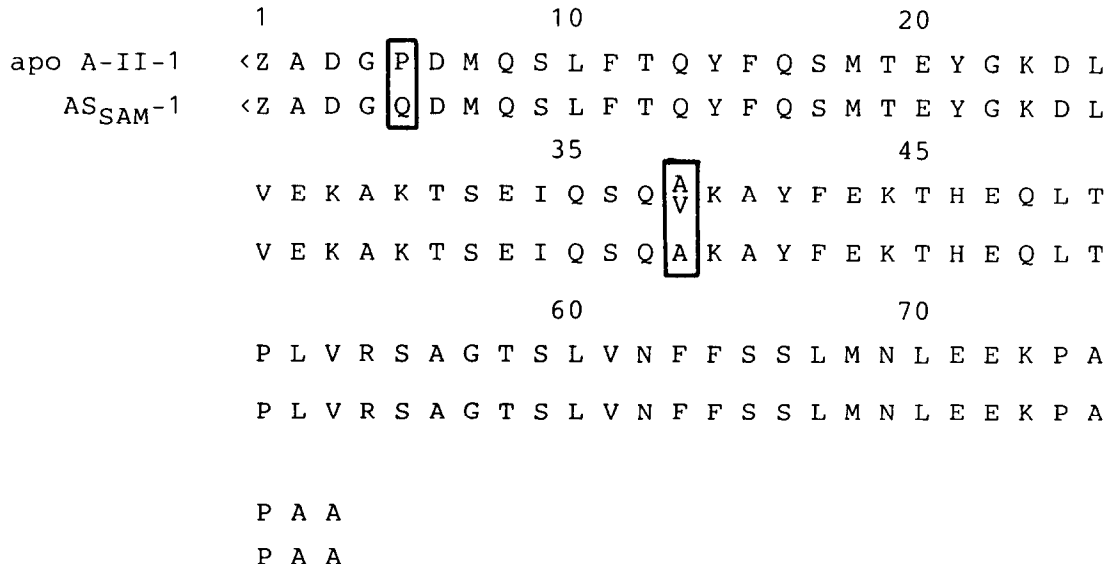

Fig.3. Comparison of the amino acid sequence between apo A-II-1 and AS$_{SAM}$ – 1 [7]. Amino acid substitutions are boxed.

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