Binding of mannose-6-phosphate and heparin by boar seminal plasma PSP-II, a member of the spermadhesin protein family

Dolores Solís[a,*], Antonio Romero[b], Marta Jiménez[a], Teresa Díaz-Mauriño[a], Juan José Calvete[a,c,*]

[a] *Instituto de Química Física 'Rocasolano', C.S.I.C., Serrano 119, E-28006 Madrid, Spain*
[b] *Centro de Investigaciones Biológicas, C.S.I.C., Madrid, Spain*
[c] *Institut für Reproduktionsmedizin, Tierärztliche Hochschule, Hannover, Germany*

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Abstract PSP-I/PSP-II, a heterodimer of glycosylated spermadhesins, is the major component of boar seminal plasma. Similarly to other spermadhesins, the PSP-II subunit is a lectin which displays heparin- and zona pellucida glycoprotein-binding activities. We have investigated the ligand binding capabilities of the heterodimer and the isolated subunits using several polysaccharides, glycoproteins, and phospholipids. PSP-II binds the sulfated polysaccharides heparin and fucoidan in a dose-dependent and seemingly-specific manner. In addition, PSP-II binds oligosaccharides containing exposed mannose-6-phosphate monoester groups and the binding is selectively inhibited by mannose-6-phosphate and glucose-6-phosphate. Inhibition experiments indicate that binding of PSP-II to sulfated polysaccharides and mannose-6-phosphate-containing oligosaccharides involves distinct but possibly overlapping binding sites. Heterodimer formation with PSP-I abolishes both the heparin and the mannose-6-phosphate binding capabilities, suggesting that the corresponding sites may be located at the dimer interface. Using the crystal structure of PSP-I/PSP-II heterodimer as a template, we have explored possible binding sites which satisfy the observed binding characteristics. In the proposed models, PSP-II Arg^{43} appears to play a pivotal role in both heparin- and mannose-6-phosphate-complexation as well as in heterodimer formation.

Key words: Boar seminal plasma; Spermadhesin PSP-II; Mannose-6-phosphate-binding lectin; Heparin-binding protein

1. Introduction

The seminal plasma, the fluid in which mammalian spermatozoa are suspended in semen, is a complex mixture of secretions originating from the epididymis and the male accessory reproductive organs. The protein composition of seminal plasmas varies from species to species but reports from several mammalian species indicate that seminal plasma contains factors that influence both the fertilising ability of spermatozoa and exert important effects on the female reproductive physiology [1–3]. In the pig, the bulk of seminal plasma proteins belong to the spermadhesin family [4,5]. Spermadhesins, a group of 12–16-kDa proteins, emerge as a novel protein family of animal lectins, which coat to the sperm surface at ejaculation and are believed to play major roles in sperm capacitation and gamete interaction (reviewed in [6–8]).

Besides the porcine, spermadhesin molecules have been so far identified in bull and stallion [9,10]. Sequence variation, glycosylation and the aggregation state of spermadhesins appear to modulate their sperm-coating and carbohydrate-recognition capabilities [11,12]. Thus, aSFP, a monomeric non-glycosylated bovine spermadhesin, shares over 50% amino acid sequence identity with its stallion and boar counterparts but neither binds to the sperm surface nor possesses lectin activity [9]. On the other hand, glycosylation of the conserved asparagine-50 abolishes the carbohydrate-binding activity of boar spermadhesins [11].

Porcine seminal plasma glycoproteins I (PSP-I) and II (PSP-II) are major components of seminal plasma which form non-covalent heterodimers [12]. The PSP-I/PSP-II heterodimer displays carbohydrate-binding activity linked to the PSP-II subunit [12]. The PSP-I/PSP-II complex does not bind to the sperm surface excluding a role in gamete interaction. Nonetheless, lectin-glycoconjugate recognition systems play a central role in other aspects of mammalian reproductive physiology, e.g. sperm-epithelial cell interactions leading to sperm capacitation during residence in the female's reproductive tract [1–3]. Glycobiological interactions in the sow's uterus mediated by non-sperm-binding boar seminal plasma lectins deserve further investigations.

The aim of this study was to investigate the binding specificities of spermadhesin PSP-I/PSP-II. PSP-II but not the PSP-I/PSP-II heterodimer specifically binds sulfated polysaccharides and mannose-6-phosphate-containing oligosaccharides at distinct but possibly interrelated binding sites. Docking of Man-6-P and of a tetrasaccharide fragment of heparin onto the crystal structure of the PSP-II subunit suggests possible binding sites for these ligands.

2. Methods

2.1. Isolation and radioiodination of proteins

The PSP-I/PSP-II heterodimer was isolated from the non-heparin-binding fraction of boar seminal plasma by gel filtration chromatography, and its subunits were purified from the heterodimer by reversed-phase HPLC [12]. Proteins (300 μg each) were labelled with 0.2 mCi of ^{125}I using Iodogen (Pierce) according to the manufacturers' recommendations. The radioiodinated proteins were indistinguishable from the corresponding unlabelled proteins by SDS-PAGE and autoradiography. The reassociation capability of isolated PSP-I and PSP-II was assessed by gel filtration of equimolar mixtures of the proteins using a Superose 12 HR 10/30 column (Pharmacia Biotech) equilibrated in 10 mM Tris-HCl, pH 7.8, 0.15 M NaCl (TBS). The flow rate was 0.5 ml/min and the elution was monitored at 280 nm. Control proteins were chromatographed under similar conditions.

2.2. Binding of ^{125}I-PSP-II to PSP-I-Sepharose

PSP-I was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Biotech) according to the manufacturers' instructions, ex-

*Corresponding authors. Fax: (34) (1) 5642431.
E-mail: d.solis@iqfr.csic.es

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cept that prior to the coupling step the gel was treated with 0.1 M HCl for 1 h at 25°C to reduce non-specific binding [13]. Aliquots of sedimented PSP-I-Sepharose (13.3 mg of PSP-I/ml gel) were resuspended in polypropylene microcentrifuge tubes to a final volume of 0.5 ml with TBS and incubated with 10 μl (35 000 cpm) of ^{125}I-PSP-II solution. After 2 h at 25°C with occasional shaking, each sample was centrifuged and the radioactivity of the supernatant solution was counted in an LKB Mini-gamma counter. Non-specific binding or trapping on the Sepharose beads was estimated in parallel experiments using unsubstituted Sepharose 6B (Pharmacia Biotech).

2.3. Microwell binding assays

The following ligands were tested: (i) the polysaccharides: heparin; fucoidan (an algal polysaccharide containing fucose sulfate); zymosan A, a *Saccharomyces cerevisiae* cell wall polysaccharide preparation containing mannan and phosphomannan polymers [14]; the glycosaminoglycan chondroitin-6-sulfate; (ii) the glycoproteins: yeast invertase, containing phosphomannosyl residues [15]; ribonuclease B (containing high-mannose oligosaccharide chains); native and desialylated (by mild acid hydrolysis or neuraminidase digestion [17]) fibrinogen and transferrin, each containing biantennary complex-type carbohydrate chains; fetuin and asialofetuin, which contain N-linked biantennary and triantennary complex-type and O-linked oligosaccharide chains; bovine seminal plasma glycoprotein PDC-109 bearing a single O-linked trisaccharide [18]; carboxypeptidase Y (CPY) from *Saccharomyces cerevisiae*, which contains mannose-rich oligosaccharide chains with one or two mannose-6-phosphate (Man-6-P) residues diesterified with mannose or mannobiose [15,16]. Mild acid hydrolysis of glycoproteins was carried out in 50 mM H_2SO_4 for 90 min at 80°C; (iii) the lipids: cholesterol-3-sulfate; ceramide; phosphatidic acid; sphingomyelin; phosphatidylethanolamine; phosphatidylserine; phosphatidylcholine; and cardiolipin.

Microtiter-plate wells (Costar) were coated with the different test compounds as follows. (i) For polysaccharides, wells were pre-coated with 50 μl of poly-L-lysine (50 μg/ml in 50 mM sodium bicarbonate buffer, pH 9.6), for 16 h at 4°C. After washing with distilled water, the different polysaccharides were immobilised onto the wells of poly-L-lysine-coated plates by fixation with 50 μl of 0.05% glutaraldehyde in 5 mM phosphate buffer, pH 7.2, 0.2 M NaCl for 10 min at 20°C. (ii) For glycoproteins, 50 μl of the protein solutions in TBS were added to the wells and kept for 16 h at 4°C. (iii) For lipids, 50 μl of the lipid solutions in methanol were added to the wells followed by evaporation to dryness at 37°C.

After immobilisation of the different test compounds, wells were blocked with bovine serum albumin and binding of the ^{125}I-labelled proteins was assayed essentially as described [17], except that the buffer was TBS. Where indicated, microtiter-plate wells coated with acid-hydrolysed CPY (100 μg/ml) were treated prior to the incubation with the labelled proteins with 50 μl of a 30-U/ml alkaline phosphatase solution from bovine intestinal mucosa (Sigma, type VII-S) in 0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, 50 mM $MgCl_2$, for different periods of time at 37°C.

For inhibition assays, microtiter-plate wells were coated with acid-hydrolysed CPY (100 μg/ml) or with heparin (300 μg/ml), and the binding of ^{125}I-PSP-II was measured in the absence or presence of different concentrations of the inhibitors.

2.4. Molecular modelling

Three-dimensional X-ray crystallographic models of (a) mannose, (b) a tetrasaccharide fragment of heparin and (c) the PSP-I/PSP-II heterodimer (PDB accession codes, 1JPC, 1BFB and 1SPP, respectively) were used. The 3D-generated molecules of Man-6-P and the heparin tetrasaccharide were manually docked into possible sites on PSP-II using the software program FRODO [19]. The resulting working models were subjected to stereochemical and geometrical refinement by energy minimisation using XPLOR [20] in order to prevent non-allowed contacts and to ensure proper geometry. The final models were regularised with a slow cooling simulated annealing molecular dynamics protocol [20].

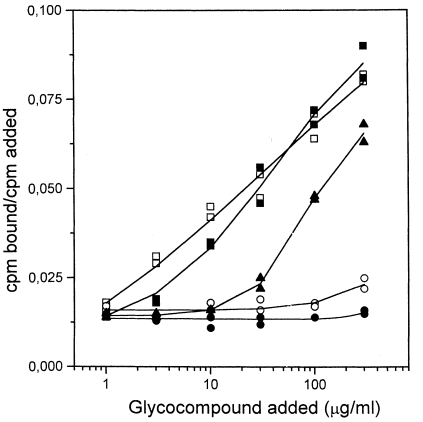

Fig. 1. Binding of ^{125}I-PSP-II to polysaccharides coated onto plastic microwells. Polysaccharides were immobilised at different concentrations onto microwells pre-coated with poly-L-lysine and binding of ^{125}I-PSP-II was assayed as described in Section 2. Fucoidan (■); heparin (□); zymosan A (▲); invertase (○); and chondroitin-6-sulfate (●).

3. Results

3.1. Isolated PSP-I and PSP-II associate into heterodimers

By gel filtration chromatography, isolated PSP-I and PSP-II glycoproteins each eluted as a single peak with apparent molecular mass of 14 kDa. When an equimolar mixture of the two proteins was chromatographed, a single peak at the elution volume of native PSP-I/PSP-II heterodimer was obtained. In addition, radioiodinated PSP-II bound in a concentration-dependent manner to PSP-I immobilised onto Sepharose beads. At subequimolar ratio, PSP-II was quantitatively retained. Maximal binding was 84 ± 2% after subtracting a background level of 16 ± 2% of non-specific binding of ^{125}I-PSP-II to unsubstituted Sepharose. The ability of isolated PSP-I and PSP-II to reassociate into heterodimers was taken as a hint that the bulk of separated glycoproteins most likely retained a conformation identical or close to their native state.

3.2. Binding studies with immobilised polysaccharides, glycoproteins and phospholipids

^{125}I-PSP-II bound to the immobilised sulfated polysaccha-

Table 1
Inhibition of binding of ^{125}I-PSP-II to hydrolyzed CPY

Compound	Maximum concentration	% Inhibition	IC_{50}
Man	100 mM	42 ± 7	> 100 mM
Man-6-P	30 mM	58 ± 2	22.4 mM
GlcNAc	100 mM	0	–
Heparin	8 mg/ml	60 ± 5	3.3 mg/ml
Fucoidan	8 mg/ml	59 ± 3	2.5 mg/ml
PSP-I	10 mg/ml	93 ± 2	0.93 mg/ml

Microtiter plate wells were coated with mild acid hydrolysed CPY (100 μg/ml) and the binding of ^{125}I-PSP-II was determined in the absence or presence of different concentrations of inhibitors. Inhibition of binding (mean of at least two data points) at the maximum concentration of inhibitor tested is shown. IC_{50}, concentration of inhibitor producing 50% inhibition.

rides heparin and fucoidan. The same binding activities have been described for other members of the spermadhesin protein family [4]. ^{125}I-PSP-II bound to a lesser extent to the polysaccharide zymosan A, no binding was observed to chondroitin-6-sulfate, and only trace binding to yeast invertase was deteced (Fig. 1). Under the same conditions no binding to any of the different polysaccharides tested was detected with ^{125}I-PSP-I or ^{125}I-PSP-I/PSP-II.

When different glycoproteins of defined oligosaccharide chains were tested (Fig. 2), there was no binding of ^{125}I-PSP-II to ribonuclease B, fibrinogen and transferrin, whether native or after desialylation by mild acid hydrolysis or by neuraminidase digestion. There was neither binding to native or desialylated fetuin, and to bovine PDC-109, and only trace binding to CPY was detected. However, mild acid hydrolysis of the phosphodiester bond of the CPY, yielding the phosphomonoester oligosaccharide [16], resulted in strong binding of ^{125}I-PSP-II (Fig. 2). The binding was progressively reduced by subsequent alkaline phosphatase digestion of the hydrolysed glycoprotein (Fig. 2, inset), demonstrating the requirement of an exposed phosphate group for binding of ^{125}I-PSP-II to CPY. However, no binding of ^{125}I-PSP-I or ^{125}I-PSP-I/PSP-II to native or mild acid hydrolysed CPY was observed (data not shown).

None of the seminal plasma proteins showed significant binding to cholesterol 3-sulfate, ceramide, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, or cardiolipin. As a positive control, ^{125}I-PDC-109 bound strongly to phosphatidylcholine and to a lesser extent to sphingomyelin as previously reported [21–23].

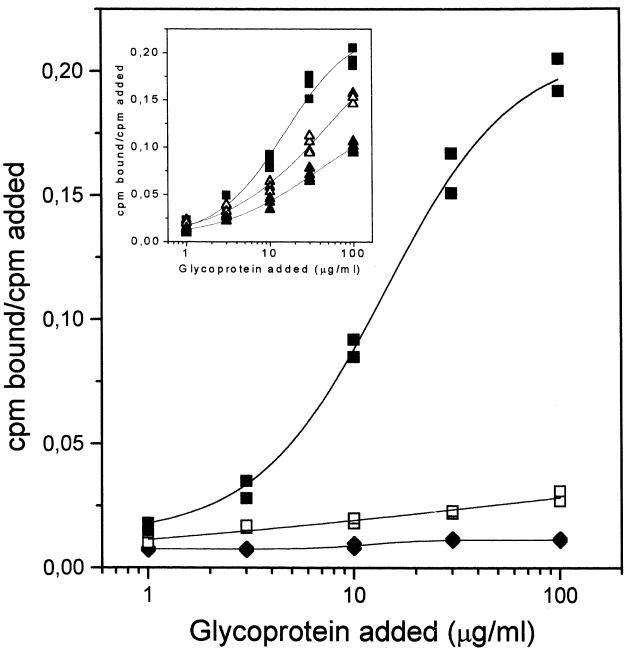

Fig. 2. Binding of ^{125}I-PSP-II to glycoproteins coated onto plastic microwells. Glycoproteins were coated at different concentrations onto microwells. Acid-hydrolysed CPY (■); native CPY (□); and other glycoproteins (♦) including acid-treated and native invertase, ribonuclease B, and the native and desialylated forms of fibrinogen, transferrin, fetuin and PDC-109. Inset: Effect of alkaline phosphatase digestion of hydrolysed CPY on the binding of ^{125}-I-PSP-II. Wells coated with hydrolysed CPY were treated with 30 U/ml alkaline phosphatase in Tris buffer, pH 9.5, for 2 h (△) or 24 h (▲), prior to the incubation with ^{125}I-PSP-II. Hydrolyzed CPY-coated wells incubated with the same buffer for 24 h (■) were used as control.

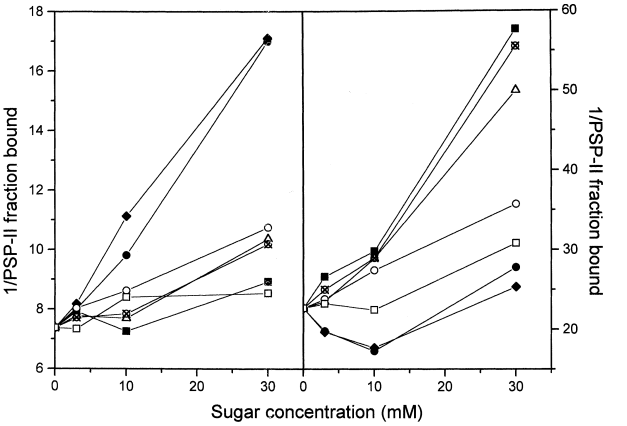

Fig. 3. Inhibition of the binding of ^{125}I-PSP-II to hydrolysed CPY (left panel) and to heparin (right panel). Hydrolysed CPY (100 μg/ml and heparin (300 μg/ml) were immobilised onto plastic microwells and the binding of ^{125}I-PSP-II was determined in the absence or presence of different concentrations of monosaccharides: Man-6-P (●); glucose-6-phosphate (♦); mannose (○); galactose-6-sulfate (△); mannose-1-phosphate (⊗); galactose-6-phosphate (■); and galactose (□).

3.3. Inhibition of PSP-II binding to immobilised acid-hydrolysed CPY and heparin

The specificity of ^{125}I-PSP-II binding to acid-treated CPY was explored by inhibition assays (Table 1). Among the three monosaccharide constituents of hydrolysed CPY oligosaccharide, Man-6-P was the most potent inhibitor of the binding of ^{125}I-PSP-II to the immobilised glycoligand, reaching 60% inhibition at 30 mM final concentration. Mannose was significantly less inhibitory, while *N*-acetylglucosamine was inactive. Heparin and fucoidan induced a dose-dependent and saturable inhibition of ^{125}I-PSP-II binding to acid-treated CPY, reaching a maximum inhibition level of 60% (Table 1). ^{125}I-PSP-I was the most potent inhibitor of the binding of ^{125}I-PSP-II to acid-treated CPY, reaching $>90\%$ inhibition at 0.9 mg/ml.

The specificity and relationship of ^{125}I-PSP-II binding to hydrolysed CPY and to heparin was further investigated using different monosaccharides as inhibitors of the binding to both glycoconjugates (Fig. 3). Man-6-P and glucose-6-phosphate were the most potent inhibitors of the binding of ^{125}I-PSP-II to hydrolysed CPY (Fig. 3, left panel). Mannose-1-phosphate, mannose, galactose-6-phosphate, galactose-6-sulfate and galactose were weaker inhibitors or almost inactive. On the contrary, Man-6-P and glucose-6-phosphate were not inhibitory of the binding of ^{125}I-PSP-II to heparin (Fig. 3, right panel). At 10 mM final concentration, these compounds even gave rise consistently to an apparent enhancement of the binding of ^{125}I-PSP-II to heparin. Mannose and galactose produced weak inhibition of the interaction of ^{125}I-PSP-II with heparin, while galactose-6-phosphate, galactose-6-sulfate and mannose-1-phosphate were the most potent inhibitors.

3.4. Docking of Man-6-P and heparin tetrasaccharide onto PSP-II

No three-dimensional structure of a lectin/Man-6-P complex is available. However, guided by the known basic fea-

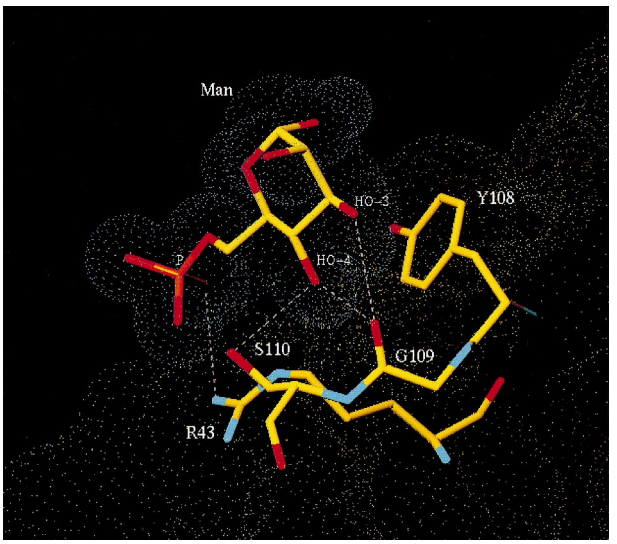

Fig. 4. Docking of mannose-6-phosphate onto the PSP-II structure. Connolly surface of the proposed complex between mannose-6-phosphate and PSP-II showing the hydrogen bonding network. For clarity, only PSP-II residues proposed to be directly involved in the binding are shown in ball-and-stick mode. Hydrogen bonds are represented by white dashed lines. The plot was made with TURBO-FRODO [35].

tures common to sugar-binding sites in a number of plant and animal lectins, i.e. van der Waals packing of a hydrophobic sugar face against an aromatic amino acid side chain and hydrogen bonding to the sugar hydroxyl groups [24], and the requirement of a suitable counter-ligand for the charged phosphate group, we have explored possible binding sites for Man-6-P on the PSP-II crystal structure [25,26]. Only one plausible site was found located at a shallow groove at the interface with PSP-I. Man-6-P could be docked into this site with the hydrophobic face of the sugar ring making stacking interaction with the aromatic ring of Tyr^{108} (Fig. 4). This model is characterized by hydrogen bonds between Man HO-4 and the Oγ of Ser^{100} (3.05 Å) and between Man HO-4 and Man HO-3 and the carbonyl oxygen atom of Gly^{109} (3.09 Å and 3.08 Å, respectively). In addition, the Nε group of Arg^{43} is involved in two salt bridges with O1 and O2 of the phosphate group of Man-6-P (3.17 Å and 3.42 Å, respectively) and Man-6-P HO-1 interacts with the NH1 group of Arg^{43} through another salt bridge (3.23 Å).

On the basis of polar interactions and maximum surface complementarity between the heparin tetrasaccharide GlcA(2-*O*-SO_3)-GlcNSO_3(6-*O*-SO_3)IduA(2-*O*-SO_3)-GlcNSO$_3$(6-*O*-SO_3) [27] and PSP-II, two possible heparin binding regions, both located in the proximity of the proposed Man-6-P recognition site, were predicted by molecular modelling. The first region extends from Arg^{43} towards Arg^{2} and the surface

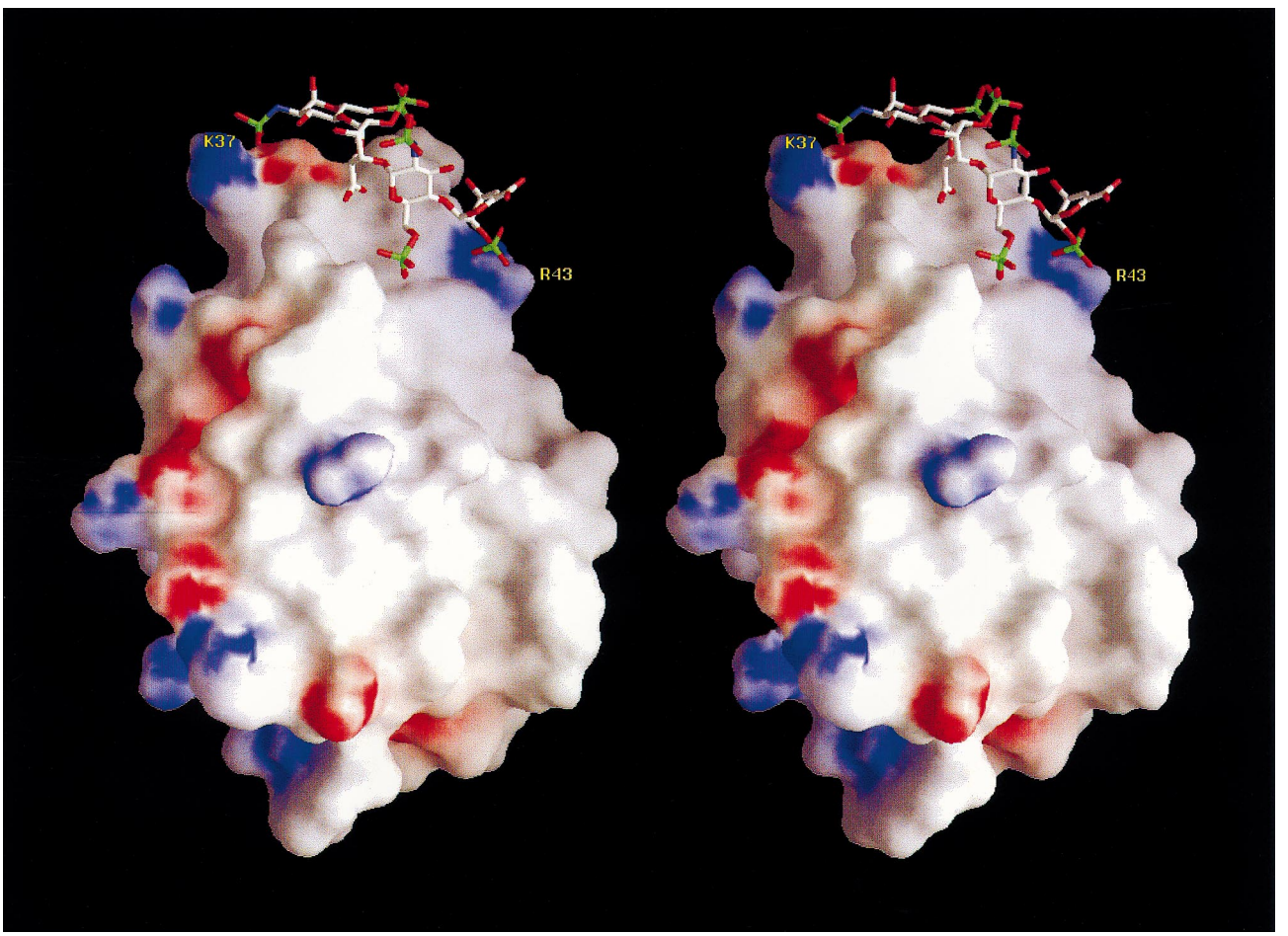

Fig. 5. Proposed docking site for a heparin tetrasaccharide on PSP-II. The molecular surface of PSP-II is colour-coded by electrostatic potential from negative (red) to positive (blue). The figure was rendered with GRASP [36].

area hidden upon docking of the tetrasaccharide onto PSP-II is 300 Å^2. The second, and most favoured region, extends from Arg^{43} towards Lys^{37}, on the opposite side of the PSP-II molecule. The binding interface shows a remarkable complementarity in the energy-minimised complex, rising up to a contact area of 450 Å^2 (Fig. 5). In the latter model, the heparin tetrasaccharide was accommodated into this region making several favourable contacts with the protein. The GlcA 2-*O*-sulfate interacts with the NH1 and NH2 groups of Arg^{43} (3.34 Å and 2.77 Å, respectively). The 6-*O*-sulfate of the internal glucosamine makes contact (2.98 Å) with the Oγ of Ser^{17}, and the *N*-sulfate of the reducing GlcN interacts with the NZ group of Lys^{37} (3.17 Å). Furthermore, the IduA carboxyl group could be involved in a water-mediated contact with Gln^{40}. Thus, the four sugar residues would be involved in the binding. The bound tetrasaccharide lies in line with the Man-6-P binding site, the two sites extending towards opposite sides of Arg^{43}.

4. Discussion

Binding studies using immobilised polysaccharides showed that iodinated PSP-II shares with other spermadhesin molecules binding specificity towards heparin and fucoidan. This binding activity appeared not to be merely related to the charge density of the polysaccharides. Thus, heparin and fucoidan, with charge densities per monomer of −1.9 and −0.3, respectively [28], were bound with similar avidity by PSP-II, while chondroitin-sulfate, with a charge density of −1.0, did not interact with PSP-II. Furthermore, binding of ^{125}I-PSP-II to heparin was inhibited by galactose-6-phosphate or mannose-1-phosphate and not by glucose-6-phosphate or Man-6-P. Thus, although apparently considerable variability in the saccharide structure (heparin vs. fucoidan) can be tolerated, the results suggest that there must be some structural and/or conformational requirements for sulfated glycan binding to PSP-II.

In addition to sulfated polysaccharides, the results revealed a novel binding capability for a spermadhesin molecule towards phosphomannose oligosaccharides. Hence, PSP-II did not interact with noticeable avidity with glycoproteins like (asialo)fetuin and PDC-109, which are good ligands of spermadhesins AWN, AQN-1, and AQN-3 [29,30], but bound to zymosan and to mild acid hydrolysed CPY. However, PSP-II did bind neither to CPY prior to the hydrolysis treatment nor to the same glycoprotein after alkaline phosphatase digestion. These results suggested that PSP-II bound to exposed Man-6-P monoester groups but not to the 'capped' phosphodiesters present in native CPY. Furthermore, the binding of PSP-II to hydrolysed CPY was selectively inhibited, although at millimolar concentrations, by Man-6-P and glucose-6-phosphate but not by other neutral and acidic monosaccharides, evidencing that the binding was specific and not only related to the acidic nature of the saccharide. On the other hand, no binding of PSP-II to either native or acid-treated invertase, a protein containing phosphomannosyl residues was detected. This strongly argues that beyond the recognition of the Man-6-P epitope, PSP-II may display a fine specificity in its binding of Man-6-P containing oligosaccharides.

PSP-II did not bind to any of the phospholipids tested, indicating that the binding activity displayed by PSP-II towards phosphorylated sugars was not related to the phospholipid binding activity exhibited by sperm-coating seminal plasma proteins like boar spermadhesins AWN-1 and AQN-3, which display affinity for phosphorylethanolamine [31] and PDC-109, a bovine phosphorylcholine-binding protein [21–23].

Recognition of Man-6-P by PSP-II was also clearly distinguishable from the binding of sulfated polysaccharides, as evidenced by the sharply different inhibitory activities exerted by acidic monosaccharides. The most potent inhibitors of the binding of PSP-II to acid-treated CPY are almost inactive on the binding to heparin, and vice versa. Heparin exerts a partial inhibition of PSP-II binding to hydrolysed CPY, however, suggesting that the corresponding binding sites may overlap or be in close spatial proximity. The location of these binding sites at the region of PSP-II involved in heterodimer formation with PSP-I was suggested by the fact that the PSP-I/PSP-II heterodimer displayed neither heparin nor phosphomannose binding activities. Furthermore, binding of PSP-II to mild acid hydrolysed CPY was completely inhibited in the presence of the PSP-I subunit. The ability of PSP-II and PSP-I to fully reassociate into a heterodimer strongly indicated that the inhibitory activity of PSP-I may be linked to reconstitution of the heterodimer, which may sterically block the Man-6-P and the heparin binding sites. Indeed, docking of Man-6-P onto the recently solved crystal structure of the PSP-I/PSP-II heterodimer [25,26] suggested that the most plausible binding sites for Man-6-P and heparin are located at the interface of PSP-II with PSP-I.

The proposed Man-6-P binding site involves a number of favourable contacts between the hydroxyl groups of the saccharide and protein main- and side-chain groups, which fully satisfy the experimental binding specificity. Binding of mannose but not of galactose to PSP-II might be explained by the involvement of the hydroxyl group at position 4 in a contact with Ser^{110}. On the other hand, the HO-2 group does not make any contact with the protein, in agreement with results showing similar affinity of PSP-II for Man-6-P and glucose-6-phosphate.

A binding site for heparin, also partly buried in the heterodimer interface, is proposed on the basis of the high complementarity with a tetrasaccharide fragment of heparin. The proposed heparin-binding site is contiguous to, and aligns with the Man-6-P recognition site, and, like the latter, implicates Arg^{43}. This residue also plays a role in the stabilisation of the heterodimeric association between PSP-II and PSP-I [25,26] by formation of a salt bridge with PSP-I Glu^{104}, and by means of water-mediated hydrogen bond interactions with Tyr^{17} and Thr^{19} of PSP-I. Thus, Arg^{43} emerges as a key residue in both ligand binding and heterodimer formation.

The participation of PSP-I/PSP-II in the fertilisation process remains enigmatic. The fact that both PSP-II heparin and Man-6-P binding activities are cryptic in the PSP-I/PSP-II heterodimer raises the question of their possible biological significance. The presence of cryptic binding sites which are exposed by different stimuli has been proposed for a number of heparin-binding proteins. For instance, denaturation/multimerisation of plasma vitronectin exposes heparin-binding sites which are also physiologically inducible by glycosaminoglycan binding or by incorporation into the terminal complement complex [32,33]. Furthermore, a cryptic Man-6-P binding activity has been described in a cell surface lectin from *Giardia lamblia* [34], a human protozoan parasite that causes diar-

rhoeal disease. This lectin is specifically activated by a host protease, trypsin, which is secreted in vivo at the site of infection, suggesting that the lectin may contribute to the host-parasite interaction. The possibility that cryptic binding sites in the PSP-I/PSP-II heterodimer could be exposed in response to a specific physiological environment cannot be ruled out and deserves further investigation.

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