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Amino Acid Structures of Multiple Forms of Amyloid-Related Serum Protein SAA from a Single Individual†

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ABSTRACT: Multiple forms of the acute-phase serum protein SAA were isolated from the lipoprotein fraction of plasma from a single individual. These protein forms were purified by size-exclusion, ion-exchange, and reverse-phase high-pressure liquid chromatography, and then the tryptic peptides were subjected to amino acid sequence analysis. A total of three distinct 104-residue proteins were identified. Two of these proteins differed only by having either an arginine or a histidine at position 71 while the third protein had seven amino acid differences. Each of these proteins has a 103-residue companion protein where the amino-terminal arginine has been removed. Two of these protein sequences match the two human SAA cDNA structures reported in the literature. The presence of three unique amino acid sequences in one individual is proof that there must be a minimum of two genes for SAA in humans.

In many chronic inflammatory and infectious diseases a severe complication is the development of systemic amyloidosis. In this condition, aggregates of fibril structures are deposited or formed in the extracellular spaces of the individual's tissue with kidney, spleen, and liver often being severely involved (Cohen, 1967; Benditt & Eriksen, 1971). The major protein constituent of these fibril structures is amyloid protein A (AA).[1] Comparison of protein AA from human and animal fibril preparations reveals polypeptides with similar molecular masses (7000–10 000 daltons) and amino acid sequences that are highly conserved, especially in the middle of the polypeptide (Hermondson et al., 1972; Levin et al., 1972; Waalen et al., 1980; DiBartola et al., 1985; Benson et al., 1985). Antibodies to amyloid protein AA have been found to cross-react with a serum α-globulin with a molecular mass of 100–200 kilodaltons which was later identified as high-density lipoprotein (HDL). Treatment of these HDL complexes with denaturing agents yielded a 12 000-dalton fraction that contained all the AA-cross-reacting material and that was named serum amyloid A (SAA) (Eriksen & Benditt, 1980). Since its identification this protein has been found to be an acute-phase protein. In an unstimulated individual, SAA serum levels are in the range of 1–5 μg/mL. However, during an infection or inflammatory episode serum levels of the protein may increase up to several hundred times the normal value and then quickly return to normal levels upon recovery. Recently, it has been shown that when human SAA is administered to a mouse undergoing casein-induced amyloid formation, the resulting fibrils contain both mouse and human AA proteins, thus

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[1] Abbreviations: AA, amyloid A protein; SAA, serum amyloid A; HPLC, high-pressure liquid chromatography; TFA, trifluoroacetic acid; ELISA, enzyme-linked immunosorbent assay; Gdn·HCl, guanidine hydrochloride; Tris, tris(hydroxymethyl)aminomethane; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone.

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confirming that SAA is the precursor molcule for AA amyloid subunit protein (Husebekk et al., 1986). Reports on the characterization of human SAA have shown that the protein is polymorphic. Initial isolation of SAA yielded two major fractions with similar amino acid compositions and amino acid sequences that were identical for the first 30 residues except that one of the fractions was missing the amino-terminal arginine residue (Eriksen & Benditt, 1980). Using DEAE-cellulose chromatography, others have isolated these two major forms and identified four minor forms (Bausserman et al., 1980). With the exception of some amino-terminal heterogeneity these six polymorphs appear to be the same size and to have similar amino acid compositions. Recently, the complete amino acid sequence of one of the major forms of human SAA isolated from pooled human serum has been reported (Parmelee et al., 1982). This protein is 104 amino acids long, and while charge homogeneous, it is polymorphic by reverse-phase HPLC. The sequence also showed both alanine and valine at positions 52 and 57. Another report determined the amino acid sequence of SAA isolated from an individual plasma donor with severe rheumatoid arthritis (Sletten et al., 1983). This protein also showed heterogeneity at positions 52 and 57 and in addition had a leucine/isoleucine polymorphism at position 58. Although this sample was from a single individual, the protein was purified by size-exclusion chromatography alone, so it was probably polymorphic in charge and hydrophobicity. These results strongly indicate that the genes for SAA are polymorphic, but they do not give a clear idea of the number and specific sequences of these proteins.

Materials and Methods

Plasma was obtained from patient HEN, a 72 year old man with Waldenstrom's macroglobulinemia and a polyneuropathy complicated by staphlococcal pneumonia. Plasma was obtained by therapeutic plasmaphoresis and was frozen at −20 °C until used for these studies. SAA plasma concentration of several fractions was aproximately 300 μg/mL as determined by ELISA. To isolate the lipoprotein complexes containing the SAA proteins, 120 mL of whole plasma was thawed and filtered and its nonprotein density raised with solid potassium bromide to 1.21 g/cm^3. The lipoproteins were then separated in a Beckman Model L Ultracentrifuge using a Ti50 rotor spun at 47 000 rpm for 22 h. All AA-immunoreactive fractions were pooled, concentrated to a 20-mL volume in an Amicon filtration system, made 6 M in guanidine hydrochloride (Gdn·HCl), and incubated at 37 °C for 2 h. The protein mixture was then fractionated by size-exclusion chromatography on a column of Sepharose CL-6B. All AA-immunoreactive fractions were pooled, dialyzed against water in Spectropore 3 tubing, and lyophilized. This mixture was fractionated by two different HPLC techniques. First, anion-exchange chromatography was performed on a Synchrom Synchropak AX-300 column with a gradient from 0.01 to 0.25 M Tris–acetate in 6 M urea at pH 7.9. Fractions were acidified with glacial acetic acid, dialyzed against 1% acetic acid and lyophilized. The second technique involved reverse-phase HPLC using a Synchrom Synchropak RP-P (C-18) column and a gradient of 27–47% 2-propanol in 0.1% TFA. Protein pools were combined and dried under vacuum. For both techniques four peaks of AA immunoreactivity were identified which accounted for about half of the starting protein.

Lyophilized SAA fractions (0.4–1.0 mg) were suspended in 0.9 mL of water, and the protein was solubilized with 50 μL of 1 M ammonium hydroxide. Excess ammonia was removed with nitrogen gas and the solution made 0.1 M in ammonium bicarbonate, pH 8.0, by the addition of 0.1 mL of a 1 M stock solution. Trypsin (TPCK treated, Worthington) was solubilized in pH 3.0 water and an aliquot (2% by weight) added to the different SAA fractions. Digestion was accomplished at 25 °C for 2 h and stopped by lyophilization. Peptide mixtures were separated by reverse-phase HPLC using an Altex Ultrasphere C-18 column (Beckman).

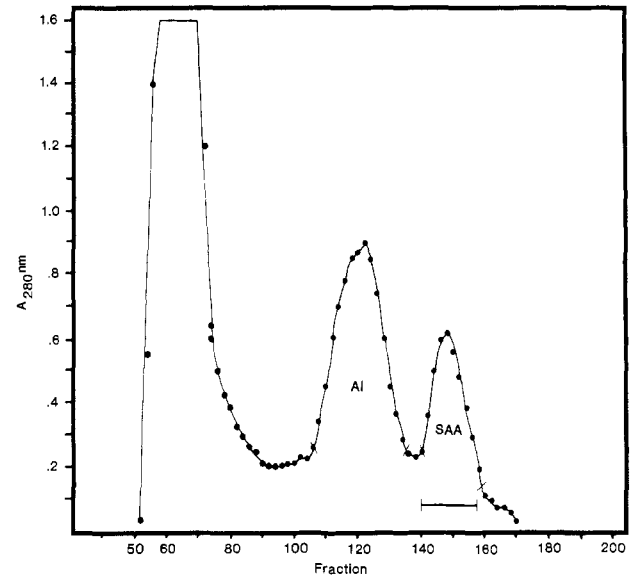

FIGURE 1: Gel permeation chromatographic separation of denatured lipoproteins from 60 mL of plasma applied to a Sepharose CL-6B column (2.6 × 92 cm) equilibrated in 4.0 M Gdn·HCl and 0.05 M Tris, pH 8.2, with a flow rate of 30 mL/h. Fractions of 2.5 mL were collected, and the absorbance at 280 nm was measured. Fractions containing AA-immunoreactive material were pooled as marked by the bar. Other apolipoproteins are identified above their corresponding peaks.

Peptide and protein samples were hydrolyzed in double-distilled 5.7 N HCl under reduced pressure at 110 °C for 20–22 h. Hydrolysates were coupled with phenyl isothiocyanate and the resultant phenylthiocarbamyl amino acids separated by reverse-phase HPLC on an Altex Ultrasphere C-18 column with 0.01 M KH_2PO_4, pH 6.5, as the buffer and an acetonitrile gradient (Scholze, 1985). Polypeptides were degraded in a liquid-phase automatic sequenator (Beckman Model 890C) using the 0.1 M Quadrol program (Brauer et al., 1975). To each sample was added 3 mg of Polybrene (Pierce) to reduce extractive losses (Tarr et al., 1978). Phenylthiohydantoin amino acid derivatives were identified by reverse-phase HPLC using a minor modification of a reported procedure (Zimmerman et al., 1977).

Results

The use of a single density gradient ultracentrifugation to isolate all lipoproteins in one fraction provides a near-complete isolation of SAA from the non-lipoprotein plasma proteins. This coupled to denaturation of the lipoprotein complexes with 6 M guanidine and separation by size-exclusion chromatography (see Figure 1) concentrated the SAA antigenic activity in a single peak. Each 120-mL fraction of plasma gave 10–20 mg of SAA-enriched protein material. This mixture was then resolved by two different HPLC techniques. Anion-exchange chromatography on a Synchropak AX-300 column gave four forms of SAA activity as seen in Figure 2. Amino-terminal sequence analysis revealed that forms I and III have the structure Arg-Ser-Phe-Phe-Ser-Phe while forms II and IV have the structure Ser-Phe-Phe-Ser-Phe-Leu. A fraction of the SAA-enriched protein material was applied to a Synchrom

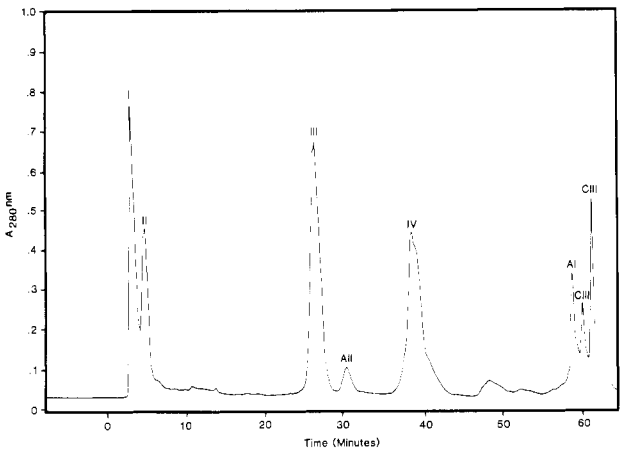

FIGURE 2: Anion-exchange chromatographic separation of 6.0 mg of SAA-enriched material isolated from the Sepharose CL-6B column applied to Synchropak AX-300 column (1 × 25 cm). The initial buffer, 6 M urea and 0.01 M Tris–acetate, pH 7.9, was maintained for 10 min before a 0–100% buffer B gradient in 60 min was started. Buffer B consists of 6 M urea and 0.25 M Tris–acetate, pH 7.9. The flow rate is 2 mL/min, and the peaks containing the four SAA forms are labeled.

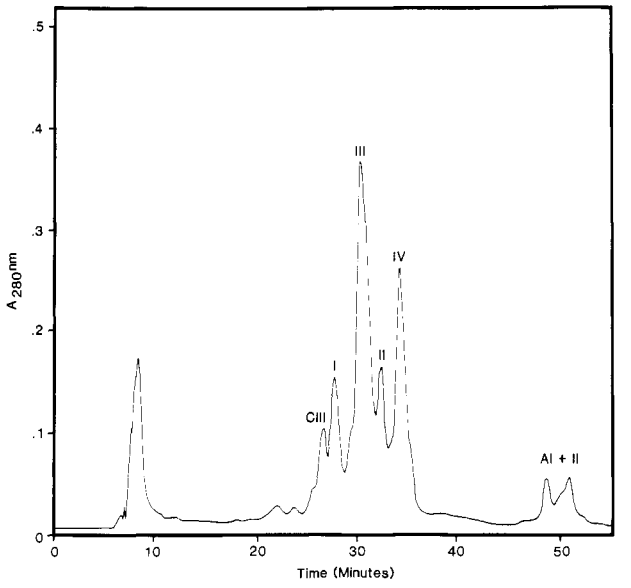

FIGURE 3: Reverse-phase HPLC separation of 3 mg of the SAA fraction isolated from the CL-6B column on a Synchropak RP-P (C-18) column (1 × 25 cm). Initial buffer (A) is 0.1% TFA water, and the final buffer (B) is 0.1% TFA and 90% 2-propanol. The sample is applied in 50% glacial acetic acid, and the proteins are eluted with a 30–50% buffer B gradient in 60 min at a 2 mL/min flow rate. The peaks corresponding to SAA forms I–IV from the AX-300 column are labeled.

RP-P column and eluted with 2-propanol as shown in Figure 3. Again four peaks were seen and numbered to correspond to the same peaks seen in Figure 2. From a 12-mg sample the average yields of fractions I, II, III, and IV isolated from the RP-P column were 0.5, 0.3, 3.1, and 2.2 mg, respectively.

A time course tryptic digest of a 100-μg sample of fraction III showed that a 2-h digestion at 25 °C provided near-maximum cleavage with minimum peptide degradation. By use of 0.3–1.0-mg samples of SAA fractions I–IV, trypsin fragmentations were performed and the resulting peptide mixtures separated by reverse-phase HPLC. Chromatograms for SAA pools I and III are shown in Figure 4. Pools II and IV gave profiles that were identical with those of pools I and III, respectively, with the only difference being that they both lacked peak T1-2. Amino acid composition data for the tryptic peptides of SAA pools I and III are shown in Tables I and II, respectively. The complete sequences of SAA forms I and III are shown in Figure 5. SAA form III shows a single amino acid sequence which is identical with the SAA structure reported from pooled human serum (Parmelee et al., 1982) without the polymorphisms at positions 52 and 57. This structure also corresponds to a reported cDNA sequence (Sipe

Table I: Amino Acid Composition of Peptides Derived from HEN SAA Forms I and II[a]

	form I whole protein	form II whole protein	T1	T2	T1-2	T3
Asp	14.5 (14)	14.6 (14)		1.3 (1)	1.2 (1)	1.1 (1)
Thr	0.8 (1)	0.7 (1)				
Ser	6.9 (7)	6.6 (7)		2.0 (2)	1.8 (2)	
Glu	8.2 (8)	8.3 (8)		1.2 (1)	1.1 (1)	
Pro	3.5 (4)	3.6 (4)				
Gly	12.1 (12)	12.4 (12)		2.1 (2)	2.2 (2)	
Ala	16.3 (16)	16.2 (16)		2.0 (2)	2.1 (2)	
Val	0.7 (1)	0.6 (1)				
Met	1.6 (2)	1.5 (2)				0.7 (1)
Ile	2.3 (3)	2.6 (3)				
Leu	4.1 (4)	4.2 (4)		0.8 (1)	0.9 (1)	
Tyr	4.6 (5)	4.7 (5)				
Phe	6.2 (6)	5.9 (6)		3.7 (4)	3.6 (4)	
His	2.6 (2.5)	2.4 (2.5)				
Lys	3.9 (4)	4.2 (4)				
Arg	11.3 (11.5)	10.2 (10.5)	1.0 (1)	0.9 (1)	1.8 (2)	1.1 (1)
Trp	+[b] (3)	+ (3)				+ (1)
position	1–104	2–104	1	2–15	1–15	16–19

	T4	T5	T6	T7	T8-9	T10	T11A
Asp	1.2 (1)	2.1 (2)		2.0 (2)	1.0 (1)	1.1 (1)	3.2 (3)
Thr							0.8 (1)
Ser	0.8 (1)	0.9 (1)			0.8 (1)		0.9 (1)
Glu		1.0 (1)			1.1 (1)	2.1 (2)	2.1 (2)
Pro					0.7 (1)		
Gly		1.1 (1)		1.1 (1)	3.1 (3)		1.9 (2)
Ala	1.1 (1)	1.0 (1)	1.0 (1)	2.0 (2)	4.0 (4)		3.8 (4)
Val					0.8 (1)		
Met	0.8 (1)						
Ile		0.9 (1)			1.1 (1)	0.9 (1)	
Leu							2.1 (2)
Tyr	1.0 (1)	0.8 (1)	0.7 (1)	0.8 (1)			
Phe			1.1 (1)				
His			0.9 (1)				1.1 (1)
Lys		1.1 (1)		1.1 (1)			1.0 (1)
Arg	1.2 (1)		1.1 (1)		2.2 (2)	0.9 (1)	
Trp					+[b] (1)		
position	20–25	26–34	35–39	40–46	47–62	63–67	68–84

	T11A1	T11A2	T11B	T12
Asp		3.1 (3)		2.0 (2)
Thr	0.8 (1)			
Ser		0.8 (1)		0.9 (1)
Glu		2.1 (2)		1.1 (1)
Pro				2.7 (3)
Gly	1.0 (1)	1.2 (1)	1.1 (1)	2.1 (2)
Ala		3.7 (4)		1.0 (1)
Val				
Met				
Ile				
Leu	1.0 (1)	1.1 (1)		1.1 (1)
Tyr				0.9 (1)
Phe				1.1 (1)
His				1.0 (1)
Lys		1.0 (1)		1.1 (1)
Arg	1.1 (1)		0.9 (1)	2.0 (2)
Trp			+[b] (1)	
position	68–71	72–84	85–87	88–104

[a] Numbers in parenthesis represent integer values determined from sequence analysis. [b] Tryptophan was observed in the amino acid analysis but was not quantitated.

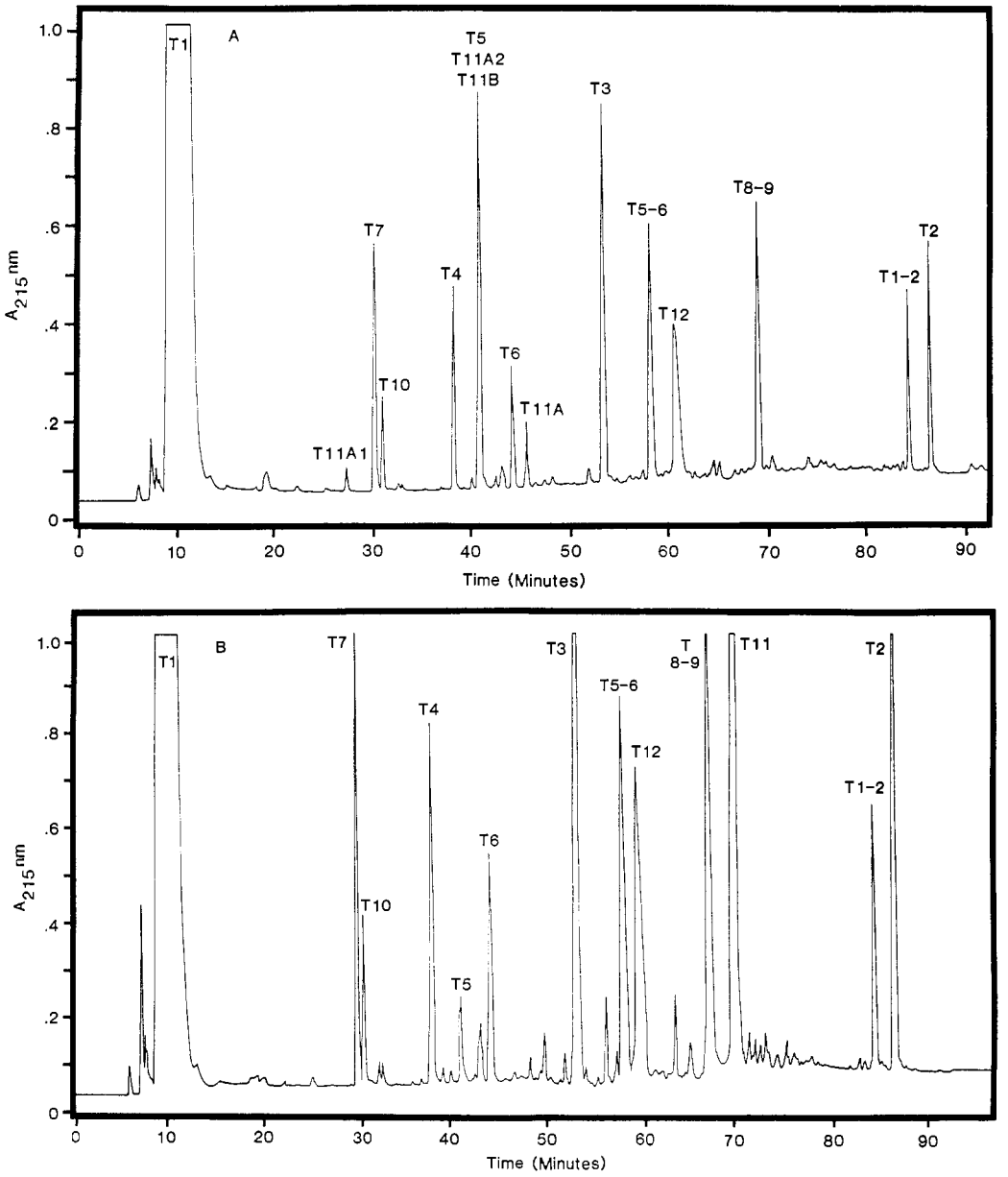

FIGURE 4: (A) Trypsin digest of 0.4 mg of SAA form I is dissolved in 0.5 mL of 50% acetic acid and applied to an Altex Ultrasphere C-18 column (1 × 25 cm) at a flow rate of 2 mL/min. First, a linear gradient of 0–30% acetonitrile in 60 min is used, followed by a 30–60% acetonitrile gradient in 30 min to elute the remaining peptides. The buffering agent was 0.1% TFA. (B) Trypsin digest of 1 mg of SAA form III. Conditions are the same as in (A).

et al., 1985). SAA form I, however, is composed of two SAA molecules differing by having either a histidine or arginine residue at position 71. Seven other differences from SAA form III are located at positions 52, 55, 60, 68, 69, 84, and 90. This amino acid structure is identical with a human cDNA structure reported by Kluve-Beckerman et al. (1986).

Discussion

Since the identification of SAA as the presumptive precursor protein of AA amyloid fibril protein, there has been a great deal of controversy as to the number and nature of its molecular forms. In an attempt to resolve these questions, we have isolated and determined the primary structure of all forms of SAA from a single individual with elevated levels of SAA. Clinical and post mortem evaluation of this patient revealed no indication of amyloid formation so that the only mechanism of SAA removal would be expected to be the normal degradative pathways. To isolate the plasma SAA in the highest yields with minimal probability for loss of specific forms, a procedure somewhat different from previously reported methods was developed. First, a single ultracentrifugation spin was performed to isolate all the lipoproteins in one fraction and avoid possible loss of specific SAA types in the low-density lipoprotein fraction. With this mixture of very low density, low-density, and high-density lipoproteins, the standard procedures of desalting, lyophilization, and delipidation by solvent extraction followed by resolubilization in guanidine buffer did not prove to be an efficient technique. The presence of B lipoprotein from the low-density lipoprotein gave a mixture from which the SAA could not easily be extracted. Instead, it was found that the lipoprotein complexes could be denatured by the addition of solid guanidine to the concentrated lipoprotein mixture. In this way, no insoluble protein material was generated, and from immunologic testing of the fractions from the Sepharose CL-6B column, all of the SAA was released from the lipoprotein complexes and migrated as a protein species of molecular mass about 12 000 daltons. Sequence analysis of this protein mixture showed that about half of the material degraded as SAA and the remainder had sequences comparable to apolipoproteins AI and CIII.

Table II: Amino Acid Composition of Peptides Derived from HEN SAA Forms III and IV[a]

	form III whole protein	form IV whole protein	T1	T2	T3	T4
Asp	14.4 (14)	14.6 (14)		1.2 (1)	1.2 (1)	1.1 (1)
Thr	0.2 (0)	0.1 (0)				
Ser	7.1 (7)	6.8 (7)		1.7 (2)		0.8 (1)
Glu	9.3 (9)	9.0 (9)		1.1 (1)		
Pro	3.5 (4)	3.8 (4)				
Gly	12.4 (12)	11.9 (12)		2.2 (2)		
Ala	16.5 (16)	16.3 (16)		2.0 (2)		1.0 (1)
Val	1.2 (1)	0.9 (1)				
Met	1.4 (2)	1.6 (2)			0.8 (1)	0.7 (1)
Ile	2.6 (3)	3.1 (3)				
Leu	3.2 (3)	3.3 (3)		1.1 (1)		
Tyr	4.7 (5)	4.8 (5)				1.1 (1)
Phe	8.3 (8)	8.4 (8)		3.8 (4)		
His	3.1 (3)	3.0 (3)				
Lys	4.3 (4)	4.4 (4)				
Arg	10.5 (10)	9.4 (9)	1.0 (1)	1.0 (1)	1.0 (1)	1.2 (1)
Trp	+[b] (3)	+ (3)			+ (1)	
position	1–104	2–104	1	2–15	16–19	20–25

	T5	T6	T7	T8-9	T10	T11
Asp	2.2 (2)		2.0 (2)	1.3 (1)	1.1 (1)	3.4 (3)
Thr						
Ser	0.8 (1)			0.8 (1)		0.9 (1)
Glu	1.1 (1)			1.1 (1)	2.1 (2)	2.9 (3)
Pro				0.8 (1)		
Gly	1.1 (1)		1.1 (1)	3.3 (3)		3.2 (3)
Ala	1.0 (1)	1.0 (1)	2.0 (2)	4.0 (4)		4.0 (4)
Val				1.1 (1)		
Met						
Ile	0.8 (1)			0.9 (1)	0.8 (1)	
Leu						1.2 (1)
Tyr	0.8 (1)	0.9 (1)	0.8 (1)			
Phe		1.1 (1)				1.9 (2)
His		1.1 (1)				1.1 (1)
Lys	1.2 (1)		1.1 (1)			
Arg		1.0 (1)		2.1 (2)	1.1 (1)	1.0 (1)
Trp				+[b] (1)		+ (1)
position	26–34	35–39	40–46	47–62	63–67	67–87

	T12		T12			T12
Asp	2.2 (2)	Val		His		0.9 (1)
Thr		Met		Lys		2.3 (2)
Ser	0.8 (1)	Ile		Arg		0.9 (1)
Glu	1.0 (1)	Leu	1.2 (1)	Trp		
Pro	3.1 (3)	Tyr	0.7 (1)	position		88–104
Gly	2.2 (2)	Phe	1.1 (1)			
Ala	0.9 (1)					

[a] Numbers in parenthesis represent integer values determined from sequence analysis. [b] Tryptophan was observed on amino acid analysis but was not quantitated.

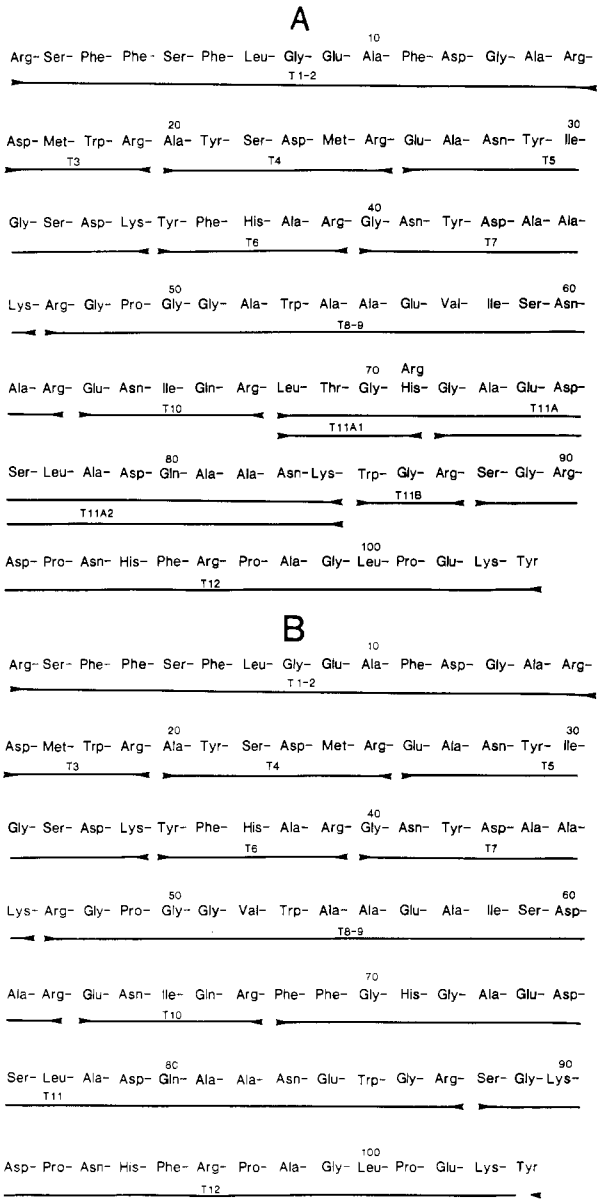

FIGURE 5: (A) Complete amino acid sequence of HEN SAA form I as well as the tryptic peptides used to prove its structure. HEN SAA form II was identical with form I except it was missing the amino-terminal arginine. (B) Complete amino acid sequence of HEN SAA form III along with the tryptic peptides used to prove its structure. Again, with the exception of its lacking the amino-terminal arginine, form IV is identical with form III.

The resolution of this mixture was then accomplished by using two HPLC techniques. The first involved anion-exchange chromatography on a Synchropak AX-300 column. The buffer system is nearly identical with that used to separate SAA fractions on DEAE-cellulose (Bausserman et al., 1980) except that acetate is used instead of chloride as the counterion. The profile shown in Figure 2 is a representative chromatogram of these separations. Chromatography on the Synchropak RP-P reverse-phase column provides a profile as shown on Figure 3. All peaks from the two columns were rerun on the other column to cross identify the peaks. The reverse-phase column did not give as good a resolution of the SAA forms as the anion-exchange column but gave much higher recovery. In general, the reverse-phase column gave near-complete recovery of protein while the anion-exchange column had about a 50–60% mass recovery, which is probably due to loss during dialysis.

Sequence analysis revealed that forms I and III have an amino-terminal arginine while forms II and IV are missing this residue and start their structure from the next residue, which is serine. Amino acid analyses of the four forms revealed that forms I and II had nearly identical compositions as did forms III and IV as shown in Tables I and II. Comparison of the two groups indicated that forms I and II contained less phenylalanine and glutamic acid with more leucine, threonine, and arginine than forms III and IV. This agrees well with data reported previously (Bausserman et al., 1980). Because of the concentration of trypsin-sensitive bonds at the carboxyl-terminal region of the molecule, a time course digest was performed to determine optimal conditions. A 2-h digest at 25 °C gave high yields of all peptides with minimum degradation of peptide 88–104 and minimum oxidation of peptide 20–25. Preparative digestions of forms I–IV gave reverse-phase HPLC profiles as shown in Figure 4 and amino acid compositions as shown in Tables I and II.

With the exception of the amino-terminal arginine, form

Table III: Comparison of Amino Acid Polymorphisms in Human SAA and Protein AA

protein	amino acid residues												
	23	52	53	57	58	60	66	68	69	71	75	84	90
SAA1	Asp	Val	Trp	Ala	Ile	Asp	Gln	Phe	Phe	His	Asp	Glu	Lys
SAA2α	Asp	Ala	Trp	Val	Ile	Asn	Gln	Leu	Thr	His	Asp	Lys	Arg
SAA2β	Asp	Ala	Trp	Val	Ile	Asn	Gln	Leu	Thr	Arg	Asp	Lys	Arg
SAA deduced from pA1 (Sipe et al., 1985)	Asp	Val	Trp	Ala	Ile	Asp	Gln	Phe	Phe	His	Asp	Glu	Lys
SAA deduced from pSAA82 (Kluve-Beckerman et al., 1986)	Asp	Ala	Trp	Val	Ile	Asn	Gln	Leu	Thr	His	Asp	Lys	Arg
SAA (Parmelee et al., 1982)	Asp	Ala/Val	Trp	Val/Ala	Ile	Asp	Gln	Phe	Phe	His	Asp	Glu	Lys
SAA (Sletten et al., 1983)	Asn	Ala/Val	Trp	Val/Ala	Ile/Leu	Asn	Gln	Phe	Phe	His	Asn	Glu	Lys
AA (Moyner et al., 1980)	Asn	Ala/Val	Trp/Arg	Ala	Ile	Asp	Glu	Phe	Phe	His	Asn		
AA (Sletten & Husby, 1974)	Asn	Val	Trp	Ala	Ile	Asp	Gln	Phe	Phe	His	Asn		
AA (Levin et al., 1972)	Asp	Ala	Trp/Arg	Val	Ile	Asn	Gln	Leu	Thr	Arg	Asp		

I gave the same tryptic peptide pattern as II. Also, forms III and IV gave identical peptide patterns which contained a number of differences from that seen for forms I and II. Amino acid sequence analysis showed identity between all corresponding peptides of forms I and II. Similarly, the corresponding peptides of forms III and IV were identical. The complete amino acid sequences of the SAA forms are shown in Figure 5.

From these findings along with published data from cDNA sequence work several observations appear clear. First, the presence or absence of arginine at the amino terminal is the result of proteolytic processing. Next, forms III and IV exactly match the cDNA sequence reported by Sipe et al. (1985) and, except for the lack of polymorphism at positions 52 and 57, match the amino acid sequence of Parmelee et al. (1982). Forms I and II are more complicated because, although they appear homogeneous by ion-exchange and reverse-phase HPLC, they have two sequences that differ by having either an arginine or histidine at position 71. The sequence with a histidine at 71 exactly matches a cDNA sequence reported by Kluve-Beckerman et al. (1986) while the structure with the arginine at 71 has not been reported for a cDNA. This finding of three amino acid sequences for SAA from one individual requires the presence of at least two and possibly three genes for human SAA. In keeping with the nomenclature derived for mouse SAA, we propose to identify the human SAA forms in the following manner. HEN SAA forms III and IV which correspond to the Parmelee et al. (1982) and Sipe et al. (1985) structures we propose to identify as SAA1 and SAA1 des Arg. This appears to be the most common form of SAA throughout the human population. HEN SAA forms I and II with the histidine at 71 we propose to call SAA2α, and SAA forms I and II with the arginine at position 71 we will call SAA2β. From the present data it is not possible to determine whether SAA2β is the product of an allele of SAA2α or a separate gene. However, studies on SAA isolated from additional individuals also show the presence of SAA2β, so that it may be the product of a very common allele or a separate gene (unpublished results).

The presence of these three forms of SAA explains a number of polymorphisms previously reported in the literature as can be seen in Table III. The presence of the alanine/valine polymorphisms at positions 52 and 57 as well as the identification of asparagine at position 60 and the leucine (position 68) and threonine (position 69) could have resulted from a mixture of SAA1 and SAA2. Although these findings explain a large number of the reported polymorphisms seen in SAA and amyloid-derived AA proteins, they do not explain them all and additional studies will be needed to identify which SAA gene products contain these substitutions. However, with the techniques reported here this is now possible by use of samples of serum available from single individuals.

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