HETEROGENEITY OF HUMAN SERUM AMYLOID A PROTEINS*

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A form of amyloidosis occurring in a variety of chronic inflammatory diseases is caused by the deposition in tissues of a fibrillar protein called amyloid A protein (AA).[1] Primary structure analyses of AA from tissues of a patient with familial Mediterranean fever (1) and from a patient with rheumatoid arthritis (2) have been described. In each case, AA was reported to be a 76-residue single-chain protein, although there are 9 discrepancies in the published sequences.

A protein that is antigenically and chemically related to AA has been identified in the serum of normals (3) and in relatively high concentrations in a number of disease states (4–7). This acute-phase reactant, designated serum AA (SAA), has an apparent molecular weight of 11,000–14,000 (8–10). SAA is less well-characterized than AA, but partial sequence studies have demonstrated a high degree of homology between SAA and AA in their NH_2-terminal regions (11, 12). Benditt and Eriksen's discovery (13), confirmed by Skogen et al. (14), that SAA is transported in association with plasma high-density lipoproteins (HDL) has greatly facilitated the purification and characterization of SAA. We have isolated SAA from the HDL of two normals after etiocholanolone-induced inflammation (15) and from the HDL of five patients, two of whom had secondary amyloidosis. Six polymorphic forms of SAA have been identified.

Materials and Methods

Sources of SAA. Written, informed consent was obtained from study subjects. 300 ml of plasma was obtained from two male volunteers before and 48 h after deep intramuscular injection of etiocholanolone, 3 mg/kg body wt (15). Plasma was also obtained from patients with Wegener's granulomatosis, Waldenström's macroglobulinemia, Goodpasture's syndrome, and systemic lupus erythematosus. A 50-yr-old man with juvenile rheumatoid arthritis or ankylosing spondylitis (HLA B27 phenotype) donated plasma after etiocholanolone-induced inflammation.

Preparation of Apolipoprotein (Apo) HDL. HDL were isolated by preparative ultracentrifugation (16) in a Beckman L5-75 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo

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[1] *Abbreviations used in this paper*: AA, amyloid A protein; apo, apolipoprotein(s); HDL, high-density lipoprotein(s); SAA, serum AA; SDS, sodium dodecyl sulfate.

Alto, Calif.). Plasma was adjusted to a solvent density of 1.080 g/ml with solid KBr and centrifuged in a Beckman 60 Ti rotor for 20 h at 10°C and 55,000 rpm (4.7×10^6 g/min average). Supernatant and infranatant fractions were recovered by tube slicing. The density of the infranatant fraction was adjusted to 1.21 g/ml and centrifuged at 59,000 rpm for 24 h (6.1×10^6 g/min average). The HDL recovered in the supernatant fraction were recentrifuged once in a 40 rotor for 48 h at 40,000 rpm (5.1×10^6 g/min average), dialyzed against 0.15 M NaCl and 2 mM EDTA, and brought to a protein concentration of ~20 mg/ml by dehydration in dialysis sacks coated with Aquacide-IIA (Calbiochem-Behring Corp., American Hoescht Corp., San Diego, Calif.). HDL were delipidated with methanol:diethyl ether (1:3) as previously described (17).

Column Chromatography. Apo HDL were initially fractionated on 2.0 × 140-cm columns of Sephadex G-100, Sephacryl S-200, or Sepharose A (0.5 M) (considered preferable) eluted with 5 M guanidine-HCl. Rechromatography of the low molecular weight (10,000–15,000) fraction on the same column was routinely performed before undertaking additional purification. 15-mg aliquots of the SAA-rich fractions were applied to 1.2 × 50-cm columns of DEAE-cellulose equilibrated with 0.01 M Tris-HCl 6 M urea, pH 8.2. Consecutive 500-ml linear gradients, 0.01–0.075 M and 0.075–0.2 M Tris-HCl were required to optimally separate and completely elute all of the SAA and C-apo applied to the columns. The urea was deionized by passage over a Rexyn 300 column (Fisher Scientific Co., Pittsburgh, Pa.) immediately before use, and chromatography was carried out at 4°C to prevent carbamylation (18). Approximately 60% of the protein applied to the DEAE columns was recovered.

Characterization of SAA. Protein concentrations were determined by the Lowry method (19). SAA concentrations were estimated by a solid-phase radioimmunoassay with plastic microtitration plates coated with affinity-purified antibodies to AA (15). Lyophilized samples for assay were treated with 10% formic acid at 37°C for 18 h before radioimmunoassay (20). Chromatography fractions were analyzed by anionic (21) and cationic (22) polyacrylamide gel electrophoresis and by Ouchterlony double diffusion. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (23) and Ferguson plots (24) were used for molecular weight estimations. Amino acid analyses were performed on a Beckman 119 CL amino acid analyzer with a single-column system (25). Proteins were hydrolyzed in sealed, evacuated tubes with either 6 N HCl for 24 h or in 4 N methane sulfonic acid for 20 h. Cysteic acid was determined after performic acid oxidation (26). Carboxypeptidases A and B were used for COOH-terminal residue identification and sequence analysis (27). Sequential Edman degradations were performed manually as described by Peterson et al. (28) except that the thiazolinone derivatives were extracted with butylacetate. Free amino acids were regenerated by hydrolysis in sealed evacuated tubes with either hydriodic acid or 0.2 N NaOH and 0.1 M dithionite (29). Neuraminidase digestion was performed as described by Morell et al. (30), and polyacrylamide gel bands were qualitatively assessed for carbohydrate with the periodate-Schiff stain (31).

Results

Plasma was obtained from two young men before and after etiocholanolone injection. Local inflammation, fever, and leukocytosis occurred in both subjects (15). The concentrations of AA-equivalent protein (herein referred to as SAA) were 131 and 249 μg/ml in the plasma obtained 48 h after etiocholanolone administration. A plasma SAA level of 175 μg/ml was induced several months later when one of these subjects received two consecutive injections of etiocholanolone in a 3-d period. SAA^+ (>30 μg/ml) plasma was also obtained from patients with Wegener's granulomatosis, Waldenström's macroglobulinemia, systemic lupus erythematosus, and Goodpasture's syndrome. Plasma from a subject with juvenile rheumatoid arthritis was studied after etiocholanolone-induced inflammation. The patients with systemic lupus erythematosus and juvenile rheumatoid arthritis both had biopsy-proven amyloidosis of the AA type, as shown by histochemical sensitivity to potassium permanganate before staining with Congo red (32). Plasma concentrations of SAA ranged from 43 to 139

μg/ml. 40–65% of the SAA proteins was recovered in the HDL density range (1.080 < density < 1.21 g/ml).

HDL from plasma obtained both before and after etiocholanolone-induced inflammation were delipidated with organic solvents and fractionated on a column of Sephadex G-100 eluted with 5 M guanidine-HCl (Fig. 1). The void-volume fraction contained only small quantities of protein. Apo A-I (M_r ~ 28,000), the major HDL apoprotein, was the principal component of peak 2 (Fig. 1, insert) and the third peak contained primarily apo A-II (M_r ~ 15,700). An obvious increase in the proportion of

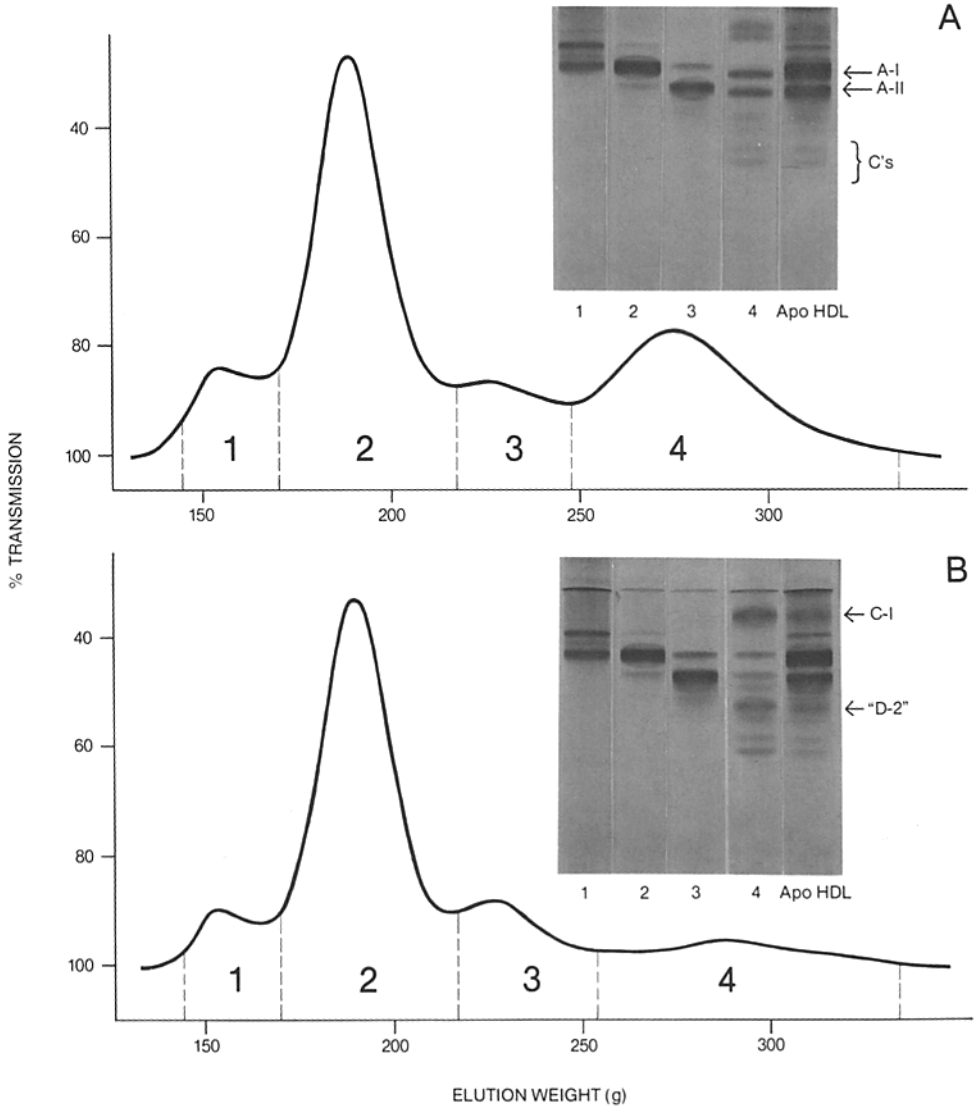


FIG. 1. Comparison of Sephadex G-100 elution profiles of apo HDL obtained from a normal donor after (A) and before (B) etiocholanolone-induced inflammation. 20 mg of protein was applied to a 2.5 × 140-cm column eluted with 5 M guanidine-HCl, and the indicated pools were analyzed by anionic (21) polyacrylamide gel electrophoresis (inserts).

protein in fraction 4 was evident in the SAA-rich apo HDL (Fig. 1 A). More than 85% of the SAA protein recovered eluted in this fraction. When the same volunteer received two injections of etiocholanolone, the proportionate increase in peak 4 was even more striking (Fig. 2), and the fraction 4 proteins comprised 24% of the total protein recovered from the column.

The HDL apo from all of the patients with SAA + plasma were fractionated on Sephacryl S-200 eluted with guanidine-HCl. The elution profiles of apo HDL from patients with Goodpasture's syndrome (Fig. 3 A) and Waldenström's macroglobulinemia (Fig. 3 B) suggested reduction of the apo A-II content (peak 3), whereas the low molecular weight protein peaks (fraction 4), which contained 24–27% of the total protein, were again proportionately large.

Anionic polyacrylamide gel electropherograms of the fraction 4 proteins from the two normals receiving etiocholanolone (Fig. 4 A) and from all five patients (Fig. 4 B) clearly demonstrated that this fraction contained several proteins not usually identifiable in SAA^- apo HDL. The proteins that normally comprise this peak include that provisionally designated "D-2" by Lim et al. (33), and the well-characterized C-apoproteins. The quantities of C-apoproteins visualized varied considerably among the study subjects. They were quite distinct in the apo HDL from the subjects with juvenile rheumatoid arthritis and systemic lupus erythematosus and present in trace concentrations in the apo HDL of the patient with Waldenström's macroglobulinemia and the volunteer who received two injections of etiocholanolone (Fig. 4 B, E-2).

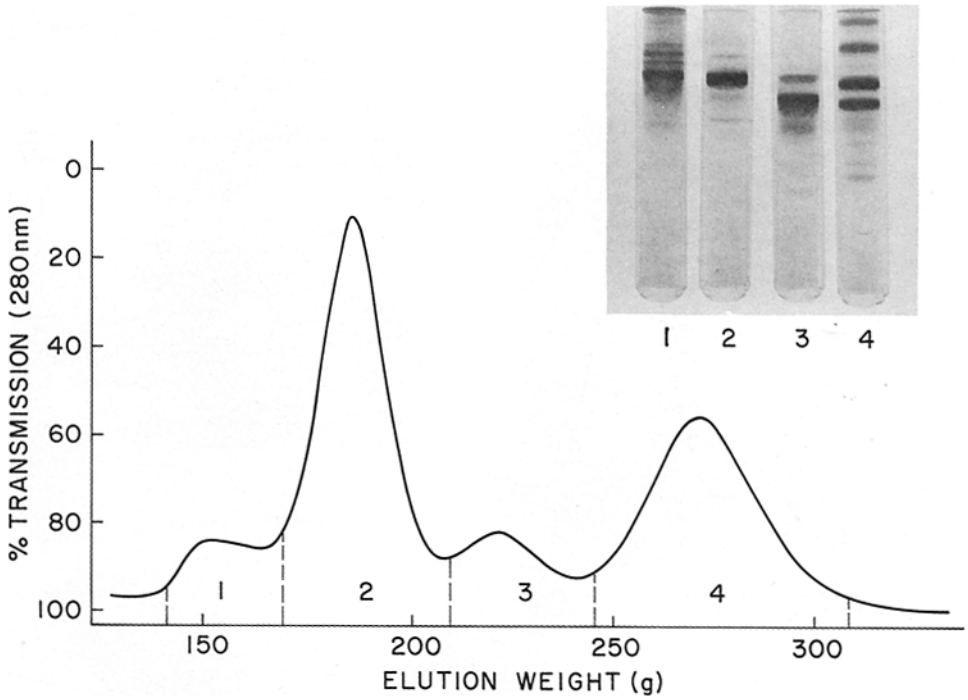


FIG. 2. Fractionation of apo HDL from a volunteer who had received two injections of etiocholanolone, 0.3 mg/kg. A 2.5 × 140-cm column of Sephadex G-100 was eluted with 5 M guanidine-HCl.

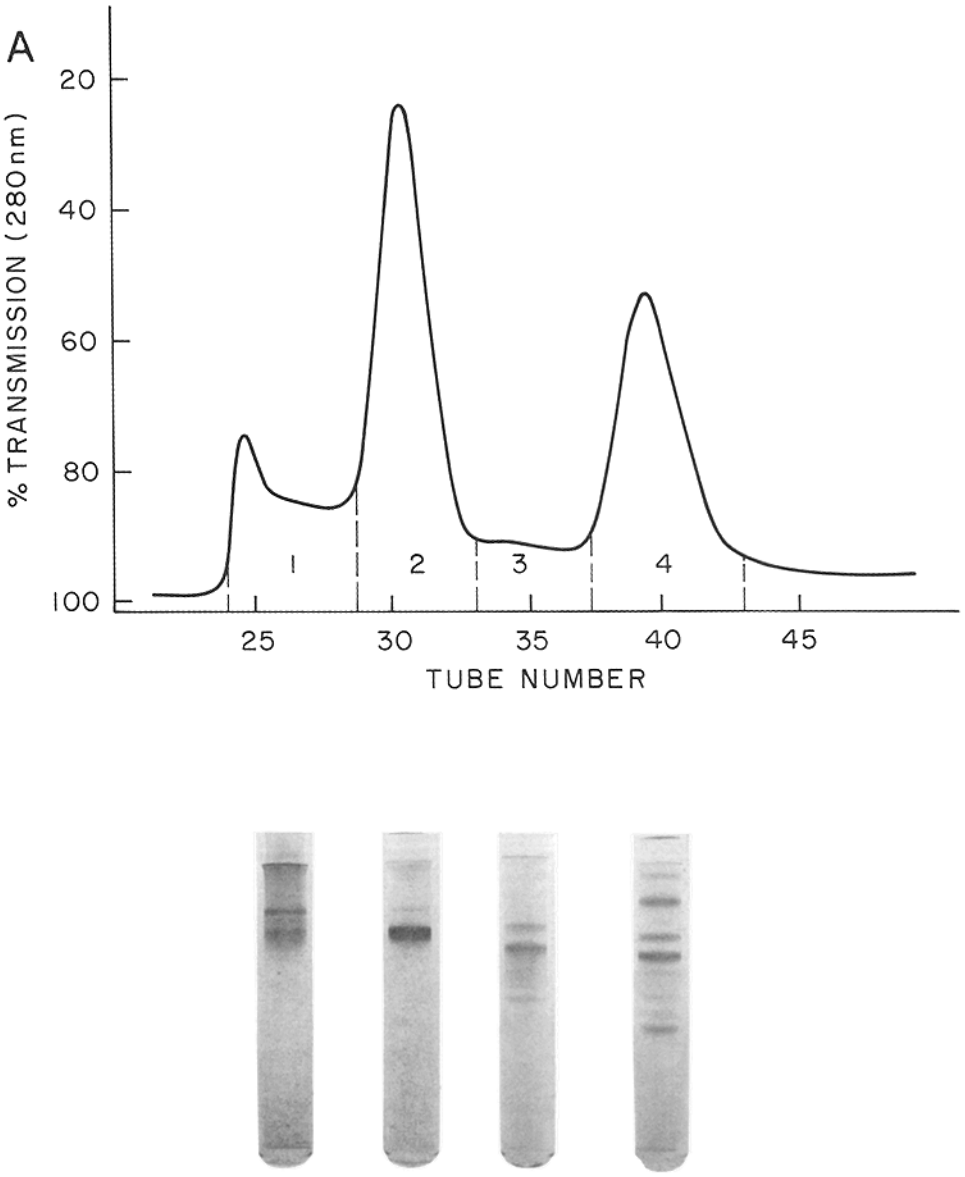
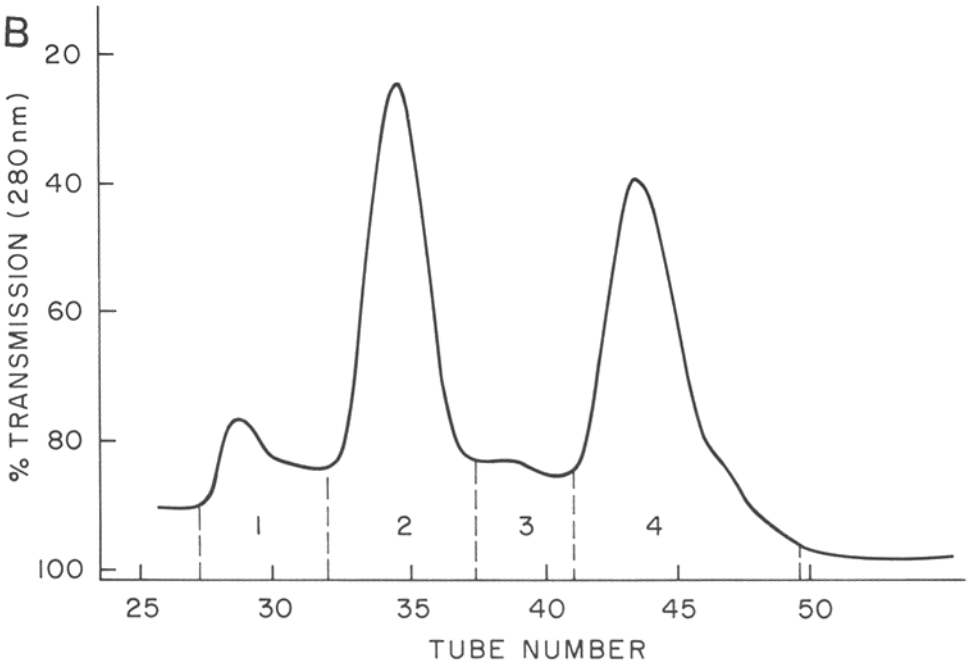


FIG. 3. Sephacryl S-200 chromatography of the SAA + apo HDL from patients with Waldenström's macroglobulinemia (A) and Goodpasture's syndrome (B). A 2.0 × 140-cm column was eluted with guanidine-HCl. Column fractions were pooled as indicated, and their apoproteins were visualized by anionic polyacrylamide gel electrophoresis (21).

Several protein species of low electrophoretic mobility were prominent in all SAA-rich fractions. The relative intensities (and presumably concentrations) of these protein bands were not the same in each patient. It is noteworthy that the quantitatively major SAA proteins had mobilities indistinguishable from apo A-I and apo A-II in the electrophoretic system employed.

Because the fraction 4 proteins were found by Ouchterlony double diffusion to be



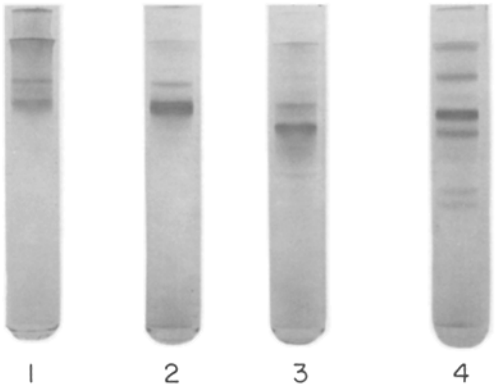


Fig. 3 B.

contaminated with apo A-I and apo A-II, they were rechromatographed on Sephadex G-100 before further purification was attempted. Apo C-I, which elutes after SAA from Sephadex G-100 and Sephacryl S-200, was also completely removed in the rechromatography step. Separation of the SAA and remaining C-apoproteins was then accomplished by DEAE chromatography. Six polymorphic forms of SAA were isolated from the fraction 4 proteins of the normal volunteers injected with etiocholanolone (Fig. 5 A) and from the patient with Waldenström's macroglobulinemia (Fig. 5 B). All of these were eluted by the gradient between apo C-I and apo C-II. The

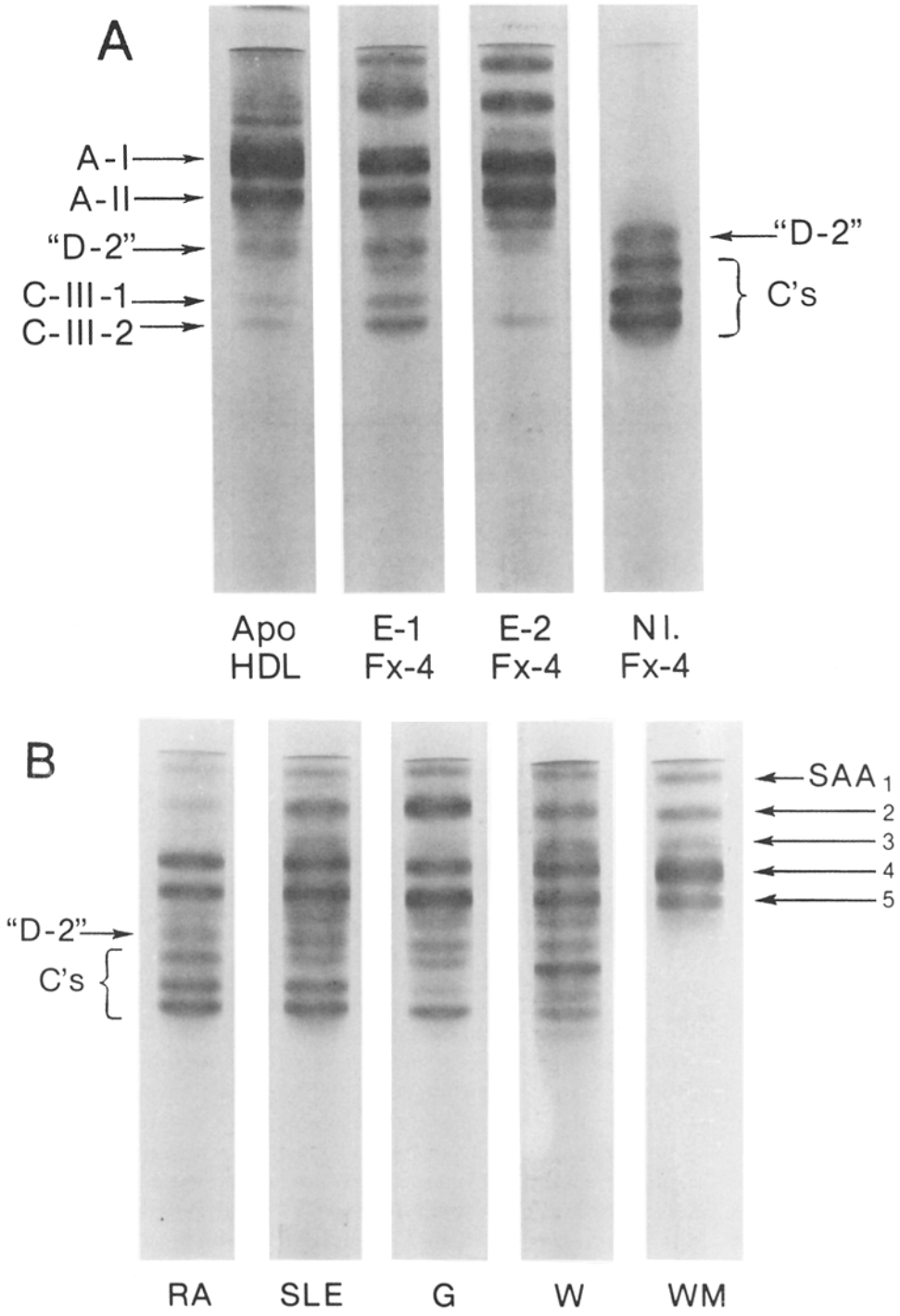


FIG. 4. (A) Comparison of the low molecular weight fractions of apo HDL from an SAA − preparation (N1 Fx-4) with those from the two normals after etiocholanolone-induced inflammation (E-1 and E-2) and with unfractionated normal apo HDL (apo HDL). Electrophoresis was performed in gels of 7.5% acrylamide monomer at pH 9.4 in 8 M urea (21). (B) Polyacrylamide gel electrophoretograms of the low molecular weight fractions of apo HDL from patients with rheumatoid arthritis (RA), systemic lupus erythematosis (SLE), Goodpasture's syndrome (G), Wegener's granulomatosis (W), and Waldenström's macroglobulinemia (WM).

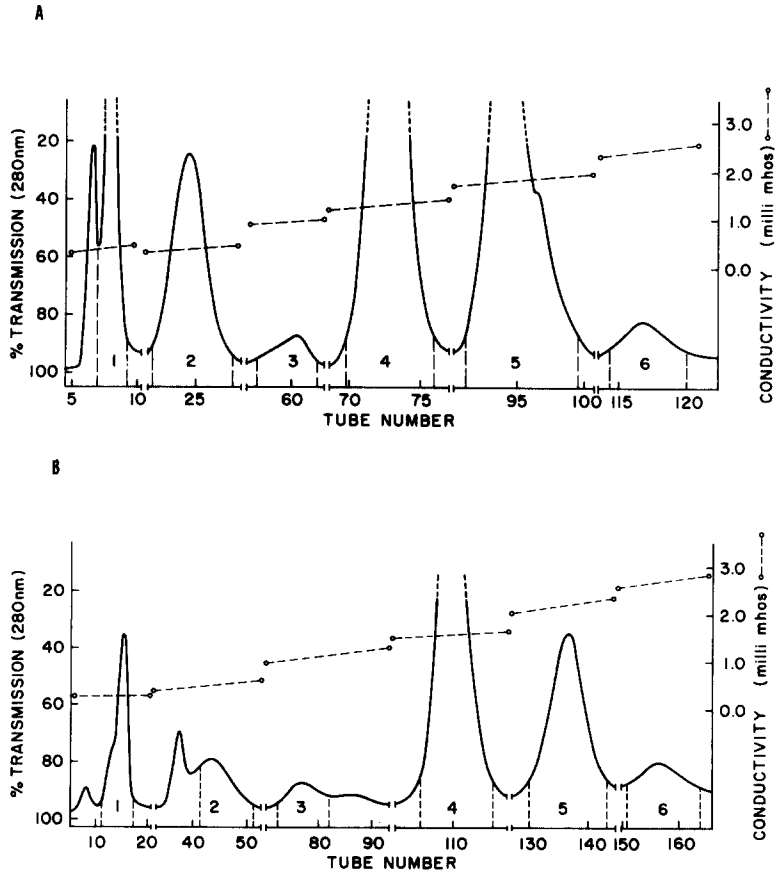


FIG. 5. DEAE-cellulose chromatography of the low molecular weight fractions of the SAA-enriched apo HDL from a normal subject after two injections of etiocholanolone (A) and from a patient with Waldenström's macroglobulinemia (B).

relative amounts of the SAA species were not identical, but the major forms eluting in the fourth and fifth peaks comprised between 63 and 68% of the total SAA in all patients and normals studied. Immunoassay indicated that SAA accounted for >95% of the protein in peaks 1–5 and somewhat less in peak 6. Homogeneity was also assessed by polyacrylamide gel electrophoresis in anionic, cationic, and SDS-polyacrylamide gel electrophoresis systems. In SDS (not shown) and at pH = 2.5 (Fig. 6) the six SAA polymorphs were indistinguishable. Consistent with their order of elution from DEAE, however, all had different mobilities in the anionic system (Fig. 6). All six fractions were quite pure except for peak 6, which contained an unidentified contaminant.

The amino acid compositions (Table I) of the six SAA forms were remarkably similar. None contained cysteine, and threonine could be confidently quantified only in fraction 1. All SAA polymorphs were rich in glycine, alanine, and arginine and were relatively deficient in glutamic acid/glutamine and leucine residues on comparison with the other apo (34).

The chemical bases of the SAA polymorphism have not yet been completely

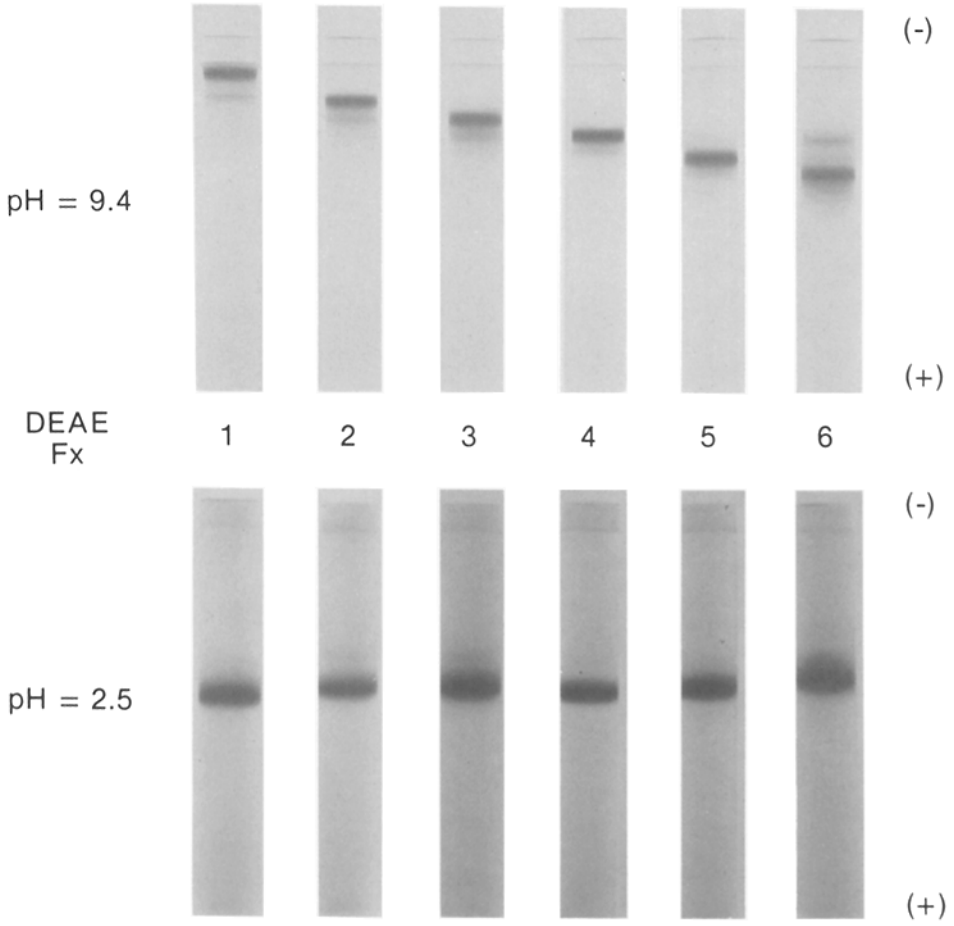


FIG. 6. Polyacrylamide gel electrophoresis of the SAA-containing DEAE fractions. Top panel: Electrophoresis in an anionic system (21) with 7.5% acrylamide and 8.0 M urea. Bottom panel: Analysis in a cationic system (22) with 5.0% acrylamide and 8.0 M urea.

defined. Incubation with neuraminidase did not alter the electrophoretic mobilities of any of the SAA polymorphs, and none stained with the periodate-Schiff reagent. Carboxypeptidase A released primarily tyrosine from all DEAE fractions. Timed carboxypeptidase A plus B digestions indicated the terminal sequence COOH-tyr-lys-phe-. Molecular weight estimates, calculated on the basis of tyrosine released, ranged from 11,100 to 11,900, consistent with the observed elution volumes from columns of Sephadex G-100. SDS-polyacrylamide gel electrophoresis demonstrated that SAA was smaller than lysozyme ($M_r \sim 14{,}300$) and slightly larger than apo C-II ($M_r \sim 9{,}000$). Because SDS-polyacrylamide gel electrophoresis does not provide good size discrimination at under 15,000 mol wt, mobilities of the various forms of SAA were compared on polyacrylamide gels with acrylamide monomer concentrations varying from 7.5 to 20% (24). All of the polymorphic forms of SAA had indistinguishable mobilities. Amino-terminal sequence analyses have been performed on the two major forms of

TABLE I

Amino Acid Composition of SAA Proteins Purified by DEAE Chromatography*

DEAE pool	1‡	2§	3‖	4§	5‖	6¶
	mol/100 mol of amino acid					
Aspartic acid + asparagine	14.1 (13.4–14.8)	14.2 (13.6–15.1)	13.9 (13.5–14.4)	13.9 (13.4–14.8)	14.2 (13.5–15.2)	13.8
Threonine	1.1 (1.0–1.2)	0.4 (0–1.6)	Trace	0.4 (0–1.0)	Trace	Trace
Serine	6.2 (5.8–6.5)	6.8 (6.6–7.1)	6.8 (5.3–8.3)	5.8 (5.7–5.9)	5.9 (5.6–6.2)	9.3
Glutamic acid + glutamine	8.5 (8.0–8.9)	8.7 (8.2–9.4)	9.2 (8.7–9.8)	9.1 (8.0–9.6)	9.4 (9.1–9.8)	11.5
Proline	3.8 (3.7–3.9)	3.9 (3.8–4.0)	3.6 (3.3–3.9)	3.9 (3.8–3.9)	3.8 (3.7–3.9)	3.7
Glycine	13.0 (12.7–13.2)	12.6 (11.9–13.8)	13.1 (12.3–14.4)	12.4 (11.6–13.4)	12.7 (12.2–13.3)	13.3
Alanine	14.5 (13.3–15.7)	15.1 (14.3–15.4)	15.6 (15.2–16.0)	15.4 (14.9–15.7)	15.4 (14.9–15.7)	14.3
Cysteine**	0	0	0	0	0	0
Valine	0.9 (0.5–1.2)	0.7 (0–1.1)	0.8 (0.7–0.9)	0.9 (0.5–1.8)	0.8 (0.6–1.0)	1.5
Methionine	1.8 (1.7–1.9)	1.9 (1.8–2.1)	1.7 (1.5–1.9)	1.9 (1.8–1.9)	1.8 (1.8–1.9)	1.6
Isoleucine	2.6 (2.3–2.8)	2.7 (2.4–3.1)	2.6 (2.5–2.6)	2.7 (2.3–3.1)	2.6 (2.2–2.8)	2.5
Leucine	4.6 (4.1–5.1)	4.4 (4.1–4.8)	3.9 (3.8–4.2)	3.6 (3.1–4.1)	3.2	3.7
Tyrosine	5.3 (5.0–5.6)	4.8 (4.3–5.1)	4.7 (4.6–4.8)	4.9 (4.8–5.0)	4.9 (4.8–5.0)	4.6
Phenylalanine	5.7 (5.4–5.9)	5.8 (5.5–6.1)	6.3 (6.0–6.7)	7.3 (5.9–7.9)	7.7 (7.5–7.9)	6.9
Histidine	2.8 (2.5–3.0)	2.8 (2.5–3.1)	3.2 (2.9–3.7)	2.9 (2.8–3.0)	2.9 (2.8–2.9)	3.6
Lysine	4.5 (4.2–4.7)	4.4 (3.9–5.4)	4.2 (4.1–4.3)	4.1 (4.0–4.2)	4.1 (4.0–4.2)	4.4
Tryptophan	1.8 (1.4–2.1)	1.8 (1.4–2.3)	1.5 (1.3–1.8)	1.8 (1.4–2.3)	2.1 (1.7–2.4)	0.9
Arginine	11.1 (10.8–11.4)	10.0 (9.5–10.7)	10.0 (9.5–10.3)	10.2 (9.3–11.4)	9.9 (9.2–10.3)	7.8

* Means (and ranges) after hydrolysis in 4 N methane sulfonic acid.

‡ Mean of two preparations from a normal subject and a patient Waldenström's macroglobulinemia.

§ Mean of four preparations from a normal subject and a patient with Waldenström's macroglobulinemia.

‖ Mean of three preparations from a normal subject and a patient with Waldenström's macroglobulinemia.

¶ Single determination from a normal subject.

** Cysteine determined as cysteic acid after performic acid oxidation (26).

SAA. Manual Edman degradation yielded the sequence NH_2-arg-ser-phe-phe- for the SAA in DEAE peak 4 and NH_2-ser-phe-phe- for the protein in peak 5.

Discussion

Segrest et al. (35) predicted, from knowledge of the primary structure of AA, that this protein could form amphipathic helixes and possibly bind lipid. An unusual AA protein of 45 residues sequenced by Ein et al. (36) was then shown to form stable complexes with dimyristoylphosphatidylcholine (35). These observations prompted Benditt and Eriksen (13) to search for SAA among the plasma lipoproteins. In studies of SAA in the plasma of a typhoid-immunized woman they found only ~2% of the SAA immunoreactivity in the fraction of density < 1.12 g/ml, which includes the very-low density, low density, and HDL_2 classes of lipoproteins. About 57% of the SAA was in the HDL_2 subfraction (1.12 < density < 1.21 g/ml), 17% in a zone of slightly higher density, and 15% was recovered with the bulk of serum proteins in the infranatant fraction (density > 1.21 g/ml).

Recognition that SAA is transported in association with HDL greatly facilitated its purification. Most potentially contaminating plasma proteins were removed by preparative ultracentrifugation. SAA proved to be of lower molecular weight than the major HDL apoproteins, and the latter were separated from SAA by molecular-sieve chromatography. DEAE cellulose chromatography in urea-containing buffers permitted isolation of relatively homogeneous preparations of six polymorphic forms of SAA. Identity of these proteins with SAA is supported both by an immunoassay employing AA standards and antiserum to AA, and by the amino acid compositions (Table I), which are very similar to those reported by others (9, 11, 12). These proteins,

moreover, have amino acid compositions very similar to two threonine-poor apo isolated by Shore et al. (37) from the sera of two men with coccidioidomycosis. One of the threonine-poor apo had an apparent molecular weight of 40,000 and dissociated into $M_r \sim 22{,}000$ subunits after mercaptoethanol treatment. The other, of nearly identical amino acid composition, had an 11,000 mol wt. These investigators attributed the enrichment of HDL with these proteins to amphotericin B treatment, but it would appear probable that they represent SAA. However, we have not observed dimeric forms of SAA, and none of the polymorphic forms reported here contain cysteine.

The SAA heterogeneity we have documented cannot be attributed to large differences in molecular weight, and carbohydration does not appear to account for the polymorphism. Variability at the COOH-terminals of the SAA proteins is unlikely because all six forms isolated had COOH-terminal tyrosine. Sufficient quantities of the major SAA species, SAA_4 and SAA_5 (Fig. 7), were available for NH_2-terminal residue analyses. SAA_5, unlike SAA_4, lacked the NH_2-terminal arginine residue. The NH_2-terminal sequence NH_2-arg-ser-phe-phe- has been reported for both AA (1, 2, 36, 38) and SAA (11, 12). Levin et al. (1), however, noted that serine, rather than arginine, was the principal NH_2-terminal residue identified in AA from a patient with familial Mediterranean fever, whereas only small amounts of arginine were present. Rosenthal et al. (11) found NH_2-terminal heterogeneity in their SAA preparation, whereas Anders et al. (12) reported that 40% of an SAA preparation they analyzed did not have NH_2-terminal arginine.

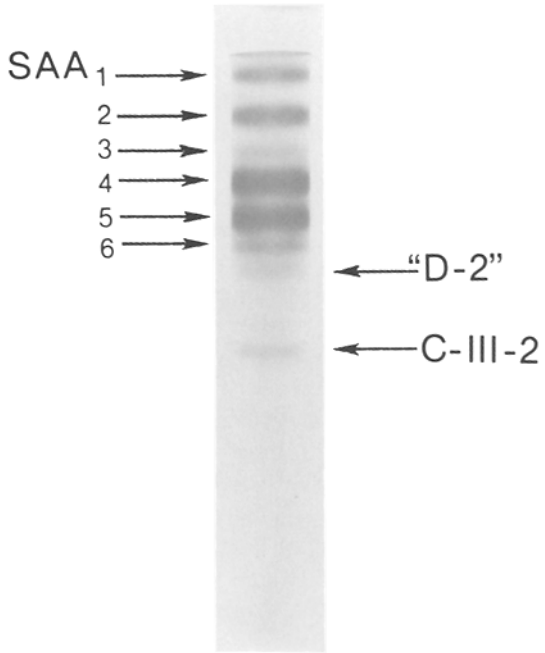


FIG. 7. Designation of the polymorphic forms of SAA identified by polyacrylamide gel electrophoresis and isolated by DEAE-cellulose chromatography (Fig. 6). Apo "D-2" (33) and C-III-2 are also visualized in this low molecular weight fraction from the apo HDL of the patient with Waldenström's macroglobulinemia.

TABLE II

Comparison of the Amino Acid Contents of SAA and AA

	SAA_4		AA*	Fragment cleaved
Aspartic acid/asparagine	(15.1)	15‡	10	5
Threonine	(0.4)	0–1	1	0
Serine	(6.3)	6	6	0
Glutamic acid/glutamine	(9.9)	10	6	4
Proline	(4.2)	4	1	3
Glycine	(13.47)	13	9	4
Alanine	(16.7)	17	12	5
Cysteine	(0)	0	0	0
Valine	(1.0)	1	1	0
Methionine	(2.0)	2	2	0
Isoleucine	(3.0)	3	3	0
Leucine	(3.9)	4	2	2
Tyrosine	(5.3)	5	4	1
Phenylalanine	(7.9)	8	5	3
Histidine	(3.2)	3	1	2
Lysine	(4.45)	4	2	2
Tryptophan	(2.0)	2	1	1
Arginine	(11.0)	11	10	1
Total residues		109	76	33

* Calculated assuming a single valine.

‡ From Levin et al. (1).

The absence of an arginine residue from SAA_4 could account for the different electrophoretic mobilities of SAA_4 and SAA_5 (Fig. 7). It is unlikely, however, that all SAA charge heterogeneity documented is attributable to NH_2-terminal differences. If the low electrophoretic mobility of SAA_1 relative to SAA_5 were a result of NH_2-terminal differences, then SAA_1 would be expected to contain about five more lysine ($pK_a \sim 9.4–10.6$) or arginine ($pK_a \sim 12.5$) residues in the NH_2-terminal region. The amino acid compositions of SAA_1 and SAA_5 (Table I) do not support the contention that such differences in basic amino acid contents exist. Deamidation of glutamine or asparagine residues during purification is similarly unlikely. SAA_1 is soluble in acidic, rather than basic, buffers, and prolonged storage in 0.1 N acetic acid has not altered its electrophoretic mobility. Moreover, artifactual polymorphism from deamidation has not been produced in other apo simultaneously purified.

The possibility that SAA microheterogeneity reflects different cellular origins of the proteins, and perhaps varying degrees of posttranslational modification (39), remains to be tested. Rosenthal and Sullivan (40) have provided evidence for a polymorphonuclear leukocyte origin of SAA, and synthesis by connective tissue and fibroblasts has been suggested (41). It has also been argued from the time-course of SAA appearance and tissue distribution that the liver is capable of SAA synthesis (42, 43). Selinger et al. (44) have recently demonstrated that SAA can be synthesized in vitro by isolated mouse hepatocytes. This SAA synthesis was induced by a soluble factor derived from stimulated macrophages, which suggests that there is immunological control of the hepatic synthesis of this apo. Gorevic et al. (45), moreover, have provided evidence that murine AA and SAA are polymorphic, thus raising the possibility that the SAA heterogeneity may be a tissue-specific phenomenon.

Assuming an 11,000–12,000 mol wt, we have calculated that SAA contains ~109 residues (Table II). If SAA and AA are related as precursor and product, then a peptide of ~33 residues must be specifically cleaved from SAA before its tissue deposition. It might be speculated that AA is protected from more extensive proteolysis because of its lipoprotein association, but it is noteworthy that a protein identical to AA has not yet been identified among the low molecular weight proteins of HDL. The plasma residence time of the SAA apoproteins, as judged from the response of the normals injected with etiocholanolone (15), is considerably less than that of apo A-I and apo A-II but may not differ greatly from that of the C-apo (46). Thus, whereas the rapid turnover of SAA may be related to biological properties not yet defined, the possibility that SAA proteins are normally catabolized like other apo will warrant investigation.

Summary

Serum amyloid A proteins (SAA), presumed precursors of the tissue amyloid A proteins (AA) characteristic of secondary amyloidosis, have been isolated from the plasma high-density lipoproteins (HDL) of normals after etiocholanolone-induced inflammation and from patients with Wegener's granulomatosis, systemic lupus erythematosis, juvenile rheumatoid arthritis, Waldenström's macroglobulinemia, and Goodpasture's syndrome. At least six polymorphic forms of SAA were identified among the low molecular weight proteins of HDL, and these comprised up to 27% of the total HDL protein. Gel and ion-exchange chromatography permitted isolation of the SAA polymorphs in homogeneous form. Their amino acid compositions were very similar, they were indistinguishable in cationic and sodium dodecyl sulfate-polyacrylamide gel electrophoresis systems, and each had the terminal sequence COOH-Tyr-Lys-Phe-. Charge heterogeneity in anionic-urea polyacrylamide gel electropherograms was unaffected by neuraminidase treatment, and none of the SAA protein bands stained with the periodate-Schiff reagent.

The two major SAA polymorphs, designated SAA_4 and SAA_5 according to their order of elution from DEAE-cellulose, had different NH_2-terminal sequences. Manual Edman degradation demonstrated NH_2-arg-ser-phe-phe- for SAA_4 and NH_2-ser-phe-phe- for SAA_5. This NH_2-terminal heterogeneity corresponds to that most frequently reported for AA and suggests that microheterogeneity in SAA may underlie that already documented in AA. Sufficient quantities of the other SAA polymorphs were not available for similar analyses, but the amino acid compositions do not indicate that NH_2-terminal heterogeneity accounts for all of the observed polymorphism. Artifactual polymorphism also appears unlikely, and the heterogeneity of SAA may reflect origin from more than one cell type with or without posttranslational modification.

We calculate from quantitative COOH-terminal analyses that SAA is of 11,000–11,900 mol wt. Primary structure studies have shown AA to be a single chain protein of 76 residues, and SAA, therefore, appears to contain a peptide of 33 amino acids that is missing from AA.

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