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Human serum amyloid P component, a circulating lectin with specificity for the cyclic 4,6-pyruvate acetal of galactose

Interactions with various bacteria

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Serum amyloid P component (SAP), a normal plasma glycoprotein, has recently been shown to have Ca^{2+}-dependent binding specificity for methyl 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (MOβDG) [Hind, Collins, Renn, Cook, Caspi, Baltz & Pepys (1984) *J. Exp. Med.* **159**, 1058–1069]. SAP was found to bind *in vitro* to *Klebsiella rhinoscleromatis*, the cell wall of which is known to contain this particular cyclic pyruvate acetal of galactose. SAP also bound in similar amounts (approx. 6000 molecules per organism) to group A *Streptococcus pyogenes*, but very much less was taken up on *Xanthomonas campestris*, which contains the 4,6-cyclic pyruvate acetal of mannose. No SAP bound to *Escherichia coli*, which contains the 4,6-cyclic pyruvate acetal of glucose, or to *Streptococcus pneumoniae* type 4, which contains the 2,3-cyclic pyruvate acetal of α- rather than β-galactopyranoside, or to other organisms (*Streptococcus agalactiae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*), the carbohydrate structures of which are less well characterized. Binding of SAP to those organisms which it did recognize was completely inhibited or reversed by millimolar concentrations of free MOβDG. SAP, a human plasma protein, thus behaves as a lectin and may be a useful probe for its particular specific ligand in the cell walls of bacteria and other organisms.

Serum amyloid P component (SAP) is a normal plasma glycoprotein that has the property of Ca^{2+}-dependent binding to particular specific ligands (Pepys & Baltz, 1983). These include amyloid fibrils, and it is the interaction of SAP with these, regardless of their chemical type, which probably underlies the universal presence of amyloid P component (AP) in amyloid deposits (Pepys *et al.*, 1979). The physiological function of SAP is not known, but it is a member of the pentraxin family of plasma proteins, the structure and Ca^{2+}-dependent ligand-binding specificities of which have been stably conserved throughout vertebrate evolution (Pepys *et al.*, 1978; Baltz *et al.*, 1982*a*). This presumably implies that they have functions worthy of preservation and that their ligand-binding capacity may be necessary for it.

Ligand binding by SAP was first demonstrated with respect to agarose (Pepys *et al.*, 1977), a linear galactan hydrocolloid from marine algae. We have subsequently identified pyruvate, a trace constituent of agarose, as the ligand therein to which SAP binds (Hind *et al.*, 1984). The pyruvate is present as the 4,6-cyclic acetal of galactose, and we have therefore synthesized methyl 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (MOβDG) (Fig. 1) and shown that it inhibits and reverses *in vitro* all human Ca^{2+}-dependent binding reactions of SAP (Hind *et al.*, 1984). However, amyloid fibrils are not glycosylated, and pyruvate acetals of galactose have not been reported in mammalian tissues, so it is improbable that interaction with this galactoside itself is of any physiological significance. It is more

Abbreviations used: NHS, normal human serum (sera); MOβDG, methyl 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside; SAP, serum amyloid P component; Tris/saline/Ca, 0.01 M-Tris-buffered 0.14 M-NaCl containing 0.002 M-$CaCl_2$, pH 8.0; Tris/saline/EDTA, 0.01 M-Tris-buffered 0.138 M-NaCl containing 0.01 M-EDTA, pH 8.0.

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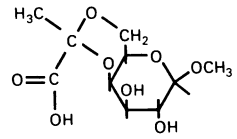

Fig. 1. *Methyl 4,6-O-(1-carboxyethylidene)-β-D-galactopyranoside (MOβDG)*

likely that the galactoside stereochemically resembles a structure present in the autologous protein ligands to which SAP binds, including fibronectin and complement-component-C4-binding protein (de Beer *et al.*, 1981) as well as amyloid fibrils (Pepys *et al.*, 1979), and that these latter reactions are of physiological and/or pathophysiological relevance. However, pyruvate acetals do occur in some micro-organisms (Kenne & Lindberg, 1983), including bacteria of clinical importance, and it was therefore of interest to determine whether SAP would bind to them. We report here that human SAP binds appreciably to bacteria known to contain the 4,6-cyclic pyruvate acetal of galactose, more weakly to others containing the mannose counterpart and not at all to species that contain the glucose counterpart or the 2,3-cyclic pyruvate acetal of galactose. Furthermore, in all cases where binding did occur, it was inhibited or reversed by MOβDG.

Methods

Bacteria

Bacterial isolates were obtained from the National Collection of Type Cultures (London, U.K.) and from the National Collections of Industrial and Marine Bacteria Ltd. (Aberdeen, Scotland, U.K.). Organisms were grown at 37°C overnight on blood agar base with 5% sterile horse blood (Tissue Culture Services, Slough, Berks., U.K.), then stored at 4°C. For quantitative binding assays, bacteria were grown in Oxoid nutrient no. 2 broth (Oxoid, Basingstoke, Hants., U.K.) at 37°C overnight, or at room temperature for 48 h (*Xanthomonas campestris*). Bacterial suspension counts were estimated by using Wellcome opacity tubes (Wellcome Reagents, Beckenham, Kent, U.K.).

SAP

In some experiments, pooled normal human sera (NHS) were used as the source of SAP. SAP concentrations were subsequently measured by electroimmunoassay (Pepys *et al.*, 1977), with monospecific antiserum to human SAP raised by immunization of sheep with isolated pure SAP prepared from NHS according to established methods (de Beer & Pepys, 1982). In other experiments, purified and radiolabelled SAP (^{125}I-SAP) was added to normal human serum and was then used as the source. It is not possible to use isolated SAP on its own, since in the absence of other proteins it precipitates in the presence of Ca^{2+} (Baltz *et al.*, 1982*b*). Human SAP was labelled with carrier-free Na^{125}I (IMS 30; Amersham International, Amersham, Bucks., U.K.) by the Iodogen method (Salacinski *et al.*, 1982). Free iodide was removed by gel filtration on Sephadex G-25 (PG10 column; Pharmacia, Milton Keynes, Bucks., U.K.) equilibrated with Tris/saline/Ca, and more than 95% of the radioactivity was then protein-bound and precipitable with trichloroacetic acid. Specific radioactivity of ^{125}I-SAP was approx. 1 μCi/μg. ^{125}I-SAP completely retained its ability to bind to agarose beads. In experiments with ^{125}I-SAP, binding was quantified by γ-radiation counting (NE 1612 instrument; Nuclear Enterprises, Reading, Berks., U.K.).

Chemicals

Methyl β-D-galactopyranoside, sodium pyruvate, D-galacturonic acid, D-galactose and phosphocholine were obtained from Sigma (Poole, Dorset, U.K.). The sodium salt of the *R*-isomer of methyl 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranoside (MOβDG) was synthesized as described previously (Hind *et al.*, 1984).

Binding of SAP to bacteria

Suitable volumes of suspensions of known numbers of bacteria were centrifuged (600*g*, 10 min) and the supernatant was removed before washing the organisms three times with Tris/saline/Ca before use. Known volumes (0.25–2.0 ml) of NHS as source of SAP, or ^{125}I-SAP in NHS, were added, mixed, and incubated at room temperature for 60 min. The bacteria were then centrifuged (600*g*, 1 min), and the supernatant removed as completely as possible before washing the pellet with 1.0 ml volumes of Tris/saline/Ca. After three washes, elution of SAP was attempted by incubation at room temperature for 10 min with known volumes (1.0 ml) of solutions of various chemicals in Tris/saline/Ca or Tris/saline/Ca only (negative control) or Tris/saline/EDTA (positive control). In other experiments, NHS or ^{125}I-SAP was first mixed with solutions of various chemicals under test in Tris/saline/Ca, or with Tris/saline/Ca alone in the controls, before being added to the bacteria. The same procedure was then adopted, except that the bacteria were eluted with Tris/saline/EDTA only. The concentration of SAP in all the supernatants and eluates was then assayed.

Results

Binding of SAP to bacteria

In the presence of Ca^{2+}, SAP in NHS or ^{125}I-SAP in NHS consistently bound to *Klebsiella rhinoscleromatis*, *Streptococcus pyogenes* and *Xanthomonas campestris*, and could then be recovered by elution with Tris/saline/EDTA (Table 1). When SAP was offered to bacteria in the presence of EDTA, there was no binding. Within the limits of these experiments the uptake of SAP on to *K. rhinoscleromatis* and *Strep. pyogenes* appeared saturable (Fig. 2), apparently with about 6000 SAP molecules binding to each bacterial cell. Among the other bacteria tested, no SAP binding was found with *Streptococcus pneumoniae*, *Strep. agalactiae*, *Staphylococcus epidermidis*, *Staph. aureus* and *Escherichia coli*.

Inhibition of SAP binding to bacteria by MOβDG

Binding of SAP to *K. rhinoscleromatis* and *Strep. pyogenes* was inhibited by the presence of MOβDG (Table 2). Methyl β-D-galactopyranoside, pyruvic acid, D-galactose and D-galacturonic acid, all at concentrations of 10–20 mM, had no appreciable

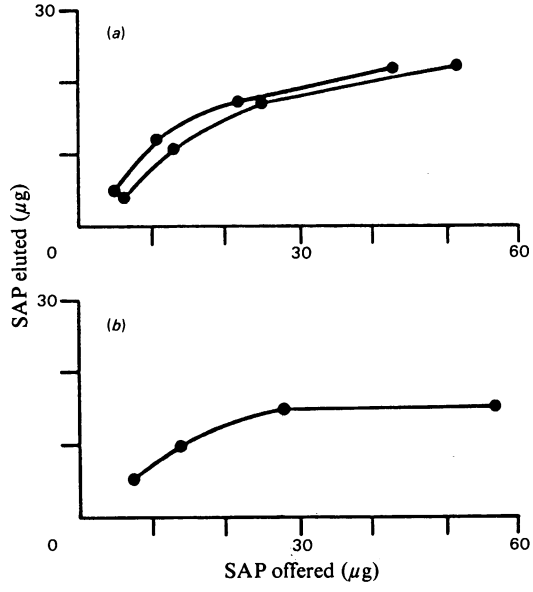

Fig. 2. *Binding of serum amyloid P component (SAP) from normal human serum to Klebsiella rhinoscleromatis (a) and Streptococcus pyogenes (b)*
In each experiment, 7.2×10^9 organisms were used.

Table 1. *Binding of SAP to bacteria*

Bacterium (and strain)	$10^9 \times$ No. of organisms	*SAP (μg) Offered	Not bound	Eluted with EDTA
Klebsiella rhinoscleromatis	7.2	29	9	16
Streptococcus pyogenes (group A, Griffith type 1)	7.2	29	8	17
Streptococcus agalactiae (group B, type 1c)	7.2	26	26	0
Streptococcus pneumoniae (type 4)	7.2	28	28	0
Staphylococcus aureus (serotype 3)	7.2	27	27	0
Staphylococcus epidermidis (type strain)	7.2	24	24	0
Escherichia coli (K29)	9.0	33	32	0
Xanthomonas campestris (N.I.C.B. 11803)	7.2	23	20	2

* From NHS.

Table 2. *Inhibition of binding of SAP to bacteria by MOβDG*

Bacterium*	SAP† bound in Tris/saline/Ca (μg)	Inhibition of SAP binding (%) Tris/saline/EDTA	MOβDG 14 mM	1.4 mM	0.14 mM
Klebsiella rhinoscleromatis	18	100	100	61	22
Streptococcus pyogenes	18	100	100	53	30

* 7.2×10^9 bacteria were used in each experiment.
† From NHS.

Table 3. *Elution of SAP† from bacteria by MOβDG*

Chemical	Concn. (mM)	SAP eluted (%)	
		*K. rhinoscleromatis**	*Strep. pyogenes**
Tris/saline/EDTA control		100	100
MOβDG	11	100	100
	1.1	45	30
	0.11	7	6

* 7.2×10^9 bacteria.
† ^{125}I-SAP in NHS.

effect on the binding of SAP. Phosphocholine, which is the ligand bound most avidly by C-reactive protein, another pentraxin closely related to SAP (Pepys & Baltz, 1983), also had no effect at 10–20mM.

Elution of SAP from bacteria by MOβDG

MOβDG eluted SAP that had already bound to *K. rhinoscleromatis* and *Strep. pyogenes* (Table 3). Methyl β-D-galactopyranoside, pyruvic acid, D-galactose, D-galacturonic acid and phosphocholine, all at 10–20mM, had no effect.

Discussion

Neither the physiological function of SAP nor its possible pathophysiological role in amyloidosis are yet known. However, following on the original finding that SAP undergoes Ca^{2+}-dependent binding to agarose (Pepys *et al.*, 1977), we have subsequently established that SAP specifically recognizes the 4,6-cyclic pyruvate acetal of galactose and, furthermore, that there are strict structural constraints in this ligand that affect binding (Hind *et al.*, 1984). Thus the α-form, the *S*-isomer, the non-cyclic acetal and the cyclic acetal in which the hydroxy residue of the carboxy group has been methylated are all notably less well bound by SAP than MOβDG itself (Hind *et al.*, 1983, 1984). Regardless of whether this carbohydrate-binding specificity itself is biologically relevant, it firmly establishes SAP as a mammalian lectin, and to complete its description as such we have previously reported the behaviour of SAP as an agglutinin (Hutchraft *et al.*, 1981; Baltz *et al.*, 1982*c*).

The present results extend to bacterial cell walls the observations of specific binding by SAP, though it was not surprising to find the interaction with *K. rhinoscleromatis* in which the presence of the 4,6-cyclic pyruvate acetal of galactose was already well established (Wheat *et al.*, 1965; Kenne & Lindberg, 1983). Of greater interest, both for fundamental reasons and because it is an organism of such considerable clinical importance, was the finding of equally good SAP uptake on to group A *Strep. pyogenes*. The chemistry of this organism's cell wall is not completely defined, and there have been no reports of the presence in it of acetals of galactose. Our observations suggest that the same 4,6-cyclic pyruvate acetal of galactose may exist in the capsule. The relatively limited binding of SAP to *X. campestris*, which had previously been thought to contain the cyclic 4,6-pyruvate acetal of glucose (Sloneker & Orentas, 1962), but was subsequently shown to contain the mannose counterpart (Jansson *et al.*, 1975), illustrates the considerable stereochemical specificity of SAP. This was confirmed by the failure of SAP to bind to *E. coli* in which the pyruvate acetal is linked to glucose (Kamei *et al.*, 1977) and to *Strep. pneumoniae*, in which there is a 2,3- rather than a 4,6-cyclic pyruvate acetal and it is linked to α- rather than β-galactopyranoside (Kenne & Lindberg, 1983). SAP may thus serve as an interesting probe for the presence of its particular specific ligand moiety in the walls of bacteria and possibly also other cells.

In the absence of any experimental evidence, the physiological significance *in vivo* of our observations *in vitro* remains a matter for speculation. It is, however, intriguing that C-reactive protein, another pentraxin with 60% homology of amino acid sequence with SAP and which shows Ca^{2+}-dependent binding specificity for phosphocholine, binds avidly to *Strep. pneumoniae*, the somatic polysaccharide of which contains this residue (Pepys & Baltz, 1983). Passive administration of human C-reactive protein to mice protects them completely from otherwise lethal infection with *Strep. pneumoniae* (Mold *et al.*, 1981; Yother *et al.*, 1982). It will therefore be interesting to determine whether SAP binds, for example, to *Strep. pyogenes in vivo* and what effect such binding may have on the course and outcome of infection.

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