Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2915–2919, May 1985
Immunology

Complete primary structures of two major murine serum amyloid A proteins deduced from cDNA sequences

(amyloidosis/cDNA cloning/sequence/acute-phase protein)

KEN-ICHI YAMAMOTO AND SHUNSUKE MIGITA

Department of Molecular Immunology, Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

Communicated by Frank W. Putnam, December 26, 1984

ABSTRACT **cDNA clones encoding two major mouse serum amyloid A proteins, SAA1 and SAA2, were isolated from a liver cDNA library of the lipopolysaccharide-stimulated BALB/c mouse, and their nucleotide sequences were determined. The insert of the SAA2 cDNA clone contained 607 nucleotides with a 5′ untranslated region of 36 nucleotides, a signal peptide region corresponding to 19 amino acids, a mature protein region corresponding to 103 amino acids, and a 3′ untranslated region of 202 nucleotides. The SAA1 cDNA insert contained 549 nucleotides specifying a part of a signal peptide region, a mature protein region, and a 3′ untranslated region. A comparision of the nucleotide and deduced amino acid sequences of SAA1 cDNA with that of SAA2 cDNA showed a high degree of homology: 95% nucleotide sequence homology in the coding region (91% amino acid sequence homology) and 90% homology in the 3′ untranslated region. One of nine amino acid differences between SAA1 and SAA2 predicted from the cDNA sequences was located in a putative proteolytic cleavage site for amyloid A protein formation: SAA2 had the Thr-Met sequence in this site, while SAA1 had the Thr-Ile sequence. This suggests that SAA1, which does not deposit as amyloid A protein, is also potentially susceptible to putative proteolytic enzymes. In addition, as compared with mouse SAA2, human SAA1, monkey and mink amyloid A protein, mouse SAA1 had two unique substitutions, which may play a role in differential deposition of mouse SAA isotypes in amyloid tissues.**

A type of amyloidosis associated with various chronic inflammatory diseases is caused by deposition in tissues of a fibrillar protein called amyloid A protein (1). Amyloid A proteins isolated from tissues of several patients are similar to each other in molecular size (8.6 kDa) and in amino acid sequences (2, 3). Several recent studies have also identified a 12-kDa protein in sera that is antigenically and chemically related to amyloid A protein (4–6). This protein termed serum amyloid A protein (SAA) is present in sera as a trace component of high density lipoproteins (HDL) (7). However, SAA is increased several hundredfold in a number of disease states (8), and increased SAA levels are believed to predispose to the accumulation of the partial proteolytic products of SAA during amyloidosis.

In several mammalian species including human, more than one form of SAA has been identified (6, 9–12). In mouse, two electrophoretically distinct SAA isotypes, SAA1 (12.6 kDa; pI, 6.35) and SAA2 (11.8 kDa; pI, 6.20), have been described: they are present in nearly equal amounts and constitute as much as 20% of the total mouse HDL protein content during acute inflammation (12). Benditt and co-workers have recently determined partial amino-terminal amino acid sequences of SAA1 and SAA2 from several mouse strains. A comparison of these sequences with those of several different mouse amyloid A protein has shown that mouse amyloid A protein contains only a single type of amino-terminal amino acid sequence, which is identical with that of SAA2, indicating that amyloid A protein is derived predominantly from SAA2 (13). However, it is not known what structural differences are responsible for selective deposition of SAA2 in amyloid tissues. In the present study, we have isolated two cDNA clones corresponding to mouse SAA1 and SAA2 mRNA and have determined the nucleotide sequences. A comparison of the amino acid sequences deduced from the cDNA sequences reveals amino acid differences in nine positions between SAA1 and SAA2. A possible significance of these amino acid substitutions to amyloidogenesis is discussed.

MATERIALS AND METHODS

Restriction endonucleases were obtained from Bethesda Research Laboratories, New England Biolabs, and Takara Shuzo (Japan). T4 polynucleotide kinase was from Takara Shuzo. M13 cloning and sequencing kits, [α-^{32}P]dATP, and [γ-^{32}P]dATP were purchased from Amersham. Wheat germ translation kits and [^{35}S]methionine were obtained from Bethesda Research Laboratories and from New England Nuclear, respectively. Oligo(dT)-cellulose (type 2) was obtained from Collaborative Research. *Escherichia coli* lipopolysaccharide (LPS) was purchased from Difco Laboratories.

Preparation and Translation of mRNA. BALB/c mice were given an intraperitoneal injection of 50 μg of LPS 24 hr before RNA preparation. Total liver RNA was prepared essentially as described by Chirgwin *et al.* (14). Poly(A)$^+$ RNA was selected by oligo(dT) cellulose column chromatography (15). mRNA was translated in a wheat germ cell-free translation system and translation products were analyzed by $NaDodSO_4$/polyacrylamide slab gel electrophoresis (16).

cDNA Cloning. A cDNA library was constructed according to the method described by Okayama and Berg (17), except that a *Hind*III/*Bam*HI fragment of pBR322 ($\approx$340 base pairs long) was used as a linker. Using 0.6 pmol of vector primer and 5 μg of poly(A)$^+$ RNA from LPS-stimulated mouse, the cDNA library containing $\approx 1 \times 10^5$ recombinants with the average insert length of 1000 base pairs was obtained.

The recombinants were screened by colony hybridization (18) with ^{32}P-labeled mRNA from LPS-stimulated mouse. The mRNA was labeled with [γ-^{32}P]dATP by T4 polynucleotide kinase after mild alkaline hydrolysis. Unlabeled mRNA from normal mouse was mixed with the probe at a ratio of 200:1 to compete with uninduced mRNA present in the ^{32}P-labeled mRNA preparation. Thus, hybridization of radioactive RNA was expected to occur preferentially to colonies containing LPS-induced sequences (19). Plasmid DNA from positive clones was further analyzed by dif-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SAA1 and SAA2, serum amyloid A proteins 1 and 2; HDL, high density lipoprotein; LPS, lipopolysaccharide.

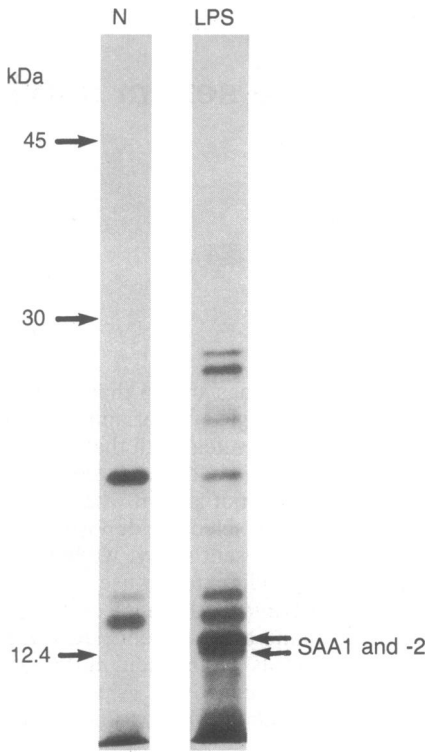

FIG. 1. NaDodSO$_4$/polyacrylamide gel electrophoretic analysis of cell-free translation products of liver mRNA from normal (N) and LPS-stimulated (LPS) mice. Poly(A)$^+$ RNA was translated in a wheat germ system, and translation products were analyzed under reducing conditions on 15% slab gels.

ferential dot blot hybridization (20) to ^{32}P-labeled normal and LPS-induced mRNA.

Other Procedures. Plasmid DNA was isolated from bacteria by the cleared-lysate method (21). DNA sequencing was carried out by the chain-termination method (22) after subcloning of restriction endonuclease fragments into M13 mp10 and mp11 phage vectors (23).

RESULTS AND DISCUSSION

Poly(A)$^+$ RNA from the liver of BALB/c mice 24 hr after LPS administration was used to construct a cDNA library. As has been reported (24), the levels of SAA mRNA increase more than 100-fold after LPS administration. The results shown in Fig. 1 also indicate that translation of mRNA from LPS-stimulated mice produced greater quantities of two polypeptides of 12.8 and 12.5 kDa, which presumably correspond to the translation products of SAA1 and SAA2 mRNA. Approximately 2000 recombinants from the cDNA library constructed as described were screened by colony hybridization with ^{32}P-labeled mRNA from LPS-stimulated mouse in the presence of an excess of unlabeled normal mRNA. Positive clones were further characterized by differential hybridization with ^{32}P-labeled normal and LPS-induced mRNA. Ten clones were strongly reactive with the LPS-induced mRNA but undetectably with the normal mRNA, and 11 clones showed greater hybridization to the LPS-induced mRNA. The rest of the clones did not show a preferential hybridization with the LPS-induced mRNA. Restriction enzyme analysis showed that the cDNA inserts of the former 10 clones contained common *Sma* I, *Eco*RI, and *Ava* I sites. However, while 4 of these 10 clones shared a unique *Stu* I site, the other 6 clones had an additional *Ava* I site as well as unique *Acc* I and *Xho* I sites. The restriction maps of the cDNA inserts of the pbSAA1.14 and pbSAA2.63 clones that contained the longest inserts among each group are shown in Fig. 2. The results of restriction-site analysis, thus, suggest that these two types of cDNA inserts were derived from two similar but distinct mRNAs. The two clones, pbSAA1.14 and pbSAA2.63, were subjected to nucleotide-sequence analysis according to the strategy indicated in Fig. 2.

The entire nucleotide sequences of the cDNA inserts of the pbSAA1.14 and pbSAA2.63 clones, excluding the poly(A) tract and poly(dG) tail, are shown in Fig. 3. The 607-base-pair cDNA sequence of pbSAA2.63 contains a single open reading frame, beginning with the ATG codon at nucleotides 37–39, followed 122 codons later by a TGA termination triplet. The 26 amino acids coded for by nucleotides 94–171 are identical with the reported amino-terminal sequence of BALB/c mouse SAA2 (13). The 19 codons preceding nucleotide 94, therefore, presumably encode a signal peptide characteristic for secreted proteins (26). The pbSAA1.14 cDNA sequence contains 549 nucleotides and begins with the codon specifying the 9th residue in the signal peptide: the 26 amino acids specified by nucleotides 34–111 and the 38 amino acids specified by nucleotides 34–147 exactly agree with the amino-terminal sequences of BALB/c mouse SAA1 and

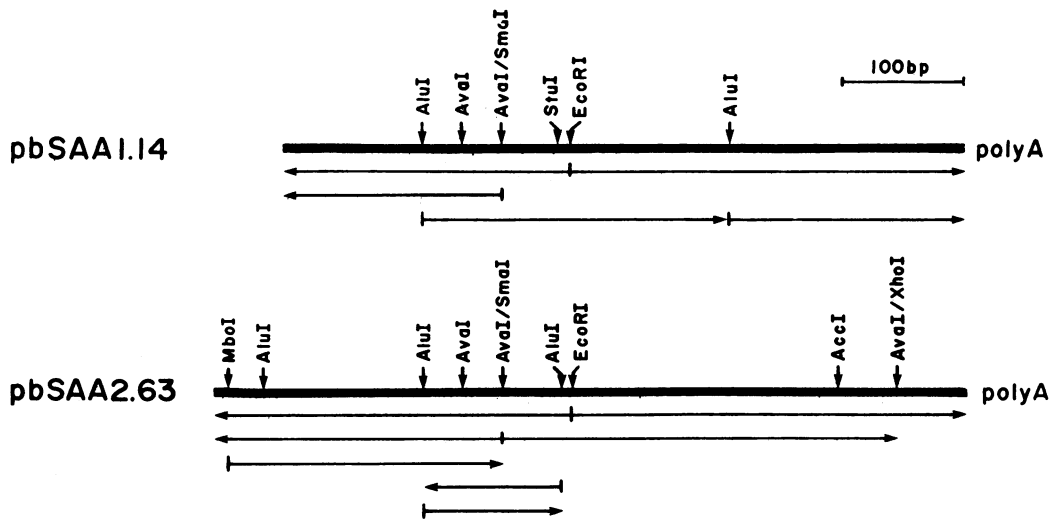

FIG. 2. Partial restriction maps and sequencing strategy for mouse SAA1 (pbSAA1.14) and SAA2 (pbSAA2.63) cDNA clones. Horizontal arrows indicate direction and extent of each sequence analysis. bp, Base pairs.

```
SAA2  ATA GAC CAC CAG ATC TGC CCA GGA GAC ACC AGC AGG ATG AAG CTA CTC ACC AGC CTG GTC  60
                                                      M   K   L   L   T   S   L   V
pRS48                                                 ATG AAG CCT TCC ATT GCC ATC ATT
                                                      M   K   P   S   I   A   I   I
                                                     -19

SAA1  TTC TGC TCC CTG CTC CTG GGA GTC TGC CAT GGA GGG TTT TTT TCA TTT GTT CAC GAG GCT 120
       F   C   S   L   L   L   G   V   C   H   G   G   F   F   S   F   V   H   E   A
SAA2  TTC TGC TCC CTG CTC CTG GGA GTC TGC CAT GGA GGG TTT TTT TCA TTT ATT GGG GAG GCT
       F   C   S   L   L   L   G   V   C   H   G   G   F   F   S   F   I   G   E   A
pRS48 CTT TGC ATC TTG ATC CTG GGA GTT GAC AGC CAA AGA TGG GTC CAG TTC ATG AAA GAA GCT
       L   C   I   L   I   L   G   V   D   S   Q   R   W   V   Q   F   M   K   E   A
                                                    -1  +1

SAA1  TTC CAA GGG GCT GGG GAC ATG TGG CGA GCC TAC ACT GAC ATG AAG GAA GCT AAC TGG AAA 180
       F   Q   G   A   G   D   M   W   R   A   Y   T   D   M   K   E   A   N   W   K
SAA2  TTC CAA GGG GCT GGA GAC ATG TGG CGA GCC TAC ACT GAC ATG AAG GAA GCT GGC TGG AAA
       F   Q   G   A   G   D   M   W   R   A   Y   T   D   M   K   E   A   G   W   K
pRS48 GGT CAA GGG TCT AGA GAC ATG TGG CGA GCC TAC TCT GAC ATG AAG AAA GCT AAC TGG AAA
       G   Q   G   S   R   D   M   W   R   A   Y   S   D   M   K   K   A   N   W   K
       10                                          20

SAA1  AAC TCA GAC AAA TAC TTC CAT GCT CGG GGG AAC TAT GAT GCT GCT CAA AGG GGT CCC GGG 240
       N   S   D   K   Y   F   H   A   R   G   N   Y   D   A   A   Q   R   G   P   G
SAA2  GAT GGA GAC AAA TAC TTC CAT GCT CGG GGG AAC TAT GAT GCT GCC CAA AGG GGT CCC GGG
       D   G   D   K   Y   F   H   A   R   G   N   Y   D   A   A   Q   R   G   P   G
pRS48 AAC TCA GAC AAA TAC TTC CAT GCT CGG GGG AAC TAT GAT GCT GCC CGG AGG GGT CCC GGG
       N   S   D   K   Y   F   H   A   R   G   N   Y   D   A   A   R   R   G   P   G
       30                                          40

SAA1  GGA GTC TGG GCT GCT GAG AAA ATC AGT GAT GGA AGA GAG GCC TTT CAG GAA TTC TTC GGC 300
       G   V   W   A   A   E   K   I   S   D   G   R   E   A   F   Q   E   F   F   G
SAA2  GGA GTC TGG GCT GCT GAG AAA ATC AGT GAT GCA AGA GAG AGC TTT CAG GAA TTC TTC GGC
       G   V   W   A   A   E   K   I   S   D   A   R   E   S   F   Q   E   F   F   G
pRS48 GGA GCC TGG GCT GCT AAA GTC ATC AGC GAT GCC AGA GAG GCT GTT CAG AAG TTC ACG GGA
       G   A   W   A   A   K   V   I   S   D   A   R   E   A   V   Q   K   F   T   G
       50                                          60

SAA1  AGA GGA CAT GAG GAC ACC ATT GCT GAC CAG GAA GCC AAC AGA CAT GGC CGC AGT GGC AAA 360
       R   G   H   E   D   T   I   A   D   Q   E   A   N   R   H   G   R   S   G   K
SAA2  AGA GGA CAC GAG GAC ACC ATG GCT GAC CAG GAA GCC AAC AGA CAT GGC CGC AGT GGC AAA
       R   G   H   E   D   T   M   A   D   Q   E   A   N   R   H   G   R   S   G   K
pRS48 CAT GGA GCA GAG GAC TCA AGA GCT GAC CAG TTT GCC AAT GAG TGG GGC CGG AGT GGC AAA
       H   G   A   E   D   S   R   A   D   Q   F   A   N   E   W   G   R   S   G   K
       70                                          80

SAA1  GAC CCC AAT TAC TAC AGA CCT CCT GGA CTG CCT GAC AAA TAC TGA GCG TCC TCC TAT TAG 420
       D   P   N   Y   Y   R   P   P   G   L   P   D   K   Y   *
SAA2  GAC CCC AAT TAC TAC AGA CCT CCT GGA CTG CCT GCC AAA TAC TGA GAG TCC TCC TAT TAG
       D   P   N   Y   Y   R   P   P   G   L   P   A   K   Y   *
pRS48 GAC CCC AAC CAC TTC CGA CCT GCT GGC CTG CCT AAA AGA TAC TGA GTT TTC TCT TCC TGT
       D   P   N   H   F   R   P   A   G   L   P   K   R   Y   *
       90                                          100

SAA1  CTC AGT AGG TTG TGC TGG GGG CCT GAG GGT GGG GTC TGG GCT TCT TCC TAC CTA GGA ACA 480
SAA2  TTC AGA AGG CTG TGT TGG GGT CCT GAG GGT GGG GTC TGG GCT --- TCC TAT CTA GGA ACA
pRS48 TGT TCC CAG TCA TGC TGC CCC CCG AGA AGA GGA GCA ACT ACT GGG TTG AGA TAT TTT CTA

SAA1  CTG AAG ATG CTC TCT GGG GAA ACA TTG TAT ATC TCT CAT GTG TGT ATC CCA CAA GGT TTC 540
SAA2  CTG AAG ATG CTC TCT GGG GCA ACA TAG TAT ACC TCT CAT GTG TGT ATC CCA CAA GGT TTC
pRS48 AAA TCT GGA TCC CTA AAC ATC CCA ATG TGC TGA ATA AAT ACT TGT GAA ATG C

SAA1  AGA ACT GAG TTA CTC TTG CAG TAG TAA CTG CTT GAG GAG GAG AGG GTA ATA AAC AGA AAC 600
SAA2  AGA ATG GAG TTA CTC GAG CAG TAG TAA CTG CTT GAG GAG GAG AGG GTA ATA AAC AGG AAC

SAA1  TTG GAA GTG
SAA2  TTG GAA GTG G
```

FIG. 3. Nucleotide sequences and deduced amino acid sequences for mouse SAA1 (pbSAA1.14) and SAA2 (pbSAA2.63) cDNA clones. For comparison, the nucleotide and deduced amino acid sequences of the SAA cDNA clone (pRS48) isolated from the cDNA library of Swiss mice (25) are also shown. The deduced amino acids (designated by standard one-letter abbreviations) are numbered sequentially from the known amino terminus of the mature SAA (the amino acids of the signal peptide are numbered -19 to -1). Nucleotide and amino acid substitutions are indicated by underlining. Dashes indicate deletions introduced in the SAA2 sequence to maximize homology in the 3′ untranslated region. The hexanucleotide sequence A-A-T-A-A-A at the 3′ end is underlined.

CBA/J mouse SAA1, respectively (13). A comparison of the nucleotide sequences of the SAA1 and SAA2 cDNAs shows a high degree of homology: 19 nucleotide substitutions and no deletion/insertion in the coding region (95% homology) and 17 substitutions and 3 insertions/deletions in the 3′ untranslated region (90% homology). Of the 19 nucleotide substitutions, all of which were located in the coding region for a mature protein, 15 nucleotide substitutions resulted in 9 amino acid changes (91% amino acid sequence homology).

For comparison, the nucleotide and derived amino acid sequences of the SAA cDNA clone (pRS48) isolated from the liver cDNA library of LPS-stimulated Swiss mouse (25) are shown in Fig. 3. This SAA cDNA sequence shows some divergence from the SAA1 and SAA2 cDNA sequences. The coding region of the pRS48 sequence shows 75% and 72% nucleotide sequence homologies with those of the SAA1 and

SAA2 sequences, respectively. There is no apparent homology in the 3′ untranslated region. Furthermore, there is only a 70% homology in the predicted amino acid sequences, excluding a signal peptide region between SAA1 and pRS48-type SAA, and a 67% homology between pRS48-type SAA and SAA2. The signal peptide region of pRS48-type SAA shows more divergence: only 7 of 19 residues are identical between pRS48-type SAA and SAA2 (37% homology). The divergence of the pRS48 sequence from the SAA1 and SAA2 sequences is unlikely to represent allelic variations. First, no allelic variations were observed between BALB/c, CBA/ksJ, and CBA/J mice in the amino-terminal 26 residues of SAA1 and SAA2 (13). Second, although the pRS48 clone was originally isolated from the cDNA library of Swiss mice, the genomic clone encoding the sequence identical with that of the pRS48 clone has been recently isolated from the genomic library of 3T3 fibroblast cell line of BALB/c origin (25). These results indicate that at least three distinct genes encode SAA in BALB/c mice. However, we have not yet been successful in isolating SAA cDNA clones of the pRS48 type. Thus, although there may be multiple genes encoding mouse SAA, it remains unclear that all SAA genes are expressed.

A comparison (Fig. 4) of the deduced amino acid sequences of mouse SAA1, SAA2, and pRS48-type SAA with the amino acid sequences of human SAA1 (27), monkey amyloid A protein (28), mink amyloid A protein (29), and duck amyloid A protein (30) reveals several interesting features. First, these seven different proteins show a striking degree of conservation at residues 15–28, 32–53, and 76–100. In particular, residues 32–53 are almost identical except for two conservative substitutions in these proteins. Second, most of the substitutions converge on a few specified regions. The amino-terminal region (residues 1–14) shows the highest degree of divergence. In addition, human SAA1, monkey amyloid A protein, and duck amyloid A protein have additional residues at the amino terminus as compared with mouse SAA. The region preceding a putative proteolytic cleavage site (residues 63–75) also shows some divergence. It is interesting to note that computer analysis predicts that the two segments (residues 1–23 and 49–73) containing the diverged regions (residues 1–14 and 63–75) form an α-helix and probably are involved in interaction with lipids in lipoproteins (31, 32), leaving the conserved regions (residues 32–50 and 76–100) with unspecified functions.

Hoffman *et al.* (13) have recently shown that amyloid A proteins from several different mouse strains have amino-terminal identity only with SAA2, indicating that amyloid A protein is derived from SAA2. They proposed several possible mechanisms for this phenomenon. First, differential gene expression during chronic inflammation may result in the preferential synthesis of SAA2, although mRNAs of both SAA1 and SAA2 were induced in a similar degree during acute inflammation, as shown in the present study as well as by other investigators (12). Alternatively, structural differences between mouse SAA1 and SAA2 may play a role in selective deposition or retention of SAA2. As shown in Fig. 3, the predicted amino acid sequences of mouse SAA1 and SAA2 differ at nine positions, and most of these substitutions are relatively conservative. However, while mouse SAA1 contains glycine at position 60, mouse SAA2, as well as the other five proteins shown in Fig. 4, all contain alanine at that position. Also, at position 7, mouse SAA1 contains histidine, but mouse SAA2, human SAA1, monkey amyloid A protein, and mink amyloid A protein contain glycine. Another substitution is located at a putative proteolytic cleavage site (residues 75–76). It has been postulated that human SAA is cleaved at the Ser-76–Leu-77 bond (75 and 76 in Fig. 4), giving rise to the 76-residue amyloid A protein. From the size of

FIG. 4. Comparison of deduced amino acid sequences of mouse SAA1, SAA2, and pRS48-type SAA with the amino acid sequences of human SAA1, monkey amyloid A protein, mink amyloid A protein, and duck amyloid A protein. The numbering system of the mouse SAA sequence is used. The amino terminus of mouse SAA corresponds to position 2 in human and monkey and to position 6 in duck. Residues that are identical at each position with the mouse SAA1 sequence are represented by dashes. Arrow indicates a putative proteolytic cleavage site for amyloid A protein formation. PCA, pyrrolidone carboxylic acid; AA, amyloid A protein. Amino acids abbreviated as in Fig. 3.

mouse amyloid A protein (8.6 kDa) (13), it is also likely that the Thr-75–Met-76 bond in mouse SAA2 is cleaved during amyloidosis. Mouse SAA1 has threonine and isoleucine in these positions and is also potentially susceptible to putative proteolytic enzymes, although it does not deposit in tissues as amyloid A protein (13). Thus, mouse SAA1 may be cleaved and form amyloid A protein, but SAA1-derived amyloid A protein may not deposit or may not be retained in tissues. More precise *in vivo* study on the behavior of SAA1 and SAA2 during amyloidosis is required.

We thank Drs. M. Nonaka, M. Takahashi, and S. Kuno for much helpful discussion and advice; Kiyoe Aikoshi for excellent technical assistance; and Hiroko Horita for typing the manuscript.

1. Benditt, E. P. & Eriksen, N. (1971) *Am. J. Pathol.* **65,** 231–252.
2. Levin, M., Franklin, E. C., Frangione, B. & Pras, M. (1972) *J. Clin. Invest.* **51,** 2773–2776.
3. Sletten, K. & Husby, G. (1974) *Eur. J. Biochem.* **41,** 117–125.
4. Levin, M., Pras, M., & Franklin, E. C. (1973) *J. Exp. Med.* **138,** 373–380.
5. Rosenthal, C. J., Franklin, E. C., Frangione, B. & Greenspan, J. (1976) *J. Immunol.* **116,** 1415–1418.
6. Anders, R. F., Natvig, J. G., Sletten, K., Husby, G. & Nordstoga, K. (1977) *J. Immunol.* **118,** 229–234.
7. Benditt, E. P. & Eriksen, N. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4025–4028.
8. Rosenthal, C. J. & Franklin, E. C. (1975) *J. Clin. Invest.* **55,** 746–753.
9. Eriksen, N. & Benditt, E. P. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6860–6864.
10. Bausserman, L. L., Herbert, P. N. & McAdam, K. P. W. J. (1980) *J. Exp. Med.* **152,** 641–656.
11. Tobias, P. S., McAdam, K. P. W. J. & Vlevitch, R. J. (1982) *J. Immunol.* **128,** 1420–1427.
12. Hoffman, J. S. & Benditt, E. P. (1982) *J. Biol. Chem.* **257,** 10510–10517.
13. Hoffman, J. S., Ericsson, L. H., Eriksen, E., Walsh, K. A. & Benditt, E. P. (1984) *J. Exp. Med.* **159,** 641–646.
14. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
15. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Okayama, H. & Berg, P. (1982) *Mol. Cell. Biol.* **2,** 161–170.
18. Hanahan, D. & Meselson, M. (1980) *Gene* **10,** 63–67.
19. Maeda, S., McCandliss, R., Gross, M., Sloma, A., Familletti, P. C., Tabor, J. M., Evinger, M., Levy, W. P. & Pestka, S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7010–7013.
20. Kafatos, F. C., Jones, C. W. & Efstratiadis, A. (1979) *Nucleic Acids Res.* **7,** 1541–1552.
21. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62,** 1159–1166.
22. Sanger, F., Nicklen, S. & Coulson, A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
23. Messing, J. & Vieira, J. (1982) *Gene* **19,** 269–276.
24. Morrow, J. F., Stearman, R. S., Peltzman, C. G. & Potter, D. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4718–4722.
25. Stearman, R. S. (1983) Dissertation (Johns Hopkins University, Baltimore).
26. Kreil, G. (1981) *Annu. Rev. Biochem.* **50,** 317–348.
27. Parmelee, D. C., Titani, K., Ericsson, L. H., Eriksen, N., Benditt, E. P. & Walsh, K. A. (1982) *Biochemistry* **21,** 3298–3303.
28. Hermodson, M. A., Kuhn, R. W., Walsh, K. A., Neurath, H., Eriksen, N. & Benditt, E. P. (1972) *Biochemistry* **11,** 2934–2938.
29. Waalen, K., Sletten, K., Husby, G. & Nordstoga, K. (1980) *Eur. J. Biochem.* **104,** 407–417.
30. Gorevic, P. D., Greenwald, M., Frangione, B., Pras, M. & Franklin, E. C. (1977) *J. Immunol.* **118,** 1113–1118.
31. Segrest, J. P., Pownall, H. J., Jackson, R. L., Glenner, G. G. & Pollock, P. S. (1976) *Biochemistry* **15,** 3187–3191.
32. Segrest, J. P. & Feldman, R. J. (1977) *Biopolymers* **16,** 2053–2065.