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Human serum amyloid P is a multispecific adhesive protein whose ligands include 6-phosphorylated mannose and the 3-sulphated saccharides galactose, *N*-acetylgalactosamine and glucuronic acid

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Carbohydrate recognition by amyloid P component from human serum has been investigated by binding experiments using several glycosaminoglycans, polysaccharides and a series of structurally defined neoglycolipids and natural glycolipids. Two novel classes of carbohydrate ligands have been identified. The first is 6-phosphorylated mannose as found on lysosomal hydrolases, and the second is the 3-sulphated saccharides galactose, *N*-acetylgalactosamine and glucuronic acid as found on sulphatide and other acidic glycolipids that occur in neural or kidney tissues or on subpopulations of lymphocytes. Binding to mannose-6-phosphate containing molecules and inhibition of binding by free mannose-6-phosphate and fructose-1-phosphate are features shared with mannose-6-phosphate receptors involved in trafficking of lysosomal enzymes. However, only amyloid P binding is inhibited by galactose-6-phosphate, mannose-1-phosphate and glucose-6-phosphate. These findings strengthen the possibility that amyloid P protein has a central role in amyloidogenic processes: first in formation of focal concentrations of lysosomal enzymes including proteases that generate fibril-forming peptides from amyloidogenic proteins, and second in formation of multicomponent complexes that include sulphoglycolipids as well as glycosaminoglycans. The evidence that binding to all of the acidic ligands involves the same polypeptide domain on amyloid P protein, and inhibition data using diffusible, phosphorylated monosaccharides, is potentially important leads to novel drug designs aimed at preventing or even reversing amyloid deposition processes without interference with essential lysosomal trafficking pathways.

Key words: Alzheimer's disease/amyloid P protein/amyloid deposition and dispersal/carbohydrate binding/glycolipids

Introduction

Amyloid P component is a serum glycoprotein of ~230 kDa composed of 10 subunits of ~23 kDa which is synthesized by hepatocytes (Skinner and Cohen, 1988). This protein of unknown physiological function has been detected in normal glomerular basement membranes (Dyck *et al.*, 1980) and in microfibrils associated with elastic tissue (Breathnach *et al.*, 1981) and is a member of a family of proteins, which includes C-reactive protein (CRP), referred to as the pentraxins (Osmand *et al.*, 1977). This protein is of considerable medical interest since it occurs in association with all types of amyloid deposits: systemic and localized forms including those associated with Alzheimer's disease (Coria *et al.*, 1988; Hawkins, 1988; Duong *et al.*, 1989). Although the precise role of serum amyloid P component (SAP) in the formation and persistence of amyloid deposits is not understood, it clearly binds in a calcium-dependent manner to amyloid fibrils (Pepys *et al.*, 1979) and to the glycosaminoglycans heparin, heparan sulphate and dermatan sulphate (Hamazaki, 1987, 1988, 1989), which are frequently associated with the fibrils (Snow *et al.*, 1987). SAP also binds to the 4,6-cyclic pyruvate acetal of galactose (Hind *et al.*, 1984). This knowledge, as well as reports that SAP may bind to certain glycoproteins and polysaccharides containing mannose residues and to the human complement glycoprotein iC3b (Hutchcraft *et al.*, 1981; Kubak *et al.*, 1988), has raised the possibility that SAP may be a calcium-dependent carbohydrate binding protein (lectin). The protein sequence (Mantzouranis *et al.*, 1985), however, is distinct from those of a major family of calcium-dependent animal lectins (Drickamer, 1988). Notable among these latter proteins is the bovine serum component conglutinin (Lachmann, 1967) and the so-called mannan binding proteins in human and animal sera (Kawasaki *et al.*, 1983, 1985; Oka *et al.*, 1988; Drickamer *et al.*, 1986; Ezekowitz *et al.*, 1988), all of which bind certain mannose-rich polysaccharides. Conglutinin in addition binds iC3b. Oligosaccharide ligands for bovine conglutinin, and the human and rat serum mannan binding proteins have been identified recently by binding studies using lipid-linked oligosaccharide probes (neoglycolipids) (Childs *et al.*, 1989; Loveless *et al.*, 1989; Mizuochi *et al.*, 1989). All three proteins are multispecific and they bind in a calcium-dependent manner to certain oligosaccharides terminating with mannose or *N*-acetylglucosamine or fucose residues. In the present study we have investigated the binding specificity of human SAP using (i) a series of polysaccharides and glycoproteins including rabbit iC3b, (ii) a series of structurally defined neoglycolipids and (iii) natural glycolipids. We report here that the binding specificity of SAP is quite distinct from those of conglutinin, the mannan binding proteins and CRP, and includes 6-phosphorylated mannose and 3-sulphated monosaccharides galactose, *N*-acetylgalactosamine and glucuronic acid, and we discuss the potential significance of these binding specificities in two important processes leading to amyloid formation: the generation of amyloid-forming polypeptides by proteolysis of precursor proteins and the formation of the multicomponent complexes in amyloid deposits.

Results

Binding studies with immobilized polysaccharides, glycoproteins and neoglycolipids

In the presence of calcium ions, [^{125}I]SAP bound strongly to the glycosaminoglycans heparin and heparan sulphate

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immobilized in microwells (Figure 1A), and to a lesser extent to dermatan sulphate and chondroitin sulphates, in accordance with previous observations using other assay conditions (Hamazaki, 1987, 1988, 1989). There was only a weak binding to immobilized keratan sulphate. Since zymosan from *Saccharomyces cerevisiae* enriched for phosphate residues (Kubak *et al.*, 1988) showed stronger binding than the unenriched preparation, while the mannose-rich polysaccharides, mannan and invertase, showed no binding (Figure 1B) [nor was there any binding to erythrocytes coated with rabbit iC3b (Figure 1B, inset)], we evaluated the binding of SAP to the mannose-containing pentasaccharide with 6-linked phosphate. SAP bound strongly in a calcium-dependent manner to the neoglycolipid prepared from this oligosaccharide, both in the plastic microwell and the chromatogram binding assays (Figures 1C and 2A). There was no binding to the neoglycolipid derived from the corresponding dephosphorylated pentasaccharide (Figures 1C and 2A), nor to those derived from high-mannose type and bi-antennary complex-type oligosaccharide chains (Figure 2A). There was also no binding to the phosphomannan core from *Hansenula holstii* (Figure 1B), where the mannose residues bearing phosphate may have different glycosidic linkages, for example 1-6 rather than 1-3 and 1-2 found in the pentasaccharide (Bretthauer *et al.*, 1973). Since 6-phosphorylated mannose residues occur on the carbohydrate chains of mammalian lysosomal enzymes (von Figura and Hasilik, 1986; Kornfeld, 1987), the binding of SAP to the lysosomal enzyme arylsulphatase A was evaluated. SAP bound to this enzyme in the microwell binding assay (Figure 1B).

The specificity of SAP binding to phosphorylated monosaccharides was further investigated by inhibition assays (Figure 3A) using the pentamannose phosphate neoglycolipid, sulphatide, heparin or heparan sulphate immobilized in microwells, and a range of monosaccharide concentrations (final concentrations 0.15−5.0 mg/ml) as inhibitors. Inhibition of binding was not observed with immobilized heparin or heparan sulphate at the highest monosaccharide concentrations tested. With immobilized sulphatide, inhibition was observed only with fructose-1-phosphate and at the highest concentration tested: 65% inhibition at 5 mg/ml final concentration (results not shown). When the pentamannose phosphate neoglycolipid was used as the immobilized ligand, fructose-1-phosphate was consistently the most potent inhibitor, although the degree of inhibition was variable from experiment to experiment. In the experiment illustrated, fructose-1-phosphate gave 58% inhibition at the lowest concentration tested; mannose-6-phosphate, galactose-6-phosphate and mannose-1-phosphate showed similar inhibitory activities (50% inhibition at 1.1, 1.2 and 2.0 mg/ml respectively). With glucose-6-phosphate inhibition (65%) was observed only at the highest concentration tested. The corresponding non-phosphorylated monosaccharides were not inhibitory (results not shown). The sulphated monosaccharide (galactose-6-sulphate) which was also tested gave a weak inhibition (25% at the highest level tested). In other experiments where the degree of inhibition by fructose-1-phosphate, mannose-6-phosphate and galactose-6-phosphate was less, mannose-1-phosphate, glucose-6-phosphate and galactose-6-sulphate were not inhibitory.

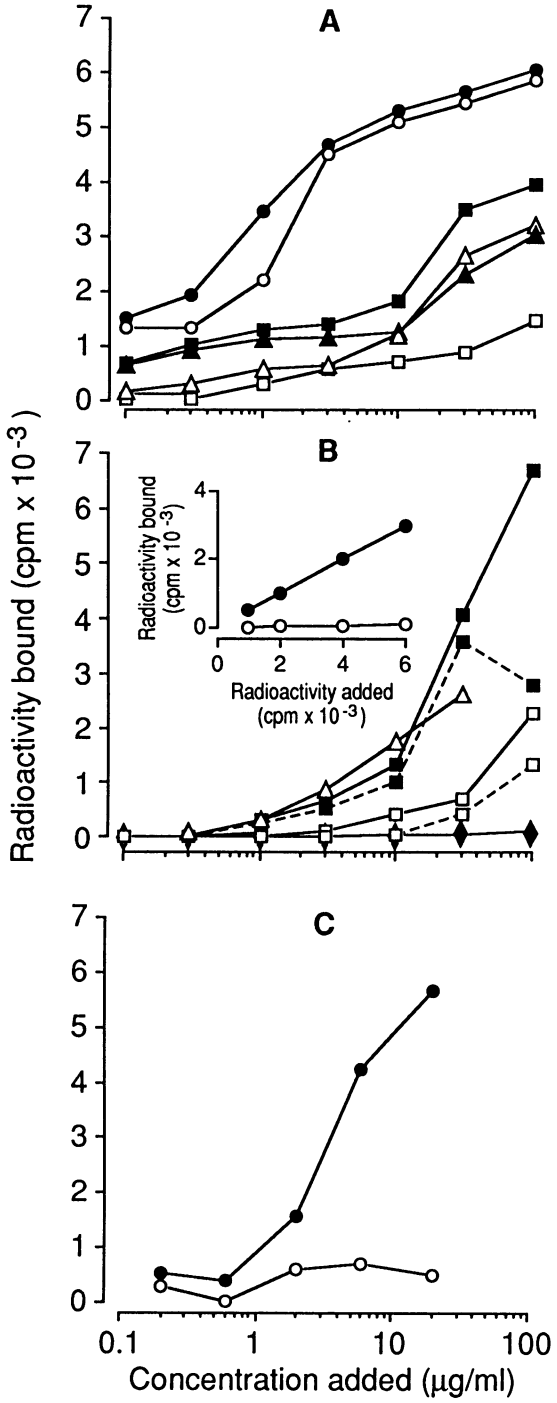

Fig. 1. Microtitre well binding assays with serum amyloid P and C-reactive protein. (**A**) Binding of [^{125}I]SAP to glycosaminoglycans: heparin (●); heparan sulphate (○); dermatan sulphate (■); chondroitin-4-sulphate (△); chondroitin-6-sulphate (▲); keratan sulphate (□). No binding was detected with [^{125}I]CRP. (**B**) Binding of [^{125}I]SAP (solid lines) and [^{125}I]CRP (broken lines) to polysaccharides and glycoproteins from *S.cerevisiae*: zymosan A (□); alkali-soluble fraction of zymosan A (■), but not to mannan, invertase or the phosphomannan core from *H.holstii* (◆). Also shown is the binding of [^{125}I]SAP to arylsulphatase A (△). Inset shows the binding of [^{125}I]conglutinin (●) and the lack of binding of [^{125}I]SAP (○) to iC3b-coated erythrocytes; binding was not detected with [^{125}I]CRP. (**C**) Binding of [^{125}I]SAP to the neoglycolipid prepared from the pentamannose phosphate oligosaccharide from *H.holstii* (●) and lack of binding to that from the corresponding dephosphorylated oligosaccharide (○).

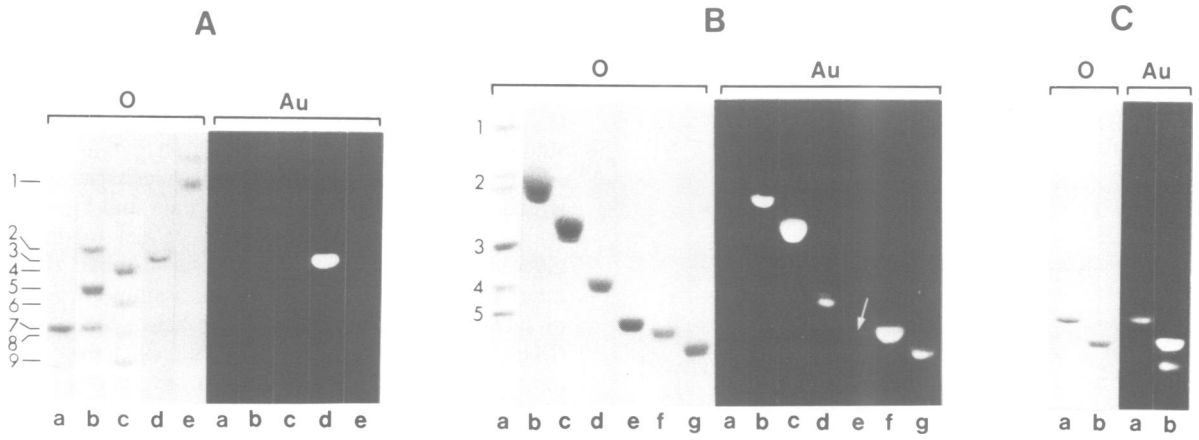

Fig. 2. Thin layer chromatogram overlay assays with [^{125}I]serum amyloid P. (**A**) Selective binding to the 6-phosphorylated pentamannose oligosaccharide. Lane a, biantennary asialo-digalactosyl oligosaccharide (1 μg) from human transferrin (migrating at position 7); lane b, biantennary agalactosyl, monogalactosyl and digalactosyl oligosaccharides (3 μg total) with core region fucose (positions 2, 5 and 7 respectively) from mouse IgG; lane c, high-mannose type oligosaccharides (3 μg total) with 5, 6, 7 and 8 mannose residues (positions 4, 6, 8 and 9 respectively) and additional minor unidentified bands, from bovine pancreatic RNase B; lane d, pentamannose oligosaccharide (1 μg) containing 6-linked phosphate (position 3) from *H.holstii*; lane e, an alkaline phosphatase-treated preparation of the phosphorylated pentamannose oligosaccharide (1 μg) from *H.holstii* (position 1): the additional minor band in this lane with a faster mobility was identified by fast atom bombardment mass spectrometry (unpublished) as a non-phosphorylated tetramannose oligosaccharide. (**B**) Selective binding to sulphated glycolipids (5 nmol per lane). Lane a, neutral glycolipid mixture (5 μg) containing galactosylceramide (position 1) lactosylceramide (duplex at position 2), trihexosylceramide (position 3), globoside (position 4) and the Forssman glycolipid (position 5); lane b, sulphatide from bovine brain; lane c, SM3 from human kidney; lane d, SM2; lane e, SM1a; lane f, SB2; lane g, SB1a. The latter four glycolipids were from rat kidney. White arrow indicates weak binding. (**C**) Binding to HNK-1 glycolipid. Lane a, SB1a (1 nmol) from rat kidney; lane b, HNK-1 glycolipid (1 nmol) from bovine cauda equina. In A, B and C, panels designated O show orcinol staining and those designated Au show the corresponding autoradiographs. In all cases, binding was abolished in the presence of 1 mM EDTA. Chromatography was upwards in solvent II (A) and solvent I (B and C).

Binding studies with natural glycolipids

Additional ligands for SAP were identified in the course of evaluating binding to naturally occurring glycolipids. Brain sulphatide (Figure 2B) and sulphoglucuronylneolactotetraosylceramide (HNK-1 glycolipid) from cauda equina (Figure 2C), which contain 3-sulphated galactose and 3-sulphated glucuronic acid respectively (Figure 4), were bound in a calcium-dependent manner, whereas cholesterol-3-sulphate, the purified brain gangliosides GM_1, GM_3, GD_{1a}, GD_{1b}, GD_3, GT_{1b} and GQ_{1b} (results not shown) and several neutral glycolipids (Figure 2B), including the non-sulphated galactosylceramides, were not bound by SAP.

The specificity of SAP binding to sulphated glycolipids was further investigated using monosulphated (SM3, SM2, SM1a) and disulphated (SB2, SB1a) glycolipids, isolated from kidney tissue, in which the sulphate residues are 3-linked to galactose and in the case of SB2 also to *N*-acetylgalactosamine (Figure 4). A preferential binding to sulphate groups on terminal galactose or *N*-acetylgalactosamine residues was clearly apparent over a range of glycolipid levels tested (0.1, 0.3, 1 and 5 nmol per lane); at 5 nmol (Figure 2B) a relatively strong binding to sulphatide, SM3 and SB1a which contain sulphated terminal galactose, and SB2 which contains a sulphated terminal *N*-acetylgalactosamine was observed. There was considerably less binding to SM2 with a sulphated penultimate galactose and only trace binding (arrowed) to SM1a with an internal sulphated galactose. At 0.1 nmol (not shown), binding only to SM3, SB2 and SB1a could be detected.

Inhibition of binding experiments with synthetic polypeptides

Inhibition of binding experiments were next performed with the synthetic 13mer polypeptide, Pep 1 (EKPLQNFTLCFRA) which represents residues 27–39 of SAP. The first 12 amino acids of this peptide constitute the sequence designated SAP-1 by Dhawan *et al.* (1990) which, when immobilized, supports adhesion of a wide variety of cell types. Pep 1 gave dose-dependent inhibition (Figure 3B) of [^{125}I]SAP binding to all five ligands tested (heparin, alkali-soluble zymosan extract, sulphatide, HNK-1 glycolipid and the pentamannose phosphate neoglycolipid). The concentration of Pep 1 giving 50% inhibition of binding was comparable (between 160 and 280 μg/ml) for all these ligands with the exception of zymosan where the inhibition curve was shallow and the concentration required for 50% inhibition was higher (1.5 mg/ml). The control peptides Pep 2 and 3 gave no inhibition of binding to the five immobilized ligands at the concentrations tested; results with Pep 2 are shown in Figure 3C. Nor was there any inhibition with two additional control peptides, Pep 4 and Pep 5, when tested with sulphatide as the immobilized ligand (results not shown).

In a further experiment, immobilized sulphatide was pre-incubated with Pep 1 and the wells washed before the addition of [^{125}I]SAP. Inhibition of binding was again observed (not shown) which was comparable with that shown in Figure 3B, and provided clear evidence for the binding of Pep 1 to this ligand.

Binding studies using CRP

With [^{125}I]CRP, significant binding only to the alkali-soluble fraction of zymosan and to a lesser extent unfractionated zymosan (Figure 1B) was observed among the polysaccharides, glycoproteins, glycolipids and neoglycolipids tested in the different assay systems.

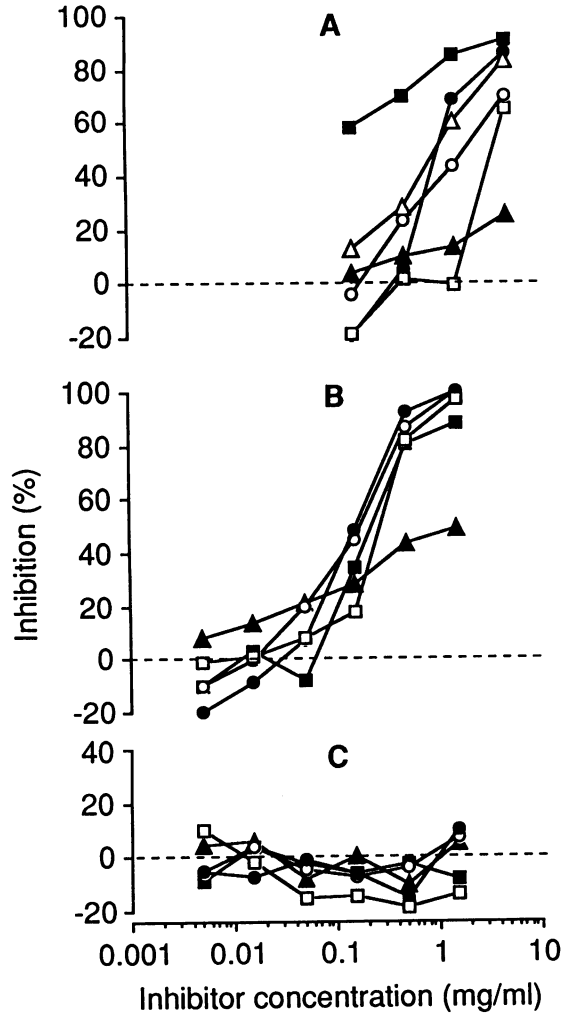

Fig. 3. Inhibition of microtitre well binding assays with serum amyloid P. (**A**) Inhibition of [^{125}I]SAP binding to the neoglycolipid derived from the *H.holstii* pentamannose phosphate by monosaccharides: fructose-1-phosphate (■), mannose-6-phosphate (●), galactose-6-phosphate (△), mannose-1-phosphate (○), glucose-6-phosphate (□) and galatose-6-sulphate (▲). (**B**) Inhibition of [^{125}I]SAP binding by the synthetic peptide, Pep 1. The immobilized ligands were: heparin (■), alkali-soluble extract of zymosan from *S.cerevisiae* (▲), sulphatide (○), HNK-1 glycolipid (●), and the *H.holstii* pentamannose phosphate neoglycolipid (□). (**C**) Lack of inhibition of [^{125}I]SAP binding by the synthetic peptide, Pep 2. Symbols for the immobilized ligands are as in (B).

Discussion

The novel findings from these studies are, first, that the saccharide binding specificity of SAP is completely different from those of the other serum proteins conglutinin, mannan binding proteins and CRP, all of which bind the yeast polysaccharide zymosan (Lachmann, 1967; Kindmark, 1972; observations with mannan binding proteins, R.W.Loveless, unpublished). SAP does not recognize the neutral oligosaccharides with terminal mannose or *N*-acetylglucosamine residues which are ligands for conglutinin (Loveless *et al.*, 1989; Mizuochi *et al.*, 1989) and the mannan-binding proteins (Childs *et al.*, 1989). Nor does SAP bind to rabbit iC3b to which conglutinin binds (Lachmann, 1967).

Second, SAP like the two mannose 6-phosphate receptors binds to an oligosaccharide which has a terminal 6-phosphorylated mannose residue and to the lysosomal enzyme, arylsulphatase A. There are some striking differences, however, in the binding specificities of the three proteins and these are discussed below.

Third, SAP binds to glycolipids with terminal galactose or *N*-acetylgalactosamine substituted with 3-*O*-linked sulphate residues. These include sulphatide and a series of acidic glycolipids with longer oligosaccharide backbones which are located notably in the brain and kidney (Stults *et al.*, 1989). Here there is preferential binding to the glycolipids with sulphate located on terminal rather than internal galactose or *N*-acetylgalactosamine residues. It is of interest to note that the amino acid sequence of SAP (Mantzouranis *et al.*, 1985) does not contain the polypeptide motif CSVTCG which is common to several other sulphatide binding proteins (Holt *et al.*, 1989).

Fourth, SAP binds to the HNK-1 glycolipid which contains terminal glucuronic acid substituted with 3-*O* linked sulphate. This glycolipid occurs on peripheral nervous tissue (Chou *et al.*, 1986), and cross-reactive antigens recognized by HNK-1 antibody occur on myelin-associated glycoprotein and on cell adhesion molecules expressed on sub-populations of astrocytes, oligodendrocytes (Schachner, 1989), as well as lymphocytes (Chou *et al.*, 1986).

Fifth, we provide evidence that the region encompassing amino acids 27–39 of SAP (Pep 1) contains contact residues that are involved in the interaction with the well-defined ligands, pentamannose phosphate, sulphatide and HNK-1 glycolipid, and with the complex polysaccharides heparin and zymosan. Snythetic Pep 1 inhibits the binding of SAP to these five types of ligand and appears to bind with considerable affinity to the ligand tested (sulphatide), thus competing for binding by SAP.

This array of binding specificities of SAP, which includes pyruvylated galactose residues as reported earlier (Hind *et al.*, 1984), is unlikely to be simply a reflection of the acidic nature of the saccharides, since keratan sulphate [rich in 6-*O* sulphated galactose and *N*-acetylglucosamine residues (Scudder *et al.*, 1986)] is only weakly bound and cholesterol-3-sulphate is not bound. Moreover, mono- and multiply sialylated glycolipids with backbone sequences similar to those of the sulphated glycolipids are also not bound by SAP.

Binding to the pentamannose phosphate and inhibition of binding in the presence of mannose-6-phosphate and fructose-1-phosphate are properties shared by SAP, both the cation-dependent and cation-independent mannose-6-phosphate receptors and the lymphocyte homing receptor, LAM-1 (Stoolman *et al.*, 1987). The amino acid sequence of SAP shows no striking homology to those of the mannose-6-phosphate receptors or LAM-1, and in particular the Pep 1 sequence which appears to contain the binding site of SAP is not present in the latter three proteins. Moreover, from the present and earlier studies (von Figura and Hasilik, 1986; Kornfeld, 1987; Stoolman *et al.*, 1987), it is clear that the fine specificities of the four proteins differ: (i) only SAP binding may be inhibited in the presence of galactose-6-phosphate, (ii) SAP binding is more strongly inhibited by fructose-1-phosphate than by mannose-6-phosphate, whereas with the mannose-6-phosphate receptors and LAM-1 the two monosaccharides are almost equally active as inhibitors, (iii) the mannose-6-phosphate receptors and LAM-1 but not SAP bind to the *H.holstii* phosphomannan core, and (iv) SAP but not the cation-independent mannose-6-phosphate receptor

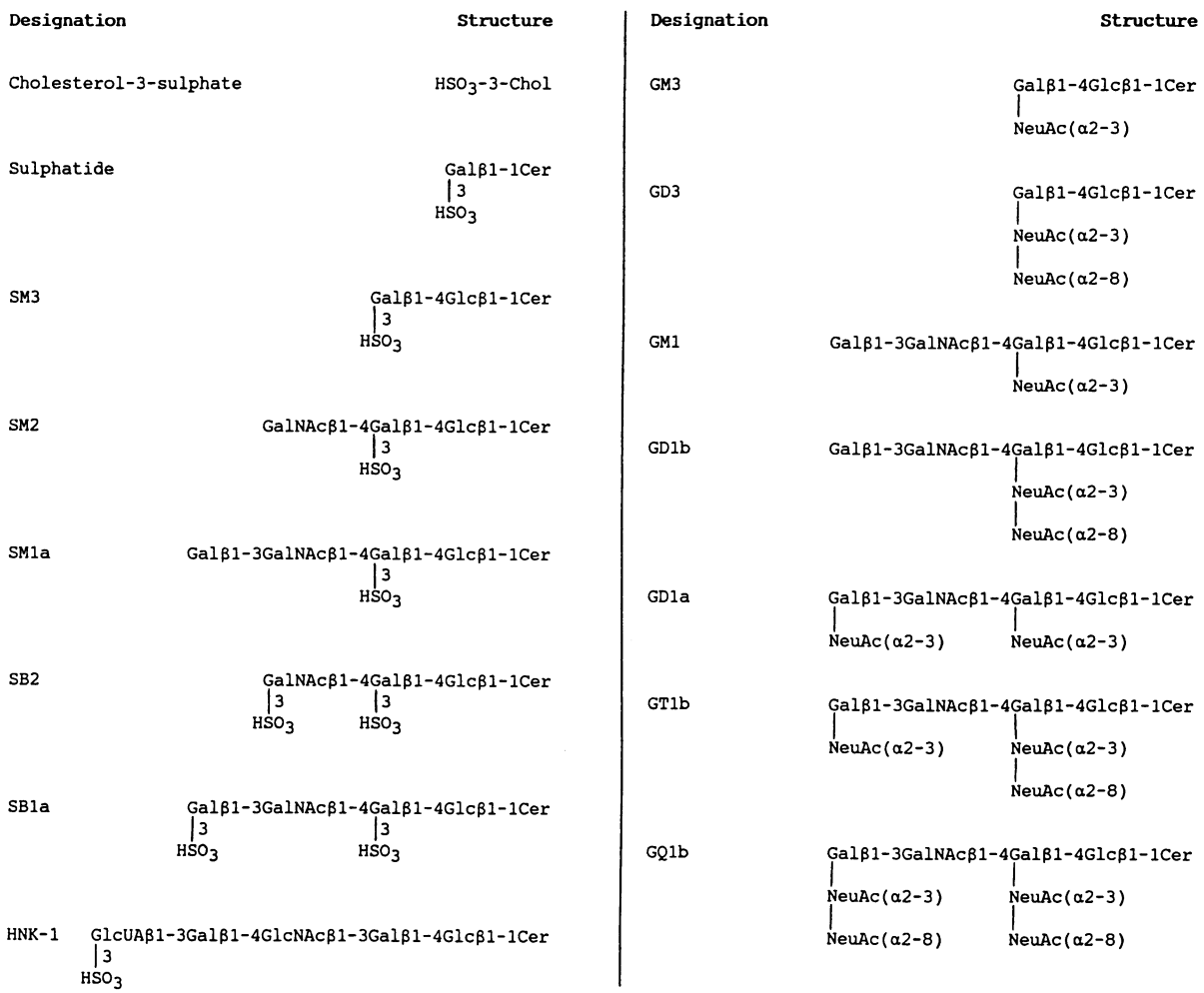

Fig. 4. The acidic glycolipids tested for binding with serum amyloid P. Abbreviations for monosaccharides and lipids: Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcUA, glucuronic acid; NeuAc, *N*-acetylneuraminic acid; Cer, ceramide; Chol, cholesterol.

binds to the alkali-soluble fraction of *S.cerevisiae* zymosan (P.Green, R.W.Loveless and T.Feizi, unpublished).

Heparin and heparan sulphate (Lindahl, 1989) contain sulphate residues linked in various ways to hexuronic (iduronic and glucuronic) acids (2-*O* linked), *N*-acetylglucosamine (6-*O* linked) and glucosamine (monosulphated 2-*N*; disulphated 2-*N*, 3-*O* and 2-*N*, 6-*O*; trisulphated 2-*N*, 3-*O*, 6-*O*). It is not yet known which of these are recognized by SAP, but from the present study it is clear that internally located *N*-acetylglucosamine with 6-*O* linked sulphate (as in keratan sulphate) is not a major determinant.

The occurrence of amyloid protein P in all forms of amyloid deposits (Coria *et al.*, 1988; Hawkins, 1988; Duong *et al.*, 1989) and its rapid extravasation and deposition into existing amyloid tissue (Hawkins *et al.*, 1988) is consistent with a role in amyloidogenesis, although the precise mechanism has not yet been defined. The new observations on the binding specificities of SAP in the present study strengthen the possibility that this protein is intricately involved in the amyloid processes: (i) indirectly, in the proteolytic cleavages that lead to aggregation and fibril formation by amyloidogenic proteins, and (ii) directly, in the assembly of multicomponent complexes characteristic of amyloid deposits. The binding of the multisubunit SAP to 6-phosphorylated mannose residues can be envisaged as a mechanism for focal concentration and sequestration of numerous lysosomal enzymes, including proteases, released either from macrophages and reticuloendothelial cells in reactive, secondary forms of amyloidosis in inflammatory disorders, or from damaged neurones and accessory cells of the brain in Alzheimer's disease. It has long been suspected that lysosomal dense bodies shed from reticuloendothelial cells and found in intimate association with amyloid fibrils may be a source of hydrolases that generate amyloid-forming polypeptides (Shirahama and Cohen, 1973). The term amyloid enhancing factor has been applied to as yet uncharacterized material released from peritoneal macrophages of amyloidogenic mice that accelerates amyloid deposition in experimental, casein-induced amyloidosis (Shirahama *et al.*, 1990). Lysosomal enzymes would seem likely candidates as the active components here. In accord with these predictions are observations that high levels of enzymatically active lysosomal proteases cathepsin B and D occur in senile plaques of Alzheimer's disease (Cataldo and Nixon, 1990). It has been proposed that these enzymes are at least partly responsible for the processing of the amyloid precursor protein in Alzheimer's disease. Although lysosomal proteases are optimally active in an acidic environment, cathepsin D still retains considerable activity at physiological pH (reviewed by Cataldo and Nixon, 1990), particularly in the presence of other factors, such as acidic glycolipids including sulphatide (Williams *et al.*, 1986),

which we now show to be among glycolipids specifically bound by SAP. Interestingly, increased levels of this glycolipid have been reported in Alzheimer brain (Majocha *et al.*, 1989).

Sulphoglycolipids of the type described in the present study, as well as glycosaminoglycans derived from degenerating neurones or renal tissues or from inflammatory foci, would be predicted to form tight aggregates in the presence of SAP. From our observations on the binding activity of the 13mer SAP peptide, Pep 1, it is predicted that even some partially degraded forms of SAP would remain complexed to these glycoconjugates. With respect to amyloid formation in Alzheimer's disease, it will be particularly interesting to investigate amyloid P protein interactions with the glycoprotein precursors of A4 (β-amyloid). These glycoproteins contain tyrosine sulphate (Schubert *et al.*, 1989; Weidemann *et al.*, 1989), which has not yet been investigated as a ligand for SAP. Moreover, the presence of additional sulphate groups on carbohydrate chains of the precursors has not been ruled out (Weidemann *et al.*, 1989).

In conclusion, further investigations are required to evaluate the part played by SAP in the tethering and focal sequestration of proteolytic enzymes that generate fibril-forming peptides from amyloidogenic proteins, and in the formation of tight multicomponent complexes that are resistant to clearance. If SAP does indeed play a central role, both types of complex would be potential targets for drugs aimed at arresting or even reversing amyloid deposition. The possibility that a pyruvate acetal of galactose which inhibits SAP binding to amyloid fibrils may have therapeutic value has been suggested (Hind *et al.*, 1986). Low molecular weight inhibitory compounds such as 6-phosphorylated galactose and other analogues tailored to inhibit effectively SAP binding to the various endogenous ligands without cross-reacting with essential processes, such as the receptor-mediated trafficking of lysosomal enzymes and lymphocyte homing, would be attractive candidates as anti-amyloid drugs.

Materials and methods

Purification and iodination of SAP and CRP

SAP was purified from 1 l of pooled heparinized plasma according to deBeer and Pepys (1982) and Ohkubo *et al.* (1986), except that, following chromatography on Sepharose 4B, protein eluted with EDTA was passed over a DEAE−cellulose column (1.6 × 30 cm) and eluted with a gradient of 0.1−0.5 M NaCl in 10 mM Tris−HCl, pH 7.6. Protein eluting between 0.2 and 0.3 M NaCl was applied to a column of zinc chelate Sepharose and eluted at 25−30 mM L-histidine. The protein (yield 8−12 mg/l plasma) gave a single band of ~29 kDa in polyacrylamide gel electrophoresis under reducing conditions (Laemmli, 1970) and was stored at −20°C. CRP was purified from 400 ml of acute phase serum according to Volanakis *et al.* (1978) by affinity chromatography using phosphocholine Sepharose 4B (1.6 × 24 cm) followed by DEAE−cellulose (1.5 × 30 cm) and Sephacryl S300 (1.6 × 60 cm) column chromatography. The protein (yield 6 mg), which gave a single band of ~25 kDa in polyacrylamide gel electrophoresis under reducing conditions, was stored at −20°C.

SAP and CRP (100 μg of each) were labelled (Salacinski *et al.*, 1981) with 0.5 mCi ^{125}I (New England Nuclear, Stevenage, Herts, UK) using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (Sigma Chemical Co., Poole, Dorset, UK). The labelled proteins (specific activity 1−3 μCi/μg) were stored at 4°C in the presence of 0.02% sodium azide.

Glycosaminoglycans, glycoproteins, mono- and oligosaccharides

The glycosaminoglycans, heparin from porcine intestinal mucosa, chondroitin-4-sulphate from bovine trachea, chondroitin-6-sulphate from shark cartilage and dermatan sulphate from porcine skin were from Sigma. Keratan sulphate (3 M NaCl fraction) was from bovine cornea (Scudder *et al.*, 1986). Heparan sulphate from bovine mast cells was a gift from Dr I.Nieduzynski, Lancaster University, UK. The lysosomal enzyme, arylsulphatase A (gift of Dr K.von Figura, Göttingen University, Germany), was purified to homogeneity from extracts of mouse L cells overexpressing the enzyme. The *S.cerevisiae* glycoproteins invertase grade VII, mannan and zymosan A were from Sigma. The alkali-soluble fraction of zymosan, enriched for mannans and phosphomannans, was prepared according to Kubak *et al.* (1988). Phosphomannan from *H.holstii* (gift of Dr M.Slodki, Agricultural Research Service, Peoria, IL, USA) was hydrolysed according to Bretthauer *et al.* (1973), by Paula Green of this department, to obtain the phosphomannan core (Slodki *et al.*, 1973) and the 6-phosphorylated pentamannose oligosaccharide (P-6Manα1-3Manα1-3Manα1-3Manα1-2Man; Bretthauer *et al.*, 1973). The corresponding non-phosphorylated pentamannose oligosaccharide was obtained by digestion with alkaline phosphatase type III from *Escherichia coli* (EC 3.1.3.1., Sigma). The monosaccharides fructose, galactose, glucose, mannose, fructose-1-phosphate, galactose-6-phosphate, glucose-6-phosphate, mannose-1-phosphate, mannose-6-phosphate and galacatose-6-sulphate were from Sigma.

Natural glycolipids

Sulphatide from bovine brain and cholesterol-3-sulphate were from Sigma. A mixture of neutral glycolipids containing galactosylceramide, Gal-Cer; lactosylceramide, Galβ1-4Glc-Cer; trihexosylceramide, Galα1-4Galβ1-4Glc-Cer; globoside, GalNAcβ1-3Galα1-4 Galβ1-4Glc-Cer, and the Forssman glycolipid, GalNAcα1-3GalNAcβ1-3Galα1-4 Galβ1-4Glc-Cer, were from Biocarb Chemicals, Lund, Sweden. Shown in Figure 4 are the structures of the gangliosides GM_1, GM_3, GD_{1a}, GD_{1b}, GD_3, GT_{1b} and GQ_{1b} from calf or bovine brain (Biocarb Chemicals); and the monosulphated glycolipids [sulphatide and SM3] from human kidney (Mårtensson, 1966; Ishizuka and Tadano, 1982), SM2 and SM1a from rat kidney (Tadano and Ishizuka, 1982a; Ishizuka and Tadano, 1982); the disulphated glycolipids SB2 and SB1a from rat kidney (Tadano and Ishizuka, 1982b; Tadano *et al.*, 1982), all gifts from Dr I.Ishizuka, Teikyo University School of Medicine, Tokyo, Japan, and sulphoglucuronylneolactotetraosylceramide (HNK-1 glycolipid) isolated from bovine cauda equina (gift of Dr F.B.Jungalwala, Eunice Kennedy Shriver Center for Mental Retardation, Waltham, MA, USA).

Neoglycolipids

Neoglycolipids prepared from high-mannose type oligosaccharides released from bovine pancreatic RNase B, asialo complex-type bi-antennary oligosaccharides derived from human transferrin and mouse IgG, were similar to those described (Stoll *et al.*, 1988; Mizuochi *et al.*, 1989). Neoglycolipids were also prepared from the pentamannose phosphate oligosaccharide and the corresponding dephosphorylated pentasaccharide by conjugation to phosphatidyl ethanolamine dipalmitoate, as described previously.

Synthetic peptides

The 13mer peptide, Pep 1 (EKPLQNFTLCFRA), which represents residues 27−39 of SAP, the unrelated decapeptide, Pep 2 (VFSYPAGNVE), and the 13mer peptide Pep 3 (LNRCAFKLEQPTF), which contains the same residues as Pep 1 but in a scrambled order, were synthesized by P. Purkiss and Dr P.Byfield of the MRC Clinical Research Centre, Harrow, UK, using Fmoc chemistry and an Applied Biosystems 431A peptide synthesizer. The peptides were further purified by HPLC and characterized by fast atom bombardment mass spectrometry and amino acid analyses. The 13mer peptide, Pep 4 (YSFQGRITTPGSC), and the 16mer peptide, Pep 5 (YVGELRHVIVGLIESL), were gifts of H.Partidos, London School of Hygiene and Tropical Medicine, London, UK.

Microwell binding assays

Binding of [^{125}I]SAP to glycosaminoglycans or glycoproteins, immobilized (without glutaraldehyde fixation) in wells of polyvinylchloride (PVC) microtitre plates (total reaction volume 50 μl), was performed essentially as described previously (Gooi *et al.*, 1985) except that poly-L-lysine coating of wells was omitted with arylsulphatase A and [^{125}I]SAP [2.5−5.0 × 10^4 c.p.m./well diluted in 10 mM Tris, 150 mM NaCl, pH 7.6, containing 1 mM Ca^{2+} and 1% bovine serum albumin (BSA-TBS-Ca^{2+})] was used instead of antibodies, and binding was measured after incubation for 2 h at 18°C. For binding to neoglycolipids or natural glycolipids, solutions in methanol were evaporated to dryness at 37°C. For inhibition assays, microwells were coated with the pentamannose phosphate neoglycolipid or sulphatide (4 μg/ml) or heparin or heparan sulphate (30 μg/ml) to give 3−6 × 10^3 c.p.m. bound. Inhibition of binding of [^{125}I]SAP was measured after 2 h incubation at 18°C in the presence of the phosphorylated or

sulphated monosaccharides (see legend to Figure 3) and their non-phosphorylated/sulphated forms, or in the presence of peptide inhibitors. In control experiments, where 1 mM EDTA was used instead of 1 mM Ca^{2+} (BSA-TBS-EDTA), binding was always $<1\%$ of radioactivity added. Binding assays under identical conditions were carried out using [^{125}I]CRP.

Binding to iC3b-coated erythrocytes

The binding of [^{125}I]SAP and of [^{125}I]CRP to sheep erythrocytes coated with rabbit iC3b was assayed as for conglutinin (Loveless *et al.*, 1989).

Chromatogram binding assays

The binding of [^{125}I]SAP and of [^{125}I]CRP to natural glycolipids and neoglycolipids on thin layer silica gel chromatograms was assayed essentially as described for concanavalin A (Stoll *et al.*, 1988), except that BSA-TBS-Ca^{2+} or BSA-TBS-EDTA for control experiments, were used as diluents, and chromatograms were soaked for 60 s in 0.05% Plexigum P28 in *n*-hexane. The solvents used for chromatography were chloroform:methanol:water 60:35:8 (by vol), solvent I, for natural glycolipids and chloroform:methanol:water 105:100:28 (by vol), solvent II, for neoglycolipids.

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