

BINDING SPECIFICITY OF SERUM AMYLOID P COMPONENT FOR THE PYRUVATE ACETAL OF GALACTOSE

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Serum amyloid P component (SAP)¹ is a normal plasma glycoprotein composed of non-covalently associated subunits arranged with cyclic pentameric symmetry in a disk-like configuration. It is a member of the pentaxin family of proteins, which includes C-reactive protein (CRP), the classical acute phase reactant, and the homologues of CRP and SAP that exist in lower animals (1, 2). Although its biological function is unknown, there are a number of observations indicating that SAP is likely to be of both physiological and pathophysiological importance. First, there are proteins that closely resemble human SAP in terms of appearance in the electron microscope, subunit composition, and amino acid sequence in the sera of all vertebrates in which they have been sought (3–5). Second, a protein that is immunochemically indistinguishable from SAP is a normal integral constituent of the human glomerular basement membrane (6) and of the peripheral microfibrillar mantle of elastic fibers throughout the body (7). Third, both in man and lower animals SAP is laid down as amyloid P component (AP), a minor but significant constituent of all forms of amyloid deposits (8) with the possible exception of the intracerebral plaques in human Alzheimer's disease and senile dementia (9).

In addition to the stable evolutionary conservation of structure in SAP of

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¹ *Abbreviations used in this paper:* $[\alpha]_D^{temp}$, optical rotation; AP, amyloid P component; BSA, bovine serum albumin; ν_{max} , frequency of infra-red absorption band; C4bp, C4-binding protein; CRP, C-reactive protein; cyclic acetal MO β DG, methyl 4,6-O-(1-carboxyethylidene)- β -D-galactopyranoside; δ , chemical shifts; Fn, fibronectin; J, coupling constants; NHS, normal human serum; NMR, nuclear magnetic resonance; non-cyclic acetal MO β DG, methyl (4 or 6)-O-[(R,S)-1'-carboxy-1'-methoxy-ethyl]- β -D-galactopyranoside; PAGE, polyacrylamide gel electrophoresis; p.p.m., parts per million; R_f , spot mobility on t.l.c.; SAP, serum amyloid P component; signal multiplicities: s, singlets; d, doublets; t, triplets; m, multiplets; t.l.c., thin layer chromatography; TMS, tetramethylsilane; Tris-saline-Ca, 0.01 M Tris-buffered 0.138 M NaCl containing 0.002 M CaCl₂ and 0.1% wt/vol NaN₃ at pH 8.0; Tris-saline-EDTA, 0.01 M Tris-buffered 0.138 M NaCl containing 0.01 M EDTA at pH 8.0.

different species, these proteins all share the capacity for specific calcium-dependent ligand binding. This property was first discovered with respect to agarose (3, 10, 11) although earlier work on a then unidentified plasma glycoprotein (12), which was later shown to be SAP (13), had demonstrated its affinity for calcium ions. It was then established that SAP binds to amyloid fibrils in a calcium-dependent interaction that probably underlies the *in vivo* deposition of AP (14). Furthermore, in the presence of calcium, aggregated, but not native, human SAP selectively binds fibronectin (Fn) and C4-binding protein (C4bp) from normal human serum (15). All these interactions are highly specific and it seems likely that ligand binding by SAP participates in its *in vivo* function. Identification of the chemical nature of the ligand(s) should therefore help to explain the biological role of SAP and may also contribute to structural characterization of the protein itself.

Although it is most improbable that binding of SAP to agarose is itself related to a physiological function of the protein, it has greatly facilitated the isolation and characterization of SAP from man and other animals and provides a model system suitable for chemical studies. Agarose is a linear galactan hydrocolloid derived from marine algae and composed of repeating units of agarobiose (1,3-linked β -D-galactopyranose and 1,4-linked 3,6-anhydro- α -L-galactopyranose) together with trace amounts of sulfate and pyruvate, the latter as the 4,6-pyruvate acetal of β -D-galactopyranose (16, 17) (Fig. 1). There are large differences in the amounts of SAP that bind to beaded agarose gels from different sources (14), suggesting that the actual ligand for SAP in agarose may be a variable trace constituent of the polysaccharide. We report here that binding of SAP to gels prepared from a range of agaroses of known composition correlates closely with their pyruvate content, but not at all with the amount of sulfate present. Methylation of the carboxylic acid of the pyruvate residues abolishes the binding of SAP. We have therefore synthesized a pyruvate acetal at the 4,6-positions of galactose, the form in which it exists in agarose, and have shown that it inhibits the calcium-dependent binding of SAP to agarose, to amyloid fibrils, to fibronectin, and to C4-binding protein. The availability of this chemically defined low molecular weight ligand should aid further work on SAP.

Materials and Methods

Agarose

Agaroses of known pyruvate and sulfate content derived from *Gelidium* and *Gracilaria* species (kindly provided by FMC Corporation, Rockland, ME) were used to prepare a 4% wt/vol gel in 0.01 M Tris-buffered 0.138 M NaCl containing 0.002 M CaCl_2 and 0.1% wt/vol NaN_3 at pH 8.0 (Tris-saline-Ca). The gels were fragmented into beads by sequential

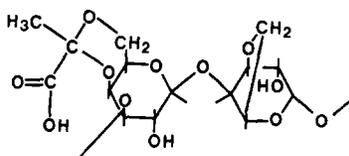


FIGURE 1. Agarobiose showing the position of pyruvic acid as the acetal 4,6-O-(1-carboxyethylidene)- β -D-galactopyranose.

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passage through 710- μm and 80- μm sieves (Endecotts Ltd., London, England), and were stored in suspension in Tris-saline-Ca at 4°C.

Diazomethane-treated Agarose

Pyruvate-rich (0.25% wt/wt) agarose beads (4 ml packed volume) were added to 30 ml of an ethereal solution of diazomethane (18) that had been diluted 1:1 vol/vol in methanol, and stirred for 60 min at room temperature. After removal of the supernatant, the beads were washed with distilled water and then resuspended in Tris-saline-Ca. Control solvent-treated beads were mixed with identical volumes of ether/methanol (1:1 vol/vol) alone.

Chemicals

Methyl β -D-galactopyranoside, methyl pyruvate, sodium pyruvate, and D-galactose were obtained from Sigma Chemical Co. Ltd., Poole, Dorset, England; ion exchange resin (IR 120 H⁺) and organic solvents from BDH Ltd., Dagenham, Essex, England and 10% palladium on charcoal from Johnson Matthey Chemicals Ltd., Royston, Herts, England.

Chemical Techniques

¹H-Nuclear magnetic resonance (NMR) spectra were usually measured in CDCl₃ with a JNM-FX200 Fourier transform NMR spectrometer (Jeol Ltd., Tokyo, Japan) or a WH-400 spectrometer (Bruker Ltd., Karlsruhe, Federal Republic of Germany) with tetramethylsilane (TMS) as internal standard. Natural-abundance ¹³C-NMR spectra were measured in CDCl₃ with JNM-FX60 or FX200 instruments (Jeol Ltd.) operating at 15 or 50 MHz, respectively. The ¹H-NMR spectra are presented as follows: chemical shifts (δ) in parts per million (p.p.m.) downfield from TMS; signal multiplicities (s, d, t, m, for singlets, doublets, triplets, multiplets); signal intensities (e.g. 3H); coupling constants in Hz (e.g. J_{1,2} 3.0 Hz); signal assignment (e.g. H-2). For the ¹³C-NMR spectra the chemical shift is reported followed, where possible, by the signal assignment.

Optical rotations ($[\alpha]_D^{temp}$) were measured in chloroform with an AA-10 polarimeter (Optical Activity Ltd., Huntingdon, Cambridgeshire, England). Infrared spectra were measured (γ_{max} recorded in cm⁻¹) with a 597 spectrometer (Perkin Elmer Ltd., Beaconsfield, Bucks, England) on samples smeared on potassium bromide disks.

Column chromatography was carried out on silica gel 230–400 mesh (Merck Kieselgel 9385, BDH Ltd.). For thin layer chromatography (TLC) 0.25-mm films of silica gel (Merck Kieselgel 60 F₂₅₄, BDH Ltd.) were used, and the spots were located with an ethanol 1% sulfuric acid spray reagent. The mobility of the spot relative to the mobility of the solvent front (R_f) was determined (19). The eluting solvents were dried over molecular sieves 4A (BDH Ltd.).

Synthesis of Acetals of Pyruvic Acid Derived from Methyl β -D-Galactopyranoside

Preparation of starting materials: Methyl pyruvate dimethyl acetal (compound I) was prepared by heating methyl pyruvate under reflux with methanol in the presence of IR 120H⁺ ion exchange resin. Methyl 2,3-di-O-benzyl- β -D-galactopyranoside (compound II) was prepared by standard procedures from methyl 4,6-O-benzylidene- β -D-galactopyranoside (20).

Condensation of methyl 2,3-di-O-benzyl- β -D-galactopyranoside with methyl pyruvate: Compound II (1 g) was dissolved in compound I (9 ml) at 20°C. The solution was cooled to 0°C and concentrated sulfuric acid (0.1 ml) added with stirring. The solution was maintained at 20°C under reduced pressure (0.1 mm Hg) for 4 h when TLC (40:60 vol/vol petroleum ether) showed several intense spots in the range R_f 0.1–0.5 and a little unchanged compound II (R_f 0.0). The acid was then neutralized by stirring the solution with an excess of anhydrous sodium hydrogen carbonate (NaHCO₃) added in portions during 3 h. Dichloromethane (1 l) was then added and the solution filtered. The filtrate was washed with aqueous NaHCO₃ and, after drying, evaporated to give a crude residue that was separated by column chromatography on silica gel with toluene, toluene/ethyl acetate (9:2 vol/vol) as an increasingly polar elutant. The major product was methyl 2,3-di-O-benzyl-4,6-O-[(R)-1-methoxycarbonylethylidene]- β -D-galactopyranoside (compound III)

(642 mg); $^1\text{H-NMR}$ (200 MHz) data: 4.17 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 3.52 (s, 3H, aglycone OCH_3), 3.78 (s, 3H, CO_2CH_3), 1.58 (s, 3H, CH_3C), 7.1–7.4 (m, 10H) and 4.65–4.92 (m, 4H) ($2 \times \text{CH}_2\text{C}_6\text{H}_5$), plus signals for six galactose protons. In a similar, larger scale experiment using 5 g of compound II, a new major product was isolated (2.3 g) by chromatography using toluene, toluene/ethyl acetate (9:2 vol/vol), ethyl acetate as increasingly polar elutants. It has been tentatively identified as methyl 2,3-di-*O*-benzyl-(4 or 6)-*O*-(*R,S*-1'-methoxycarbonyl-1'-methoxy-ethyl)- β -D-galactopyranoside (compound IV). Details of the ^1H and $^{13}\text{C-NMR}$ results may be obtained from the authors.

Hydrogenolysis of methyl 2,3-di-O-benzyl- β -D-galactopyranoside acetals of methyl pyruvate: Compound III (642 mg) in ethyl acetate (60 ml) containing 10% palladium on charcoal (150 mg) was shaken for 2 h under 2.5 atmospheres of hydrogen. The catalyst was filtered off, and the filtrate evaporated to give methyl 4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]- β -D-galactopyranoside (compound V) (415 mg), $^1\text{H-NMR}$ (200 MHz) data: 3.80 (s, 3H, CO_2CH_3), 3.56 (s, 3H, aglycone OCH_3), 1.63 (s, 3H, CCH_3). The mixed, non-cyclic acetal (compound IV) (228 mg) was similarly hydrogenolyzed to give methyl (4 or 6)-*O*-[(*R,S*)-1'-methoxycarbonyl-1'-methoxy-ethyl]- β -D-galactopyranoside (compound VI) (141 mg). Details of the ^1H and $^{13}\text{C-NMR}$ results may be obtained from the authors.

Saponification of methyl β -D-galactopyranoside acetals of methyl pyruvate: The cyclic acetal methyl ester (compound V) (225 mg) was placed in 1.0 M aqueous sodium hydroxide (0.4 ml) at 23°C for 24 h, and then the pH adjusted to 7.4 with 1.0 M hydrochloric acid. This solution of the sodium salt of the acetal was used without further characterization. The mixed non-cyclic acetal methyl ester (compound VI) (141 mg) was similarly treated.

Proteins

Isolated pure SAP (21) and Fn (22) were prepared from normal human serum (NHS) and citrated human plasma, respectively, according to established methods. Isolated pure C4bp (23) was the generous gift of Dr. V. Nussenzweig, New York University, New York. Bovine serum albumin (BSA) was obtained from Sigma Chemical Co. Ltd.

Serum

Fresh NHS was obtained by allowing venous blood from laboratory volunteers to clot at room temperature for 2–4 h before centrifugation. In some experiments, serum from a single donor was used, in others different sera were pooled. Acute phase mouse serum was obtained by exsanguination of MRL mice 48 h after subcutaneous injection of 0.5 ml 2% wt/vol AgNO_3 solution. Pooled normal plaice serum (*Pleuronectes platessa* L.) was kindly provided by Dr. Thelma C. Fletcher, NERC, Institute of Marine Biochemistry, Aberdeen.

Antisera

Monospecific antisera to human SAP and Fn, and to mouse and plaice SAP were raised by immunization of sheep and rabbits with the isolated pure proteins (24, 25).

Assay of SAP and Fn

Specific protein concentrations were measured by electroimmunoassay (26) using standards of the isolated pure proteins.

PAGE

Gradient PAGE analysis of native proteins was performed in 4–30% gradient gels (Pharmacia, Uppsala, Sweden), run exactly according to the manufacturer's instructions (15).

Immobilized Proteins

Human SAP was coupled to CNBr-activated Sepharose 4B (Pharmacia) (SAP-Sepharose, 10 mg/ml) in pH 9.0 carbonate buffer precisely as described elsewhere (15). Control BSA-Sepharose beads (10 mg/ml) were prepared as before (15).

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Isolation of Amyloid Fibrils

Fibrils from the spleen of a patient with reactive systemic (AA) amyloidosis were isolated (27) and lyophilized from distilled water.

Binding of SAP to Agarose Beads and to Amyloid Fibrils

Known amounts of agarose beads and, in different experiments, of amyloid fibrils were equilibrated with Tris-saline-Ca and then tested for SAP binding from serum exactly as described elsewhere (14). Elution with the chemicals under test was attempted using solutions in Tris-saline-Ca. In other experiments these solutions were added to serum before it was offered to either pyruvate-rich agarose beads (0.25% wt/wt) or fibrils. Tris-saline-EDTA was used as a control for complete elution or inhibition of binding of SAP.

Binding of Fn and C4bp by Immobilized Human SAP

The experimental procedure for demonstration of binding of Fn and C4bp from NHS by immobilized SAP has been described previously (15). Solutions in Tris-saline-Ca of the chemicals under test were used to either elute bound Fn and C4bp or to inhibit their uptake. Eluates in Tris-saline-EDTA from SAP-Sepharose columns were tested by gradient PAGE and by specific electroimmunoassay for Fn.

Results

Amount of SAP Binding to Different Agaroses of Known Composition. The quantity of SAP from human, mouse, and plaice serum that bound to different agaroses correlated closely with the pyruvate content of the agarose (human, $r = 0.92$, $n = 11$, $P < 0.001$; mouse, $r = 0.95$, $n = 4$, $P < 0.01$; plaice, $r = 0.98$, $n = 4$, $P < 0.001$; Fig. 2). There was no significant correlation between SAP bound and the sulfate content (human, $r = 0.26$, $n = 11$; mouse, $r = 0.73$, $n = 4$; plaice, $r = 0.68$, $n = 4$). Very little SAP bound to agarose gels that contained extremely low levels (0.01% wt/wt) of pyruvate.

Effect of Methylation of the Carboxylic Acid Residues in Agarose. Prior treatment of the agarose beads with diazomethane completely abolished their capacity to bind SAP (Table I). Treatment of beads by solvents alone had no effect.

Synthesis of Pyruvate Acetals of Methyl β -D-Galactopyranoside. Methyl 4,6-O-(1-

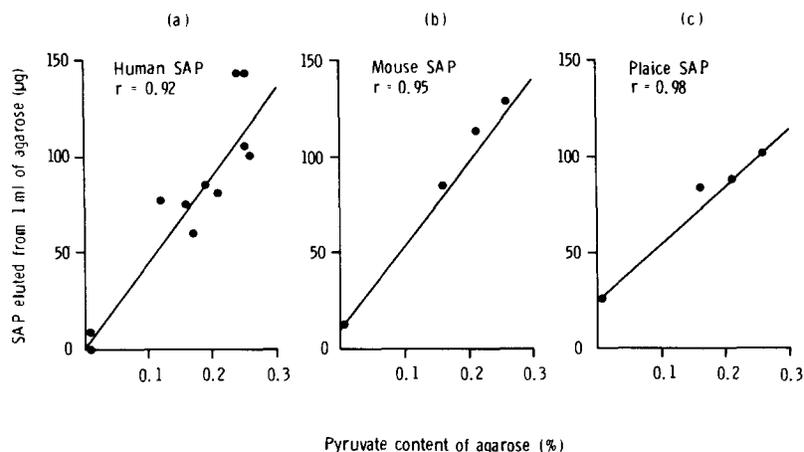


FIGURE 2. Capacity of different agaroses of known pyruvate content to bind human (a), mouse (b), and plaice (c) SAP.

TABLE I
Binding of Human, Mouse, and Plaice SAP to Pyruvate-rich Agarose Beads*

Agarose	Human SAP	Mouse SAP	Plaice SAP
	<i>mean ± SD,</i> <i>n = 12</i>		
Control	100 ± 4	104	88
Solvent treated	118 ± 8	128	80
Diazomethane treated	0	0	0

* Expressed as μg SAP per 1 ml of packed agarose beads.

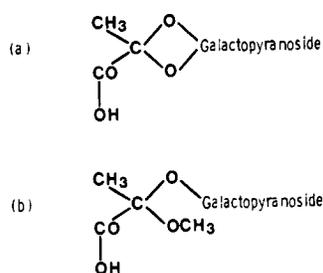


FIGURE 3. Comparison of the structures of the cyclic acetal MO β DG (a) and the non-cyclic form (b).

methoxycarbonylethylidene)- β -D-galactopyranoside was synthesized and identified as the R isomer (compound V) on the basis of its ^1H -NMR spectrum (28). This was then saponified to give methyl 4,6-O-(1-carboxyethylidene)- β -D-galactopyranoside (the cyclic acetal MO β DG; Fig. 3a), the form in which the pyruvate acetal of galactopyranose exists in agarose. In another experiment, a mixed non-cyclic methyl pyruvate acetal of methyl β -D-galactopyranoside was synthesized and partially identified as compound VI on the basis of its ^1H and ^{13}C -NMR spectra. This was also saponified to give methyl (4 or 6)-O-(1'-carboxy-1'-methoxy-ethyl)- β -D-galactopyranoside (the non-cyclic acetal MO β DG; Fig. 3b). The solutions of these acetals were then corrected to pH 7.4. In the subsequent binding experiments, these solutions were used either in this form or further diluted in Tris-saline-Ca.

Inhibition of SAP Binding to Agarose by Synthesized Galactosides. Binding of human, mouse and plaice (Table II) SAP to pyruvate-rich agarose was inhibited by the presence of MO β DG. The R isomer of the cyclic acetal MO β DG was more potent than the non-cyclic MO β DG. For all further experiments with human SAP the R isomer of the cyclic acetal MO β DG was therefore used. Methyl β -D-galactopyranoside, pyruvic acid, and D-galactose, all at concentrations of $1-2 \times 10^{-2}$ M, had no appreciable effect on the binding of human, mouse, or plaice SAP.

Elution of SAP from Agarose by Synthesized Galactosides. MO β DG eluted human, mouse, and plaice (Table III) SAP that had already bound to pyruvate-rich agarose. Again, the R isomer of the cyclic acetal MO β DG was more potent than the non-cyclic MO β DG. Methyl β -D-galactopyranoside, pyruvic acid, and D-

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TABLE II
Inhibition of Binding of SAP to Agarose by MO β DG

Chemical	Concentration	Inhibition of SAP binding	
		%	
		<i>Human</i>	
MO β DG, R isomer	7.63×10^{-3} M	95	
	7.63×10^{-4} M	60	
	7.63×10^{-5} M	0	
Non-cyclic MO β DG	6.82×10^{-3} M	52	
	6.82×10^{-4} M	0	
		<i>Mouse</i>	<i>Plaice</i>
MO β DG, R isomer	5×10^{-3} M	100	73
	5×10^{-4} M	60	15
	5×10^{-5} M	0	0

TABLE III
Elution of SAP from Agarose by MO β DG

Chemical	Concentration	SAP eluted	
		%	
		<i>Human</i>	
Tris-saline-EDTA control		100	
MO β DG, R isomer	7.75×10^{-3} M	91	
	7.75×10^{-4} M	66	
	7.75×10^{-5} M	0	
Non-cyclic MO β DG	2.04×10^{-2} M	66	
	2.04×10^{-3} M	42	
	2.04×10^{-4} M	0	
		<i>Mouse</i>	<i>Plaice</i>
Tris-saline-EDTA control		100	100
MO β DG, R isomer	3.8×10^{-2} M	100	100
	5.4×10^{-3} M	74	21
	5.4×10^{-4} M	20	0
	5.4×10^{-5} M	0	0

galactose, all at $1-2 \times 10^{-2}$ M, did not elute human, mouse, or plaice SAP that had already bound to agarose.

Effect of the Cyclic Acetal MO β DG on Binding of Fn and C4bp by SAP-Sepharose. When added to fresh NHS before mixing with SAP-Sepharose, the R isomer of the cyclic acetal MO β DG inhibited the binding of both Fn and C4bp. This was shown by immunoassay of Fn (Table IV) and gradient PAGE analysis of the subsequent Tris-saline-EDTA eluate from the SAP-Sepharose. In other experiments, the R isomer of the cyclic acetal MO β DG eluted Fn (Table V) and C4bp that were already bound by SAP-Sepharose, the gradient PAGE profile of these eluates being identical to that of the Tris-saline-EDTA eluates (15).

Effect of the Cyclic Acetal MO β DG on Human SAP Binding to Amyloid Fibrils. When increasing amounts of the R isomer of the cyclic acetal MO β DG were added to NHS before being offered to AA amyloid fibrils, there was a progressive

TABLE IV
Effect of the Cyclic Acetal MO β DG on the Capacity of SAP-Sepharose to Bind Fn

	Total Fn eluted*	
	SAP-Sepharose	BSA-Sepharose
	μ g	
Control:		
Tris-saline-Ca	45	0
MO β DG, R isomer (7.66×10^{-3} M)	0	0

* NHS was offered to SAP-Sepharose in the presence of MO β DG, or Tris-saline-Ca as control, and after washing with Tris-saline-Ca was eluted with Tris-saline-EDTA.

TABLE V
Elution of Fn from SAP-Sepharose by the Cyclic Acetal MO β DG

MO β DG, R isomer concentration	Fn eluted: percent of amount eluted by Tris-saline-EDTA
1.15×10^{-2} M	100
2.23×10^{-3} M	85
1.15×10^{-3} M	85
2.3×10^{-4} M	67

TABLE VI
Effect of the Cyclic Acetal MO β DG on the Binding of Human SAP to Amyloid Fibrils

MO β DG, R isomer concentration	Human SAP eluted with Tris-saline-EDTA
	%
0	100
5.5×10^{-5} M	78
5.5×10^{-4} M	56
5.5×10^{-3} M	50

reduction in the amount of SAP bound (Table VI). In other experiments, the R isomer of the cyclic acetal MO β DG eluted human SAP that had already bound to the AA amyloid fibrils (Table VII). No elution was observed with methyl β -D-galactopyranoside, pyruvic acid, or D-galactose at concentrations between 10^{-4} and 2×10^{-2} M.

Discussion

The present results show clearly that the amount of SAP that binds to agarose gels is directly related to their pyruvate content. SAP scarcely binds at all to gels that either have been prepared from pyruvate-free agarose or in which the carboxylic moiety of the pyruvate has been methylated by treatment with

TABLE VII
*Elution of Human SAP from Amyloid Fibrils by the Cyclic Acetal
 MO β DG*

Chemical	Concentration	SAP eluted off 1 mg fibrils %
Control:		
Tris-saline-EDTA		100
MO β DG, R isomer	1.15×10^{-2} M	65
	1.15×10^{-3} M	35
	1.15×10^{-4} M	14

diazomethane. The pyruvate residues in agarose exist as the acetal 4,6-*O*-(1-carboxyethylidene)- β -D-galactopyranose. In order to test the hypothesis that this material is in fact the ligand in agarose recognized by SAP and to obtain a defined low molecular weight ligand for SAP, we synthesized methyl 4,6-*O*-(1-carboxyethylidene)- β -D-galactopyranoside (the cyclic acetal MO β DG) from methyl 4,6-*O*-(1-methoxycarbonyl ethylidene)- β -D-galactopyranoside (compound V), which we obtained by a shorter route than the one previously reported (29). Analysis of $^1\text{H-NMR}$ spectra of this product showed it to be identical to the R isomer of compound V as reported by Garegg et al. (28). Subsequent saponification of this material yielded the cyclic acetal of MO β DG. This product was able both to inhibit binding of human, mouse, and plaice SAP to pyruvate-rich agarose and to elute SAP that was already bound. The effective concentration (10^{-3} – 10^{-2} M) was comparable to the calculated concentration of pyruvate residues in the pyruvate-rich agarose gel (1.66×10^{-3} M). The importance of the fixed stereochemistry of the cyclic acetal ring in these binding experiments is emphasized by the lower potency of the non-cyclic MO β DG. The inherent flexibility of this non-cyclic form (Fig. 3) presumably results in a poorer stereoalignment for SAP to bind. Pyruvate or D-galactose alone had no inhibitory effects on SAP binding, nor did methyl β -D-galactopyranoside. Further work is required to determine which parts of the structure of these acetals of MO β DG are most important for its recognition by SAP, but the methylation experiments suggest that the carboxyl group of the pyruvate residue may be critical.

The binding of SAP to agarose is presumably fortuitous and not in any way directly related to the physiological function of the protein. Binding to amyloid fibrils (14) and the more recently discovered interaction between aggregated SAP and Fn and C4bp (15) are probably of greater physiological and pathophysiological importance. It is therefore of considerable interest that millimolar concentrations of the R isomer of the cyclic acetal MO β DG inhibited all these reactions. The ligands for SAP expressed by autologous proteins apparently share some structural similarity with agarose and with MO β DG, but the resemblance does not necessarily reside in a carbohydrate moiety, since amyloid fibrils consist exclusively of nonglycosylated polypeptide chains (30). A possible candidate for the polypeptide structure recognized by SAP is the so-called β -turn, which is thought to exist in amyloid fibrils at the point where the fibril subunit

proteins, whether of AA, AL, prealbumin, or other origin, fold back on themselves to form the anti-parallel β -pleated sheets that characterize amyloid (31). The β -turn forms when there are many glycine residues appropriately distributed among bulky hydrophobic L-amino acids, and it is a 10-atom hydrogen-bonded ring inserted into the polypeptide backbone so that the end peptide moiety has its peptide acyl oxygen directed nearly perpendicular to the plane of the other atoms (32). This is an exposed position in which it is capable of binding cations, and sequences that form β -turns bind calcium ions with high selectivity over other metallic cations (32). Amyloid fibrils are known to bind calcium (33) as does elastin, the first protein in which β -turns were described (32). It is therefore notable that in man normal elastic fibers throughout the body bear SAP on their surface (7). Both Fn and C4bp bind calcium ions and it is possible that their structures also contain β -turns.

The functional significance of the existence throughout vertebrate evolution of a plasma protein equipped under certain circumstances to recognize and bind β -turns in other proteins and/or the pyruvate acetal of galactose in carbohydrates of autologous or extrinsic origin remains obscure. However, identification of MO β DG as a low molecular weight ligand for SAP should assist investigation of this question. Furthermore the complete amino acid sequences of both human (34) and mouse SAP (35) have recently been elucidated and it should be possible to use MO β DG to localize the binding sequence in SAP.

Summary

Serum amyloid P component (SAP) is a normal plasma protein that is of interest because of its presence in amyloid deposits, its presence in normal human glomerular basement membrane, and its stable evolutionary conservation. It has calcium-dependent ligand-binding specificity for amyloid fibrils, fibronectin (Fn), C4-binding protein (C4bp), and agarose. Although the binding to agarose, a linear galactan hydrocolloid derived from some marine algae, is unlikely per se to be related to the physiological function of SAP, it does provide a model system in which to explore the precise ligand requirements of SAP. We report here that the amount of SAP from human, mouse, and plaice (*Pleuronectes platessa* L.) serum able to bind to agarose from different sources reflect precisely their pyruvate content. Methylation with diazomethane of the carboxyl groups in the pyruvate moiety of agarose completely abolishes SAP binding to agarose.

The pyruvate in agarose exists as the 4,6-pyruvate acetal of β -D-galactopyranose. We have therefore synthesized this galactoside, using a novel procedure, established its structure by analysis of its nuclear magnetic resonance spectra, and shown that it completely inhibits all known calcium-dependent binding reactions of SAP. The R isomer of the cyclic acetal, methyl 4,6-O-(1-carboxyethylidene)- β -D-galactopyranoside (MO β DG) was effective at millimolar concentration and was more potent than its noncyclic analogue, while pyruvate, D-galactose, and methyl β -D-galactopyranoside were without effect.

The autologous protein ligands of SAP presumably, therefore express a structural determinant(s) that stereochemically resembles MO β DG. Availability of this specific, well-characterized, low molecular weight ligand for SAP should facilitate further investigation of the function of SAP and its role in physiological

and pathophysiological processes.

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