

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 5
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
ALVARO CORREA ORDOÑEZ

Asunto: Radicación: 11-176414- -5
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 11181
Folios: 5

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2010/037542				
Fecha de Presentación Internacional	04/06/2010				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 90 a 159	21/Diciembre/2011
Reivindicaciones:	Folios 160 a 176	21/Diciembre/2011
Dibujos:	Folios 178 a 185	21/Diciembre/2011
Secuencias:	Folios 186 a 189	21/Diciembre/2011
Prioridad:	US 61 217 931	04/Junio/2009

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. Para la presente solicitud, se estudió el capítulo reivindicatorio completo.

Título de la solicitud: “Derivados de Suero Amiloide P, su preparación y uso”.

El problema planteado en esta solicitud consiste en la necesidad de desarrollar agentes terapéuticos en cantidades suficientes, útiles para tratar diversos trastornos como fibrosis, mucositis, trastornos autoinmunes y trastornos inflamatorios causados por infecciones microbianas.

Para resolver este problema técnico la solicitud en estudio proporciona polipéptidos glicosilados Amiloide P de suero humano (SAP) y métodos para su producción.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K 14; A61K 38/17; A61P 29/00.

4. Excepción a la Patentabilidad (Art 20 D 486 literales (c) - (d))

El objeto de las reivindicaciones 30 – 35 y 100, las cuales se refieren a un método de tratamiento en humanos, no es patentable de acuerdo con el artículo citado.

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio carece de claridad y concisión, por cuanto:

- 1) Un polipéptido glicosilado debe ser reclamado por su estructura, es decir, por su secuencia de aminoácidos, identificada con un número de identificación SEQ ID N°, y por la estructura tridimensional de la cadena de oligosacáridos (reivindicación 1);
- 2) Las expresiones “en donde al menos...”, “tiene al menos 50% menos...”, “termina con una parte de...”, “termina con partes de...”, “comprende al menos una...”, “está sustancialmente libre...”, son imprecisas por lo cual no permiten delimitar la materia que se quiere proteger. Se sugiere sustituirlo por términos concretos (reivindicaciones 1 – 4, 9 – 16);
- 3) Se sugiere indicar las modificaciones hechas a la cadena de oligosacáridos del polipéptido glicosilado para que ésta tenga una estructura diferencial bi-, tri-, tetra- o penta-antenaria (reivindicaciones 5 – 8);
- 4) En las reivindicaciones 15, 52, 76 y 91, las cuales hacen referencia a un residuo glicosilo modificado de la cadena de oligosacáridos, se debe especificar el tipo de modificación reclamada, para tener claridad sobre el alcance de la invención. De igual manera en las reivindicaciones 16, 53, 77 y 92 se debe especificar los conjugados empleados;
- 5) Un polipéptido no debe definirse por porcentajes de identidad ya que esto no permite diferenciar la invención del estado de la técnica (reivindicaciones 18 y 19);
- 6) La reivindicación 20, la cual hace referencia al polipéptido como una proteína de fusión que comprende uno o más dominios heterólogos, no es admisible puesto que no se especifican las características esenciales de dichos dominios, y por lo tanto, no se puede tener claridad sobre el real alcance de la invención;
- 7) Las reivindicaciones 21, 24, 25, 45, 61, 78, 93, 95, 96 y 99 están expresadas en términos de un resultado a alcanzar, lo cual no es limitativo para la invención, puesto

que la solución propuesta incluiría no solo la invención aquí descrita, sino toda aquella realización que llegue a esos mismos resultados. Se sugiere hacer las aclaraciones necesarias, invocando las características esenciales de la invención que le permiten la consecución de dichos resultados;

- 8) En la reivindicación 22 se deben especificar las modificaciones y las posiciones en la secuencia de aminoácidos;
- 9) Una composición farmacéutica debe estar definida por su estructura en términos de sus elementos esenciales (compuesto activo y excipientes) y sus proporciones (reivindicaciones 27 y 28);
- 10) Las reivindicaciones de procedimiento deben referirse a unas actividades organizadas en una serie de pasos de manera determinada. El método para hacer un polipéptido SAP humano, de las reivindicaciones 36, 37 y 94 corresponde a un resultado a alcanzar, puesto que se refieren a la obtención de un polipéptido aislado, sin indicar la técnica para expresar el polipéptido en una célula, las condiciones necesarias para el cultivo de dicha célula, ni los procedimientos para aislar el polipéptido de la célula. Se sugiere caracterizar dicho método a partir de las etapas específicas que posee;
- 11) Las reivindicaciones de procedimiento deben referirse a unas actividades organizadas en una serie de pasos de manera determinada. El método para hacer un polipéptido SAP humano, de las reivindicaciones 62 y 79 corresponde a un resultado a alcanzar, puesto que se refieren a la obtención de un polipéptido, sin indicar la forma y las condiciones en las que se va a suministrar un polipéptido SAP, ni los procedimientos para lograr la alteración enzimática o química para producir dicho polipéptido. Se sugiere caracterizar dicho método a partir de las etapas específicas que posee.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en 4 de Junio de 2009, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1 ¹	Pepys <i>et al.</i> 1994	"Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure"	Junio 1994
D2 ²	Mantzouranis <i>et al.</i> 1985	"Human serum amyloid P component. cDNA isolation, complete sequence of pre-serum amyloid P component, and localization of the gene to chromosome"	Junio 1985
D3 ³	AU 712819 B2	"Pharmaceutical composition comprising serum amyloid P component for prophylactic or therapeutic treatment of virus infections and kit for detecting binding of compositions to virus components"	09/Octubre/1997

7. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

- La reivindicación 1 consiste en un polipéptido glicosilado Amiloide P de suero humano (SAP) que comprende una cadena de oligosacáridos N-ligados, en donde al menos

1 Pepys *et al.* 1994. "Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure". Proc. Natl. Acad. Sci. 91: 5602-5606.

2 Mantzouranis *et al.* 1985. Human serum amyloid P component. cDNA isolation, complete sequence of pre-serum amyloid P component, and localization of the gene to chromosome 1. J Biol Chem 260(12):7752-6.

3 http://www.patentlens.net/patentlens/patents.html?patnums=AU_712819_B2

una rama de la cadena de oligosacáridos termina con una parte de ácido siálico α2,3-ligada.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Polipéptido glicosilado Amiloide P de suero humano (SAP)	Proteína SAP (Pág. 5602)
cadena de oligosacáridos N-ligados	Que contiene una sola cadena de oligosacáridos ligada (Pág. 5604 resultados)
al menos una rama de la cadena de oligosacáridos termina con una parte de ácido siálico α2,3-ligada	Puede contener dos terminaciones de ácido siálico o ser monosiliada siendo α2,3 o α2,6 (Pág. 5603 figura 1, Pág. 5604 resultados)

Las características esenciales del polipéptido de la reivindicación 1 habían sido divulgadas previamente en el documento D1, el cual da a conocer la glicoestructura del componente SAP humano que consiste en un oligosacárido N-ligado biantenarico (Resumen) que puede contener dos terminaciones de ácido siálico o ser monosiliada siendo α2,3 o α2,6 (Pág. 5603 figura 1, Pág. 5604 resultados).

En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 3, 5 y 9 – 13 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

De igual manera, las características esenciales de la reivindicación 2, que consiste en un polipéptido glicosilado Amiloide P de suero humano (SAP) que comprende una cadena de oligosacáridos N-ligados, en donde la cadena de oligosacáridos tiene al menos 50% menos de partes de ácido siálico α2,6-ligadas, ya habían sido divulgadas en el documento D1.

Por lo tanto, la reivindicación 2 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 4 – 5 y 9 – 13 no mencionan alguna característica que haga nueva a la composición de la reivindicación 2, por lo cual se considera que no son nuevas.

- La reivindicación 62 consiste en un método para hacer un polipéptido SAP, que comprende a) suministrar un polipéptido SAP glicosilado, que comprende una cadena de oligosacaridos N-ligados, y b) alterar enzimáticamente o químicamente la cadena de oligosacáridos del polipéptido SAP.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Método para hacer un polipéptido SAP	Modificaciones a un polipéptido SAP silvestre (Pág. 5602)
Etapas a).	Proveer un polipéptido SAB que comprende una cadena oligosacáridos N-ligados (Pág. 5602)
Etapas b).	Alteración enzimática de la cadena de oligosacaridos de SAB con enzimas glicosidasas (Pág. 5602)

Las características esenciales del método de la reivindicación 62 habían sido divulgadas previamente en el documento D1, el cual da a conocer modificaciones de SAP humano

que consisten en la remoción de los ácidos siálicos de las terminaciones de las ramas de la cadena de oligosacaridos N-ligados, la desgalactosilación y la resialilación (Pág. 13).

Por lo tanto, la reivindicación 62 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones 63 – 65, 68 – 70, 74 – 76, 79 – 80, 83 – 85, 89 - 91 no mencionan alguna característica que haga nueva al método de la reivindicación 62, por lo cual se considera que no son nuevas.

Nivel inventivo (Art18 D486)

- Las reivindicaciones 18 y 19 consisten en un polipéptido glicosilado que comprenden respectivamente una secuencia de aminoácidos por lo menos 85% o 95% idéntica a la SEQ ID N° 1.

La secuencia SEQ ID N° 1 de la presente solicitud se encontró 99% idéntica a la secuencia de aminoácidos al componente de suero amiloide P humano aislado de la naturaleza, según reposa en GenBank: AAA60302.1 con fecha de ingreso a la base de datos de 9 de enero de 1995 y divulgado en el documento D2.

Por lo tanto, modificaciones de aminoácidos que resulten en una secuencia casi idéntica a un polipéptido tal como se encuentra en la naturaleza, no representa nivel inventivo, puesto que modificaciones o mutaciones de aminoácidos pueden ocurrir *invivo* durante los procesos moleculares naturales de la célula.

En consecuencia, las reivindicaciones 18 y 19 no cumplen con el requisito de nivel inventivo.

- La reivindicación 26 reclama una preparación farmacéutica para uso en mamíferos que comprende el polipéptido SAB de las reivindicaciones 1 a 25 y un portador farmacéuticamente aceptable.

Las características esenciales de la reivindicación 26 ya habían sido divulgadas previamente en el documento D3, el cual enseña una composición farmacéutica que comprende un componente SAP nativo o recombinante y un vehículo farmacéuticamente aceptable que es formulada para administración nasal (Pág. 1 líneas 5 - 30).

De este modo, la reivindicación 26 carece de nivel inventivo. Las reivindicaciones dependientes 27 – 29 no adicionan características a la composición enseñada en el documento D3, por lo tanto tampoco cumplen con el requisito de nivel inventivo.

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Pepys <i>et al.</i> 1994	1 – 5 y 9 – 13	Novedad
D2	Mantzouranis <i>et al.</i> 1985	63 – 65, 68 – 70, 74 – 76, 79 – 80, 83 – 85, 89 - 91	Novedad
D1	Pepys <i>et al.</i> 1994	18 y 19	Nivel inventivo
D3	AU 712819 B2	26 - 29	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-07-02

Elaboró: Edna Carolina Bonilla Leon

Revisó: Consuelo Leguizamon Leguizamon

Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure

(glycoprotein metabolism/mass spectrometry/scintigraphy)

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Communicated by David Weatherall, March 14, 1994 (received for review January 7, 1994)

ABSTRACT Human serum amyloid P component (SAP) is a normal plasma protein and the precursor of amyloid P component (AP), a universal constituent of the abnormal tissue deposits in amyloidosis, including Alzheimer disease. We show here that its single N-linked biantennary oligosaccharide does not display the microheterogeneity usually characteristic of glycoproteins. The protein and the glycan structures of AP were also invariant, their resistance to degradation suggesting a role in persistence of amyloid deposits. Asialo-SAP was rapidly cleared from the circulation in mice by a mechanism dependent on terminal galactose residues and was catabolized in hepatocytes. However blockade of this pathway did not affect the clearance of native SAP. Rapid hepatic uptake and catabolism of human asialo-SAP in man were also directly demonstrated. The protein and glycan homogeneity of SAP and the integrity of AP suggest that the complete glycoprotein structure is important for the normal and the pathophysiological functions of this molecule.

Human serum amyloid P component (SAP) is a normal plasma protein (1) and the precursor of amyloid P component (AP) (2), a universal constituent of the abnormal tissue deposits in amyloidosis (3), including Alzheimer disease (4, 5). AP remains in amyloid deposits for prolonged periods without protein catabolism (6) and might thereby contribute to the persistence which underlies their pathogenicity. However, the structure of AP has not been well characterized. AP from only one individual has been completely sequenced at the protein level (7), and the two reports of the SAP cDNA sequence (8, 9), as well as preliminary reports of the glycostructure (10, 11, 37), are conflicting. We have therefore compared the complete covalent structures of SAP and AP, with particular emphasis on the oligosaccharide and its role in turnover of the SAP glycoprotein.

MATERIALS AND METHODS

Proteins. SAP was isolated from the serum (13) of two healthy subjects, from two large pools of normal serum containing 1-ml aliquots from each of >5000 different healthy volunteer blood donors, and from a pool of malignant effusion fluids containing material from >300 donors. AP was isolated from two spleens containing AA amyloid, two spleens and two livers with AL amyloid, one spleen containing [Arg⁶⁰]apolipoprotein A-I amyloid, and the spleen, liver, heart, kidney, and adrenals of an individual patient with AA amyloidosis. Amyloidotic tissue was homogenized in 0.01 M Tris/0.14 M NaCl/0.01 M EDTA, pH 8.0, and the superna-

tant after centrifugation at 50,000 × *g* for 30 min was dialyzed extensively into 0.01 M Tris/0.14 M NaCl/0.002 M CaCl₂, pH 8.0, before extraction of AP precisely as described for SAP from serum (13). All SAP/AP preparations were >99% pure in heavily overloaded SDS/polyacrylamide gradient gels (Excelgel, Pharmacia) run under reducing conditions and stained with brilliant blue R-350 or with silver.

Glycan Structure Analysis. Oligosaccharides attached to SAP and AP were released by hydrazinolysis and radiolabeled with sodium borotritide at the reducing terminus, which incorporates a single label into each chain independently of its structure, so that the relative molar proportions of all glycans present can be estimated directly (14). These oligosaccharides were analyzed by high-voltage paper electrophoresis in pyridine/acetic acid water (3:1:387 by volume) at pH 5.4, 80 V/cm. They were then converted to neutral glycans by neuraminidase digestion and were analyzed on a high-resolution 1.5 cm × 2 m Bio-Gel P-4 (<400 mesh) column by elution at 55°C with water (14). The structure was determined by sequential exoglycosidase digestion in combination with permethylation and GS-MS (14). Heterogeneity of sialic acid linkage in the A-1 and A-2 structures was determined by digestion with neuraminidase from Newcastle disease virus, specific for α2-3 bonds, and neuraminidase from *Arthrobacter ureafaciens*, which cleaves α2-3(6) bonds, followed by selective exoglycosidase digestion and product analysis using permethylation and GC-MS (14).

Electrospray (ES)-MS. Purified SAP and AP were lyophilized after dialysis into pure water, dissolved in 2% acetic acid, diluted with acetonitrile to give a 50:50 water/acetonitrile mixture at a concentration of about 20 μM, and analyzed by electrospray ionization (15) on the VG BIO-Q quadrupole mass spectrometer (Fisons, Altrincham, Cheshire, U.K.). Data were acquired at 10 sec per scan over the *m/z* range 1000–1900 after introduction of 5 μl of sample into a solvent flow at 3 μl/min. The mass scale was calibrated with the multiply charged ions from a separate introduction of 5 μl of 20 μM bovine trypsinogen.

Oligosaccharide Modification. Bovine α₁ acid glycoprotein (AGP) (Sigma) was desialylated by incubation at 80°C for 1 hr at 2–5 mg/ml in 12.5 mM H₂SO₄ (16), resulting in the expected alteration in electrophoretic mobility in 1% agarose gel in pH 8.6 Veronal buffer. Human SAP was desialylated by incubation for 4 hr at 37°C at 10 mg/ml in pH 5.0 acetate buffer, with *Arthrobacter ureafaciens* neuraminidase, 5 units/ml, free of other glycosidase or proteinase activity

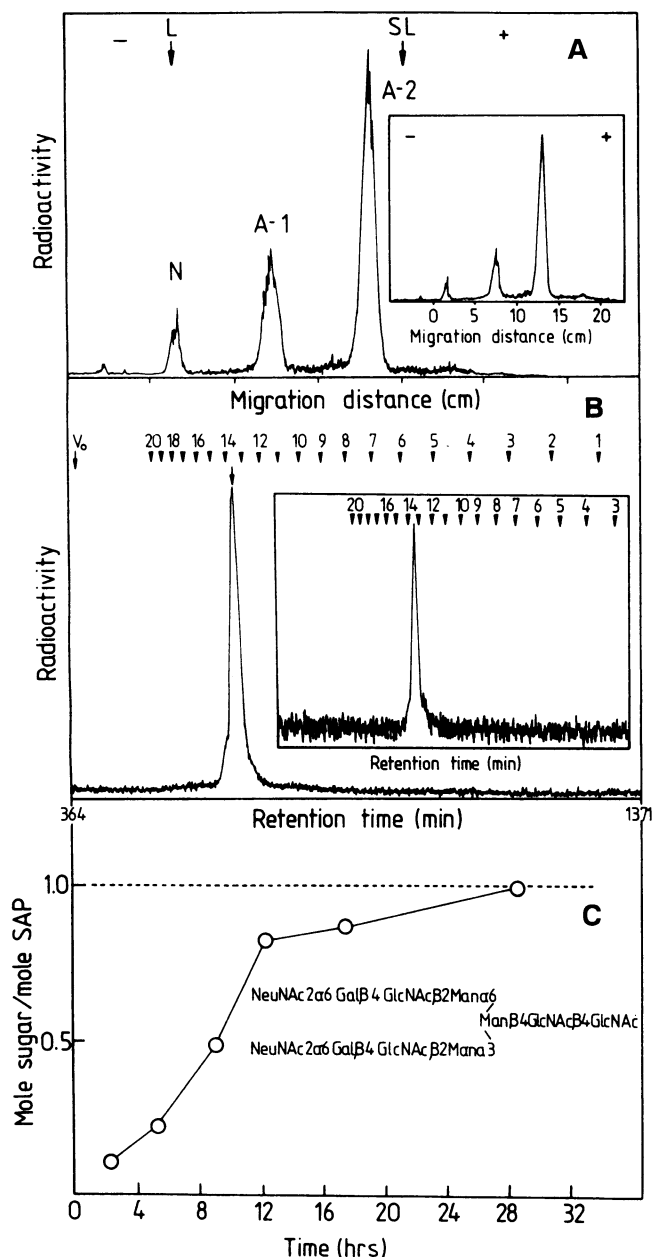


FIG. 1. Glycostructure of SAP. (A) Electrophoretic separation of oligosaccharides released from SAP isolated from normal sera, or, *Inset*, SAP from malignant effusion fluids; AP preparations from amyloidotic tissues gave identical results. A-2, structures containing two terminal sialic acid; A-1, monosialylated structures; N, neutral structures. Lactitol (L) and mixed α 2-6/ α 2-3 sialyl-lactitol (SL) were run as markers on the same paper. (B) Size-exclusion chromatography of the neutral glycan derived from the released oligosaccharides following neuraminidase digestion. Typical profiles of glycans from a preparation of normal SAP and, *Inset*, SAP from malignant effusion fluids are shown. Numbered arrows indicate the elution positions of glucose oligomers coinjected with the radiolabeled oligosaccharides. A single peak at 13.5 glucose units was found in all samples. All attempts to generate alternative sequences by nonsequential exoglycosidase digestions were unsuccessful. V_0 , void volume. AP gave identical results. (C) Release of glycan as a function of time in hydrazine. The structure of the fully sialylated form of the oligosaccharide is indicated.

(Oxford GlycoSystems, Abingdon, Oxon, U.K.). Complete removal of sialic acid was confirmed by electrophoresis and by ES-MS analysis (see Fig. 2) demonstrating that the molecular mass of the major component was 24,882.3 Da. This also confirmed the integrity of the remaining glycopro-

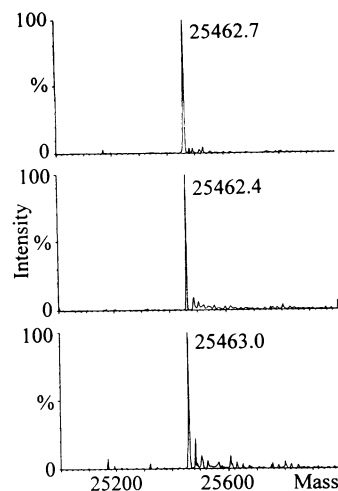


FIG. 2. ES-MS. (Top) SAP from the pooled serum of >5000 normal donors. (Middle) AP from the spleen of an individual dying with AL amyloid. (Bottom) AP from the spleen of an individual dying with AA amyloid.

tein, in particular the absence of any further degradation products of lower mass. Asialo-SAP at 8 mg/ml in 0.1 M acetate/2 mM EDTA, pH 6.0, was degalactosylated by incubation with *Streptococcus pneumoniae* β -galactosidase (Oxford GlycoSystems) at 80 units/ml. Samples taken at 4, 24, and 48 hr all showed the same anodal electrophoretic mobility shift compared with the asialo-SAP starting material. Degalactosylation was confirmed by ES-MS analysis, which demonstrated the molecular mass of the major component to be 24,553.7 Da, consistent with removal of two sialic and two galactose residues from each SAP glycoprotein protomer. For resialylation, asialo-SAP at 17 μ M with respect to glycoprotein subunits was incubated at 37°C for 12 hr with 140 μ M cytidine 5'-monophospho-*N*-acetylneuraminic acid (Sigma), bovine serum albumin (830 μ g/ml), and rat liver sialyltransferase (33 milliunits/ml) (Sigma) in 50 mM sodium cacodylate/0.5% (vol/vol) Triton CF-54 at pH 6.0. Acquisition of sialic residues was confirmed by complete restoration of the normal anodal electrophoretic mobility of the SAP.

Turnover Studies in Mice. SAP was oxidatively iodinated with 125 I (2–5 MBq/mg) by using *N*-bromosuccinimide (17). Female CBA/Ca mice (Bantin & Kingman, Hull, U.K.), 6–12 weeks old, each received 2–10 μ g of 125 I-SAP in 200 μ l intravenously. In experiments with AGP and asialo-AGP, this was administered simultaneously. Precisely measured tail bleeds (0.01–0.04 g) were taken at the time intervals shown and trichloroacetic acid-precipitable radioactivity was counted in the same run at the end of each experiment together with standard aliquots of the injected tracer.

Trapped Catabolism Studies. 125 I-tyramine cellobiose (125 I-TCB) was synthesized and conjugated to SAP as described (18). Both intact SAP and asialo-SAP labeled by covalent attachment of 125 I-TCB retained in full their *in vitro* ligand-binding capacity (13), and the plasma clearance of 125 I-TCB-labeled intact SAP was identical with that of directly iodinated intact SAP. Since 125 I-TCB labeling did not alter the properties or behavior of the protein, the site of catabolism of the 125 I-TCB-SAP complex therefore indicates the site of catabolism of native SAP. After intravenous injection of 125 I-TCB-labeled protein and the usual plasma clearance study, mice were killed at 24 hr, the organs were removed for measurement of radioactivity individually, and histological autoradiographs of the livers were prepared (19).

Turnover and Imaging Studies in Man. SAP for use in man was isolated from the plasma of a single normal accredited

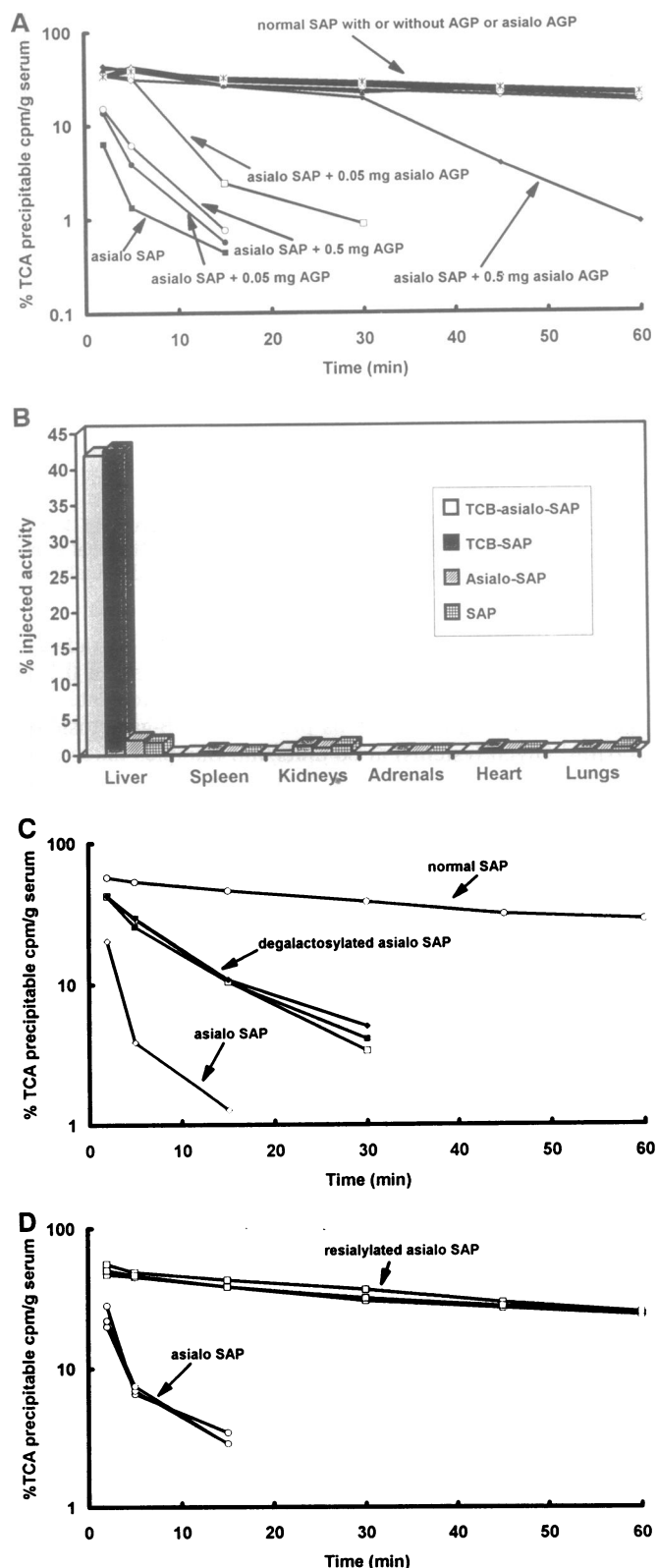


FIG. 3. *In vivo* clearance of human SAP from the circulation in mice. (A) Plasma clearance of ^{125}I -labeled normal and asialo-SAP and the effect of simultaneous injection of AGP or asialo-AGP. $\blacksquare$, Asialo-SAP; $\bullet$, asialo-SAP plus 0.05 mg of AGP; $\circ$, asialo-SAP plus 0.5 mg of AGP; $\square$, asialo-SAP plus 0.05 mg of asialo-AGP; $\blacklozenge$, asialo-SAP plus 0.5 mg of asialo-AGP. The remaining, overlapping lines represent normal SAP with or without 0.05 mg or 0.5 mg of AGP or asialo-AGP. Each point is the mean of three mice in each group; for all points SD was $<10\%$ of the mean. TCA, trichloroacetic acid. (B) Organ distribution of radioactivity 24 hr after injection of normally sialylated SAP or asialo-SAP labeled either directly by oxida-

tion and was oxidatively iodinated with ^{125}I (2–5 MBq/mg) or ^{123}I (110 MBq/50 μg of protein) by using *N*-bromosuccinimide, all as previously described (6, 20, 21). Asialo-SAP for human *in vivo* use was prepared by using *Arthobacter ureafaciens* neuraminidase which had been coupled under sterile conditions to CNBr-Sepharose (Pharmacia). It was sterile with no bacterial growth at 5 days; quantitative *Limulus* amoebocyte lysate assay (Coatest, KabiVitrum, Stockholm) detected only 230 pg of endotoxin per ml. Desialylation was confirmed by electrophoresis. Clearance of ^{125}I -labeled normal and asialo-SAP was studied in three normal healthy male volunteers aged 32, 33, and 36 years, as previously described (6), except that for asialo-SAP, frequent early samples were collected as shown. After injection of ^{123}I -asialo-SAP, data were acquired and processed on an IGE Starcam γ camera (IGE Medical Systems, Slough, U.K.).

RESULTS

Glycostructure of SAP and AP. Release of glycan as a function of time in hydrazine in the presence of an internal standard demonstrated that each protomer of SAP and AP contained only a single oligosaccharide, analysis of which showed the same single structure at all time points (Fig. 1). Exoglycosidase sequencing combined with size-exclusion chromatography (Fig. 1B) and permethylation analysis using GC-MS were consistent with this structure having the formula $\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 6(\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 3)\text{-Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc-ol}$, the fully sialylated form of which is shown in Fig. 1C. Over 70% of the structures in most preparations contained two terminal sialic residues; about 25% were monosialylated and the remainder were neutral (Fig. 1A). ES-MS analysis usually showed the same molar ratios of differently sialylated forms in the intact glycoproteins, indicating that these existed in the starting samples and were not produced by loss of sialic acid during hydrazinolysis. The general pattern of sialic acid loss is consistent with that expected during protein isolation, and greater losses were sometimes observed when SAP was isolated from extremely small quantities of serum. However, in a typical SAP preparation isolated from one of the large pools of normal serum the abundance of various disialylated structures, expressed as sialic acid bond(mannose 6 arm)-sialic acid bond(mannose 3 arm), was 60% 6(6)-6(3), 4% 6(6)-3(3), 3% 3(6)-6(3), and 1% 3(6)-3(3); among monosialylated structures there were 17% 6(3), 8% 6(6), $<1\%$ 3(3), and $<1\%$ 3(6). A closely similar pattern was found in SAP from malignant effusion fluid. Base-sensitive substitutions of sialic acid could not be determined following hydrazinolysis.

ES-MS. ES-MS (Fig. 2) of 3 separate preparations of SAP and 12 of AP revealed a single predominant molecular species with mass of $25,462.5 \pm 1.1$ Da (mean $\pm$ SD; range, 25,459.6–25,463.8 Da). The mass calculated for the known amino acid sequence of SAP (9) together with the oligosaccharide structure reported here is 25,462.4 Da. Traces of other species with masses of approximately 25,171 Da and 24,880 Da were sometimes present, corresponding to loss of one or both of the terminal sialic residues (mass, 291 Da). Neither in pools

tive ^{125}I iodination or indirectly with ^{125}I -TCB (18). The 24-hr timepoint is so much greater than the plasma half-life of both normal SAP and asialo-SAP that all the tracer had been cleared—hence the same liver retention of ^{125}I -TCB in both cases. (C) Plasma clearance of degalactosylated ^{125}I -SAP in mice. $\circ$, Normal SAP; $\diamond$, asialo-SAP; $\blacklozenge$, $\square$, and $\blacksquare$, asialo-SAP incubated with β -galactosidase for 4, 24, or 48 hr, respectively. Each point is the mean of three mice in a group; for all points SD was $<10\%$ of the mean. (D) Plasma clearance of ^{125}I -labeled asialo-SAP ($\circ$) and resialylated asialo-SAP ($\square$). Each point is the value in a single mouse. The plasma half-life of enzymatically resialylated SAP, ≈ 3 hr, was the same as that of normal, untreated SAP.

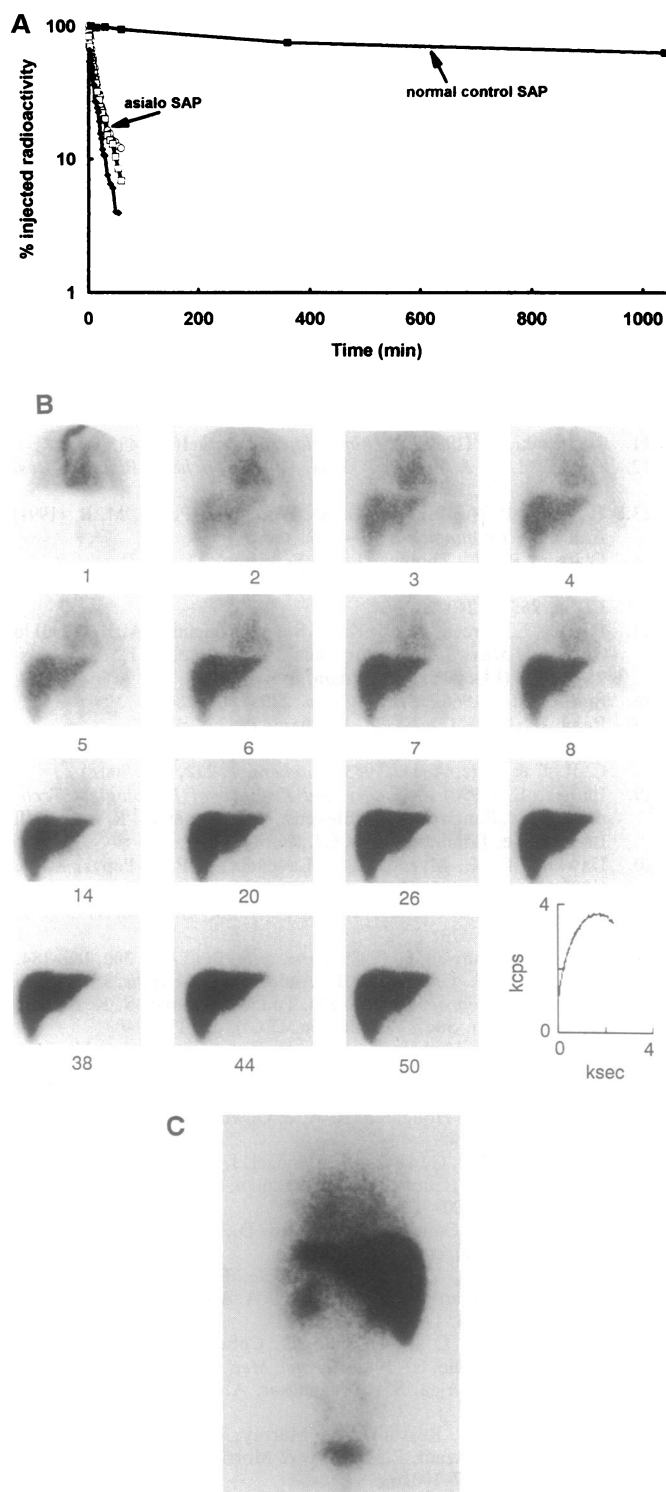


FIG. 4. (A) Plasma clearance of ^{125}I -human SAP in men. ■, Normal SAP (each point is the mean of 3 individuals and for all time points the SD was $<10\%$ of the mean); □, ○, and ♦, asialo-SAP (each symbol corresponds to one of the same three volunteers who had previously received normal SAP). The plasma half-life of normal SAP was 24 hr, as previously reported (6), compared with the extremely rapid clearance of asialo-SAP in the same subjects. (B) Anterior scintigraphic images of the torso taken at 40-sec intervals after intravenous injection of ^{125}I -asialo-SAP into a human volunteer. Initially (frame 1) there was activity only in the bloodstream, but the liver rapidly became as radioactive as the heart (frames 2 and 3), and then (from frame 4 onward) radioactivity in the liver increased progressively as all the tracer was cleared from the circulation. (Inset) Graph shows acquisition of radioactivity (kilocounts per second, keps) in the liver as a function of time (kiloseconds, ksec).

nor in SAP or AP from individuals have we observed a species of 25,469 Da, the mass expected if there was proline (7, 8) rather than serine (9) at residue 82. More importantly there was no difference between AP and normal SAP, nor any evidence of postsecretory modification of AP.

Effect of Oligosaccharide Modification on SAP Turnover in Mice. Desialylated human ^{125}I -SAP, labeled directly by oxidative iodination, was extremely rapidly cleared from the circulation in mice (Fig. 3A) and completely catabolized with prompt urinary excretion of all radioactivity in trichloroacetic acid-soluble form. When SAP was labeled with ^{125}I -TCB, a nondegradable tracer which is retained in the lysosomes of the cells in which the protein is catabolized (18), both fully sialylated and asialo forms of human SAP were taken up significantly only in the liver (Fig. 3B). Retained activity at 24 hr, detected by autoradiography, was confined to hepatocytes. In contrast, virtually all the activity from directly iodinated SAP was excreted after prompt catabolism of the protein, and there was no significant retention in the tissues (Fig. 3B). Degalactosylated asialo-SAP was cleared at a rate intermediate between that of normal and desialylated SAP (Fig. 3C) and was also taken up in the liver, although the cellular location was not determined. Simultaneous administration of asialo-AGP temporarily normalized clearance of asialo-SAP, in a dose-dependent manner (Fig. 3A). Enzymatic resialylation of asialo-SAP also restored its clearance to normal (Fig. 3D). Desialylated human SAP is thus apparently cleared by binding to the hepatocyte asialoglycoprotein receptor (22). However, this is evidently not the normal catabolic pathway for intact native human SAP, since the plasma half-life was unaffected by complete receptor blockade (Fig. 3A). The usual route may thus involve protein rather than carbohydrate recognition.

Clearance of Asialo-SAP in Man. Asialo-SAP was cleared extremely rapidly from the circulation by the liver in normal healthy men (Fig. 4A). Scintigraphic imaging after injection of ^{125}I -asialo-SAP showed immediate blood pool distribution followed promptly by uptake in the liver. Activity there peaked after 40 min and then fell as radioactive breakdown products appeared in the urinary tract (Fig. 4B). There was no uptake elsewhere in the body. In contrast, no local uptake is demonstrable after injection of normal SAP, and breakdown products appear in the urine much later (6, 20, 21).

DISCUSSION

The absence in the oligosaccharides of SAP or AP of the microheterogeneity characteristic of other glycoproteins is remarkable (23), especially in the SAP isolated from malignant effusion fluids (Fig. 1B) and in AP from reactive (AA) amyloid. These molecules had been produced during sustained acute-phase responses, conditions in which the quantity and complexity of glycosylation of other plasma glycoproteins of hepatic origin are most likely to be altered (24). Further, both the ES-MS results and the structural analysis of the released glycans showed that the SAP molecules which are deposited in amyloid and remain there as AP are completely unaltered. This demonstrates that the notable proteinase resistance of SAP, previously reported *in vitro* (25, 26), prevails *in vivo* and also shows that the SAP glycan is not degraded. These findings support the concept that SAP/AP may contribute to persistence of amyloid deposits *in vivo* (27).

The unvarying homogeneity of the SAP glycan suggested that it might be structurally and/or functionally important. We therefore investigated in mice the *in vivo* metabolic effects of oligosaccharide modifications and found that removal of the

(C) Posterior scintigraphic image of the torso of the same individual taken at 4 hr. In addition to the strong liver signal, tracer was present in the kidneys, ureters, and bladder, demonstrating the pathway of excretion of iodinated breakdown products of the labeled SAP.

terminal sialic residues led to immediate clearance and catabolism via the hepatocyte asialoglycoprotein receptor. We also observed similarly accelerated hepatic uptake and breakdown of human asialo-SAP in man. Although specific hepatic localization of ^{99m}Tc -labeled galactosyl neoglycoalbumin (28) and asialo-orosomucoid (29) has been reported previously, we have directly demonstrated the carbohydrate-dependent catabolism of an autologous plasma glycoprotein in man.

Apart from rat C-reactive protein (CRP) (30), the main oligosaccharide of which is closely similar or identical to that described here for human SAP (31), such high-efficiency uptake by the asialoglycoprotein receptor has not been observed before, either *in vivo* or *in vitro*, with biantennary oligosaccharides, as, for example, on transferrin (22). This uptake probably reflects the multimeric assembly of the protomers of SAP and of rat CRP (32), both of which belong to the pentraxin family of plasma proteins, since binding and uptake via this receptor are directly related to the degree of branching of oligosaccharides with terminal galactose or N-acetylgalactosamine residues (22).

However, the clearance of normally sialylated SAP was unaffected by total blockade of the asialoglycoprotein receptor, indicating that normal SAP turnover may involve a protein rather than a carbohydrate-recognition step. Indeed, human CRP, which is not glycosylated, is cleared even more rapidly than intact sialylated SAP, both in man (33) and in mouse (34), in which it is also exclusively catabolized by hepatocytes (unpublished observations). The existence of such an efficient clearance mechanism for asialo-SAP suggests that integrity of the oligosaccharide may be important or that altered SAP may be harmful.

Our results are thus of interest with regard both to the metabolism of plasma glycoproteins in general and to the role of SAP in particular. It is not clear whether presence in plasma of the single invariant glycoform of SAP reflects a unique biosynthetic pathway (for which, incidentally, there is little precedent) or results from a postsecretory skimming process. If SAP molecules alternatively glycosylated during biosynthesis were preferentially cleared, like the asialo-SAP generated *in vitro*, or were more susceptible to proteolysis, then only the intact proteinase-resistant glycoform would be demonstrable.

Asn³², the N-glycosylation site of SAP (7), is on a β -strand lying under the single α -helix on the face of the protomer opposite to the calcium and ligand binding site (35). The protomers are arranged as two pentameric discs interacting face-to-face, and since the native decamer displays maximal calcium-dependent ligand binding (1), the glycans must be attached to the promoter faces involved in the pentamer-pentamer interaction. However, the dimensions of the oligosaccharide are $55 \text{ \AA} \times 0.5 \text{ \AA} \times 24 \text{ \AA}$ (36) and it must therefore extend outward from each 20- $\text{\AA}$ protomer. Desialylated SAP retained fully the capacity to bind *in vitro* to amyloid fibrils (13) and to DNA (12), and it also remained decameric (data not shown). Nevertheless, the presence of glycan on the promoter surfaces involved in pentamer-pentamer association may contribute to decamer assembly during biosynthesis. Studies of this question and of the effects of the oligosaccharide on stability and proteinase resistance of SAP could have implications for the clinical use of modified glycoforms of SAP in diagnosis and therapy of amyloidosis (20).

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Human Serum Amyloid P Component

cDNA ISOLATION, COMPLETE SEQUENCE OF PRE-SERUM AMYLOID P COMPONENT, AND LOCALIZATION OF THE GENE TO CHROMOSOME 1*

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Complementary DNA clones corresponding to the human serum amyloid P component (SAP) were isolated, and the complete nucleotide and derived amino acid sequence of preSAP was determined. PreSAP biosynthesis is directed by a 1.1-kilobase mRNA. Synthesis and postsynthetic processing of preSAP in *Xenopus* oocytes result in secretion of a protein with mobility similar to native purified SAP when analyzed by sodium dodecyl sulfate gel electrophoresis. The human SAP gene is on chromosome 1, probably closely linked to the gene for C-reactive protein which encodes the related acute phase reactant found in human plasma.

The pentraxins, a family of proteins found in the sera of many species, share primary amino acid sequence homology and display a characteristic discoid arrangement of five non-covalently bound subunits (1, 2). Human serum contains two pentraxins: serum amyloid P component (SAP)¹, a precursor of the protein amyloid P component found in nearly all amyloid deposits (3, 4), and C-reactive protein (CRP). Although these two proteins are similar, they are distinguished by (a) ligand binding specificity, (b) the fact that SAP is glycosylated and CRP is not, (c) SAP is decameric (two pentameric discs face to face) and CRP is pentameric, and (d) SAP serum concentrations are relatively constant (20–40 µg/ml) while CRP concentrations rise from normal (1–2 µg/ml) to more than 100 µg/ml during an acute phase response (reviewed in Ref. 5).

Recently, cDNA clones corresponding to human CRP have been isolated and used to localize the CRP gene to human chromosome 1 (6), as well as to analyze the molecular regulation of CRP gene expression (7). In the present report, we describe the isolation of human SAP-specific cDNA clones, provide the nucleotide and derived amino acid sequences for the SAP primary translation product, and compare the biosynthesis and postsynthetic processing of human SAP and CRP. In addition, evidence is presented that the SAP gene is also on chromosome 1, probably closely linked to CRP, pro-

viding further support for the hypothesis that these are products of a gene duplication event.

MATERIALS AND METHODS

Preparation of Synthetic Oligonucleotides—A mixture of 256 different 20-nucleotide long oligonucleotides, containing all sequences that could code for the amino acids of human SAP between residues 102 and 108 (residue number according to Ref. (8)), was synthesized by a solid phase phosphotriester method using a library of dimer anions (9). In addition, three unique sequence 18-mer oligonucleotides were synthesized by a modified phosphotriester method on a Biosearch Sam One automated nucleotide synthesizer, for use as primers during nucleotide sequencing.

Isolation of SAP cDNA Clones—An adult human liver cDNA library containing more than 200,000 recombinant clones (10) was screened using a modification of the Grunstein-Hogness method (11). The library was plated at high density on 82-mm nitrocellulose filters and probed with the oligonucleotide mixture, 5' end-labeled with adenosine [γ -³²P]triphosphate (New England Nuclear) and T4 polynucleotide kinase (Bethesda Research Laboratories) at a concentration of 50 ng/ml in 0.9 M NaCl, 90 mM Na citrate, 1 × Denhardt's solution, 0.05% Na pyrophosphate, 100 µg/ml tRNA (12). Filters were washed repeatedly with 0.9 M NaCl, 90 mM Na citrate, 0.05% Na pyrophosphate at 20 °C and once at 42 °C for 10 min in the same buffer. Positive hybridization signals were visualized by autoradiography.

Plasmids were prepared by the alkaline lysis method (13), and the SAP-specific cDNA inserts were isolated by agarose gel electrophoresis after restriction endonuclease digestion with *Pst*I (New England Biolabs, Beverly, MA). Nucleotide sequencing of SAP cDNA was accomplished by the method of Maxam and Gilbert (14) and by the dideoxynucleotide chain termination method using inserts subcloned into the M13 vector mp11 (15).

Messenger RNA Isolation and Analysis—Human hepatic RNA was obtained as previously described (10) from a cadaver organ transplant donor, and the polyadenylated poly(A⁺) fraction was purified by affinity chromatography on oligo(dT)-cellulose (16). Poly(A⁺) mRNA was size fractionated by agarose-formaldehyde gel electrophoresis and transferred to nitrocellulose filters (17, 18). Following nick translation (19), the 613-base pair (bp) *Pst*I fragment of SAP cDNA clone (pSAP1) was used as a hybridization probe. SAP mRNA was visualized by autoradiography on Kodak XAR-5 film following prehybridization, hybridization, and washing using standard conditions (20).

RNA Translation—The mRNA was translated in the presence of [³⁵S]methionine (500 µCi/ml) (New England Nuclear) in a rabbit reticulocyte lysate system (21) and in *Xenopus* oocytes (22). Oocytes were each injected with 50 µg of poly(A⁺) hepatic RNA and incubated at 20 °C in Barth's medium (10 µl/oocyte) containing [³⁵S]methionine (500 µCi/ml) in microtiter wells each containing four oocytes. Media from separate wells were pooled, 48 h after injection, and the oocytes from wells containing four to eight oocytes in a total volume of 40–80 µl were pooled, washed with phosphate-buffered saline, and homogenized in lysis buffer (phosphate-buffered saline containing 1% Triton X-100, 0.5% Na deoxycholate, 10 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride, pH 7.6). The homogenate was centrifuged at 12,000 × g for 5 min at 4 °C, after which an equal volume of

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¹ The abbreviations used are: SAP, serum amyloid P component; CRP, C-reactive protein; kb, kilobase; bp, base pairs.

1% sodium dodecyl sulfate in phosphate-buffered saline was added. The culture media were mixed with an equal volume of lysis buffer containing sodium dodecyl sulfate.

Immunoprecipitation and Analysis of Translation Products—Reticulocyte lysate translation products were diluted 6-fold with 0.5 × lysis buffer with 5 mM EDTA and incubated with 4 µl of antiserum to human SAP (obtained from Atlantic Antibodies, Scarborough, ME) at 4 °C overnight. Oocyte lysate and culture media were analyzed using the same antiserum. Fifty microliters of a 10% suspension of protein A (IgG Sorb, Enzyme Center, Cambridge, MA) in lysis buffer with 5% bovine serum albumin was added to each sample and incubated at 0 °C for 1 h. Complexes were sedimented by centrifugation at 12,000 × *g* for 1 min, washed in lysis buffer, solubilized by boiling in Laemmli sample buffer (23), and analyzed by sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis. The radiolabeled products were visualized by autoradiography.

Purified human CRP and SAP isolated using previously described techniques (24) were electrophoresed in parallel with immunoprecipitates of translation products and visualized after staining with Coomassie Blue.

Cell Lines—The somatic cell hybrids were derived from fusions of the hypoxanthine-guanine phosphoribosyltransferase-deficient Chinese hamster cell E36 or mouse cell RAG with white blood cells or fibroblasts from three unrelated individuals. Two of the white blood cell donors were female carriers of reciprocal X/19 translocation chromosomes: the X/19W translocation t (X;19) (q23-25;q13) (25) and the X/19B translocation t (X;19) (q1;p13) (26). The fibroblast donor was a karyotypically normal male. The hybrid clones were characterized for human chromosome complements by both isozyme and cytogenetic techniques (27). In addition, the DNAs used in the present study were analyzed with cloned DNA probes for 21 of the human autosomes and the X chromosome.

Isolation and Analysis of Human, Rodent, and Somatic Cell Hybrid DNA—High molecular weight DNA from human peripheral blood lymphocytes, mouse RAG cells, hamster E36 cells, and somatic cell hybrids was prepared (28). After digestion with various restriction endonucleases, the DNA was size-fractionated by agarose gel electrophoresis, alkali-denatured, and then transferred to nitrocellulose filters (29). The resulting blots were hybridized under standard conditions (30) with the large *Pst*I fragment of pSAP1, which had been radiolabeled by nick translation. Filters were washed in 30 mM NaCl, 3 mM Na citrate at 65 °C for 1 h. Hybridization signals were visualized by autoradiography.

RESULTS

Isolation and Sequence Analysis of SAP cDNA—The oligonucleotide mixture (Fig. 1) was used to screen 25,000 recombinant colonies in duplicate. Sixteen positive clones were identified of which two (pSAP1 and pSAP11) were selected for further analysis. Fig. 2 shows the relative sizes of these two clones and the sequencing strategy employed. Clone pSAP1 contains 734 bp, extending from the codon specifying amino acid residue 30 for SAP through the stop codon followed by 150 bp of the 3' untranslated region and a 20-bp poly(A) tail. Clone pSAP11 is 919 bp in length and contains 38 bp of the 5' untranslated region, the codons for a 19 amino acid signal peptide, the 204 amino acid residues of the mature SAP protein, the 3' untranslated region, and a poly(A) tail. The complete nucleotide sequence of preSAP and the 3' untranslated region is shown in Fig. 3.

	102							108
Amino Acid	Gln	Gly	Tyr	Phe	Val	Glu	Ala	
mRNA	5' CA ^A _G	GG ^U _C	UA ^U _C	UU ^U _C	GU ^U _C	GA ^A _G	GC ^U _A	3'
Synthetic Oligonucleotides	3' GT ^T _C	CC ^A _G	AT ^A _G	AA ^A _G	CA ^A _G	CT ^T _C	CG	5'

FIG. 1. Composition of the synthetic oligonucleotide mixture corresponding to amino acid residues 102–108 (8) used as hybridization probe to screen the adult human liver cDNA library.

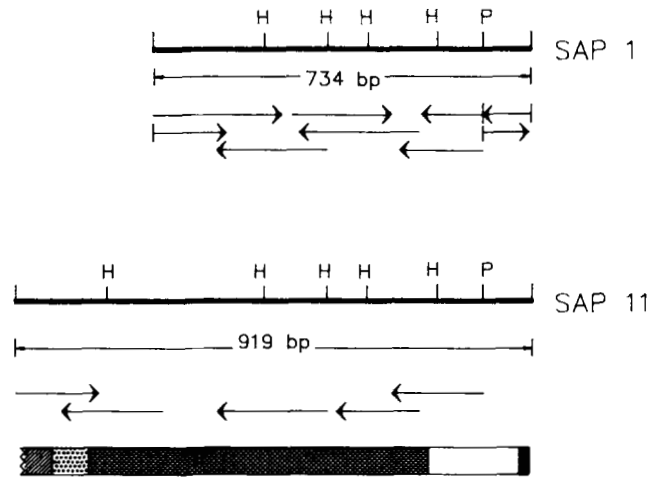


FIG. 2. Size comparison and nucleotide sequencing strategy for pSAP1 and pSAP11. Restriction endonuclease recognition sites are designated P, (*Pst*I) and H (*Hinf*I). Arrows start at priming sites and indicate the length of sequence generated. Arrows beginning with bars indicate fragments sequenced by the method of Maxam and Gilbert (14) and others by the method of Sanger *et al.* (15). The lower panel shows a schematic representation of the organization of the nucleotide sequence for pSAP11 (striped bar, 5' untranslated region; dotted bar, signal peptide region; hatched bar, coding sequence; open bar, 3' untranslated region; solid bar, poly(A) tail). Sequence was generated for 64% of the coding strand and 84% of the complementary strand of pSAP1 and 63% of the anti-coding strand and 9% of the coding strand of pSAP11. Overlapping sequence was determined for the entire coding region of SAP at least two times.

Biosynthesis and Postsynthetic Processing of SAP—A 1.1-kilobase (kb) SAP-specific mRNA species was identified in RNA isolated from human liver when Northern blots were probed with the large *Pst*I (613 bp) fragment of pSAP1 (Fig. 4a). Hybridization of the same filter with a human CRP-specific cDNA probe (pCRP5) revealed a 2.2-kb mRNA species (data not shown) in agreement with a previous study (7). Under the conditions of this experiment, no hybridization was detected between SAP mRNA and pCRP5 or CRP mRNA and pSAP1.

Under cell-free conditions, human hepatic RNA directed synthesis of a single chain protein (apparent *M_r* ~ 26,000) precipitable with antiserum to human SAP (Fig. 4b, lane 1). A diffuse radiolabeled band (Fig. 4b, lane 2) (apparent *M_r* ~ 28,000) with an electrophoretic mobility similar to native purified SAP (Fig. 4b, lane 4) was detected in lysates of *Xenopus* oocytes injected with human hepatic mRNA. Proteins with similar mobility (three distinct bands) (Fig. 4b, lane 3) were found in the oocyte extracellular medium after immunoprecipitation with an aliquot of the same antiserum to SAP. Lysates and media from oocytes injected with water served as controls, and no SAP-specific bands were detected (data not shown).

Chromosomal Localization of the Human SAP Gene—Somatic cell hybrids between human leukocytes and mouse RAG or hamster E36 cells (6, 25–27), which carried a full complement of rodent chromosomes and a limited but varied human chromosome complement, were analyzed for the presence of the human SAP gene. DNA was isolated from the peripheral blood lymphocytes of eight unrelated individuals, digested with the restriction endonuclease *Bam*HI (New England Biolabs), and subjected to Southern blot analysis, using the radiolabeled pSAP1 as a hybridization probe. A fragment of approximately 4.8 kb was detected for each individual (data not shown). DNA isolated from somatic cell hybrid lines was

In this report, we describe the isolation and nucleotide sequence of cDNA clones corresponding to human serum

TABLE I

Correlation of human chromosome content with the presence of the SAP gene

Data are summarized from hybridization of pSAP1 to DNA from somatic cell hybrids (Fig. 5). Concordant: ++ indicates presence of the 4.8-kb human SAP-specific band and presence of the chromosome; -- indicates the absence of both. Discordant: +- indicates presence of the 4.8-kb band and absence of the chromosome; -+ indicates absence of the 4.8-kb band and presence of the chromosome.

Human chromosome	Hybridization of probe				Total	
	Concordant		Discordant		Concordant	Discordant
	++	--	+-	-+		
1	9	8	0	0	17	0
2 ^a	2	6	6	2	8	8
3 ^a	6	5	3	2	11	5
4	6	5	3	3	11	6
5	4	4	5	4	8	9
6	5	6	4	2	11	6
7	5	5	4	3	10	7
8	2	2	7	6	4	13
9	2	6	7	2	8	9
10	7	3	2	5	10	7
11 ^a	6	4	3	3	10	6
12 ^a	7	2	2	4	9	6
13 ^a	3	4	5	3	7	8
14	5	4	4	4	9	8
15	5	6	4	2	11	6
16	5	4	4	4	9	8
17 ^a	4	4	5	3	8	8
18 ^a	7	3	2	4	10	6
19 ^{a,b}	6	0	1	7	6	8
20	5	2	4	6	7	10
21	5	3	4	5	8	9
22	5	6	4	2	11	6
X ^{a,c}	6	6	3	1	12	4
Y	1	8	8	0	9	8

^a Clones with a chromosome present in less than 15% of metaphases or in which a chromosome-specific isozyme or DNA probe exhibited only a weak signal were excluded from analysis for that chromosome. Clones with a rearranged chromosome were likewise excluded from the analysis.

^b The chromosome 19 line includes the intact 19 and the 2 different 19q⁺ translocation chromosomes.

^c The X line represents the intact X and the 2 Xq⁻ translocation chromosomes.

amyloid P component. The complete nucleotide sequence for the SAP primary translation product was generated from two SAP-specific clones. The larger of these clones (pSAP11) contains a 919-bp SAP-specific insert but does not span the entire SAP mRNA (estimated size, 1.1 kb) since it lacks a portion of the 5' untranslated region. This clone was used to determine the nucleotide and derived amino acid sequence of the signal peptide for preSAP. This sequence for preSAP is similar to the sequences of signal peptides for other secreted proteins (31).

The amino acid sequence of native SAP derived from the nucleotide sequence is similar to the primary sequence of SAP protein reported by Anderson and Mole (8), but differs in several respects. The most important of these differences is noted in the region between residues 65 and 98 (number according to Ref. 8), corresponding to residues 65 and 116 in the sequence reported here. The cDNA sequence defines a realignment of the peptide specified by Anderson and Mole between residues 67 and 80 (67-99 in the derived sequence) and two regions (78-85 and 100-116) not identified at the protein level. We believe the cDNA sequence represents the authentic SAP sequence and is not a result of a cloning

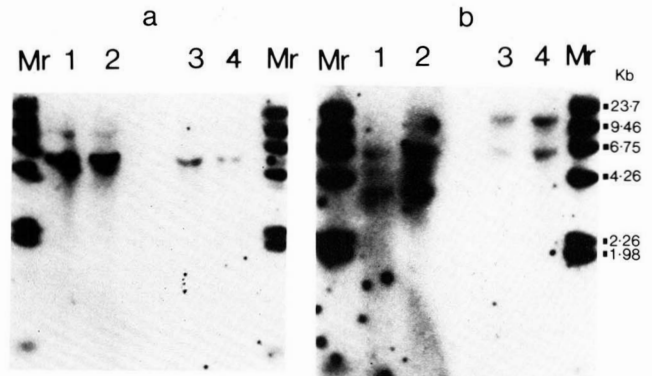


FIG. 6. Southern blot analysis of human DNA from peripheral blood cells probed with pCRP5 (lanes 1 and 2) and pSAP1 (lanes 3 and 4). DNA samples (15 µg/slot) from two unrelated individuals digested with *Xba*I (panel a) and *Kpn*I (panel b) are shown, as well as DNA from one individual in lanes 1 and 2 and the other in lanes 3 and 4. Filters were cut following transfer hybridized with the respective cDNA probes and rematched or autoradiography. *Hind*III-digested λ phage served as size markers in flanking lanes. Common bands 5.3 and 6.0 kb, respectively, for *Xba*I and *Kpn*I digests were visualized when probed with pCRP5 and pSAP1.

artifact for several reasons: (a) two SAP cDNA clones demonstrate identity of structure based on restriction mapping and partial nucleotide sequencing of pSAP11 and complete sequence analysis of pSAP1; (b) the SAP cDNA sequence aligns with the sequence of a related pentraxin, the Syrian hamster female protein, determined by sequence analysis of a clone isolated from a hamster liver cDNA library (32); and (c) the previously reported protein sequence was based on alignment of peptides (8) by analogy with human CRP (33). No overlapping peptides were reported in the region in which the sequence derived from the cDNA and the protein sequence differ. Several single residue differences are noted elsewhere when the sequences determined from SAP-specific cDNA and the protein are compared. These could be due either to technical considerations in amino acid sequencing or represent microheterogeneity or allotypic variants. Overall, the amino acid sequence of human SAP derived from the nucleotide sequence analysis of the cDNA shows 54% homology with human CRP and 71% homology with Syrian hamster female protein.

SAP synthesis is directed by a messenger RNA species approximately one-half the size of the mRNA for CRP (7), although the monomeric subunit of each is similar in size. Hence the 3' and/or 5' untranslated regions for SAP are smaller than corresponding regions in the CRP mRNA. Native SAP is glycosylated (2, 4) so that, although it is likely that the signal peptide of preSAP is cleaved during postsynthetic processing, the mobility of the mature intracellular and secreted SAP monomers is slower than that of the primary translation product.

Availability of the SAP cDNA clones and a panel of human-rodent hybrid cell lines permitted localization of the SAP gene to chromosome 1. Moreover, both SAP and CRP cDNA probes hybridized to restriction endonuclease fragments of identical size in DNA from two individuals. Several possible explanations for this finding were considered: (a) the similarity in size of the fragments detected with the two probes was coincidental; (b) the DNA within the boundaries defined by both enzymes which contain coding regions for CRP and SAP are sufficiently conserved, so that identical fragments are generated even though the genes are not closely linked; and (c) both probes recognize a common fragment suggesting close

linkage of the SAP and CRP genes. The third possibility is most likely but cannot be established with certainty until genomic clones have been isolated and characterized.

Analysis of genomic structure of human SAP and CRP and a comparison with the structures of the corresponding genes in other species should provide insight into the molecular basis for the differences in regulation of SAP and CRP gene expression during the acute phase response. Comparison with the structure of the Syrian hamster female protein gene will be of particular interest because expression of this homologous gene is regulated by sex steroids as well as mediators of the acute phase response.

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