Studies on the carbohydrate-binding characteristics of human pulmonary surfactant-associated protein A and comparison with two other collectins: mannan-binding protein and conglutinin

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The surfactant-associated protein A (SP-A) belongs to the collectin family, a group of C-type lectins encompassing also surfactant-associated protein D, mannan-binding protein (MBP) and conglutinin. These proteins all have carbohydrate-recognition domains joined to collagen stalks. It seems likely that SP-A, like MBP and conglutinin, may mediate anti-microbial activity through binding to carbohydrates on the micro-organisms and collectin receptors on phagocytic cells. We have studied the influence of carbohydrates on the binding of SP-A, MBP and conglutinin to mannan in an enzyme-linked lectin-binding assay. All sugars were of D-configuration, except fucose of which both L- and D-configurations were tested. The order of inhibiting potency on the binding of SP-A was: *N*-acetylmannosamine > L-fucose, maltose > glucose > mannose.

The following sugars were non-inhibitory: galactose, D-fucose, glucosamine, mannosamine, galactosamine, *N*-acetylglucosamine, and *N*-acetylgalactosamine. The best inhibitor of MBP was *N*-acetylglucosamine. Otherwise MBP showed a selectivity similar to that of SP-A. Conglutinin binding was inhibited by all the sugars examined except *N*-acetylglucosamine. For conglutinin, as for MBP, the best inhibitor was *N*-acetylglucosamine. Normal human SP-A, alveolar-proteinosis SP-A purified by ion-exchange chromatography, and alveolar-proteinosis SP-A purified by n-butanol extraction showed no difference in sugar selectivity. The influence of pH and of the calcium concentration was also examined. Organic solvent-extracted SP-A from patients suffering from alveolar proteinosis and normal SP-A showed different sensitivity profiles.

INTRODUCTION

Pulmonary surfactant-associated protein A (SP-A) is a glycoprotein synthesized by the type-II alveolar epithelial cells and secreted into the alveoli as the most abundant surfactant-associated protein [1]. The SP-A molecule is assembled from 18 polypeptide chains of slightly varying molecular masses of approx. 34 kDa. Each polypeptide chain consists of a short N-terminal sequence, followed by a collagen-like sequence, and a C-terminal region showing sequence similarity with the carbohydrate-recognition domain (CRD) of C-type lectins and containing an N-linked glycosylation site [1–4]. C-type lectins exhibit calcium-dependent binding to carbohydrates. Another surfactant-associated protein, SP-D, as well as the plasma proteins, mannan-binding protein (MBP) and conglutinin, also contain both a collagenous region and a C-terminal CRD. The name collectin has been suggested for these collagen-lectin hybrid molecules [5]. The overall structure of SP-A resembles a bouquet of tulips similar to the structure of MBP and the complement component C1q [6].

As predicted from the presence of a CRD sequence characteristic of C-type lectins, SP-A was shown to bind calcium-dependently to various sugars [7]. SP-A showed binding to all the agarose-linked carbohydrates tested: D-mannose, L-fucose, D-galactose, and D-glucose. The results do not indicate the relative carbohydrate-binding selectivity of SP-A.

Furthermore, it has not been investigated whether SP-A purified from normal human alveolar lavage fluid and SP-A purified from patients with pulmonary alveolar proteinosis show the same carbohydrate-binding characteristics.

SP-A is involved in the regulation of surfactant secretion and re-uptake by the type-II alveolar epithelial cells, as well as in the formation of tubular myelin in concerted action with surfactant phospholipids and the hydrophobic surfactant-associated proteins SP-B and SP-C [8,9].

SP-A possibly plays a role in the defence against lung infections. Malhotra et al. [10] have shown that SP-A, as well as MBP and conglutinin, binds to the C1q receptor (C1qR) on phagocytic cells and the interaction of SP-A with the U937 cells results in up-regulation of the number of C1qR molecules [5]. The binding of SP-A to carbohydrates on micro-organisms could conceivably lead to enhanced phagocytosis of micro-organisms via the C1qR present on macrophages. MBP has been shown to have such an opsonizing effect on *Salmonella montevideo* [11], but such activity has not yet been demonstrated for SP-A. Van Iwaarden et al. [12] found that pre-incubation of rat alveolar macrophages with SP-A increased the subsequent phagocytosis of serum-opsonized *Staphylococcus aureus*. This anti-bacterial effect appeared to be due to a stimulatory action of SP-A on the macrophages, as pre-incubation of the bacteria with SP-A did not promote phagocytosis. In another study the same group found evidence for an opsonizing effect of SP-A on herpes simplex virus [13].

Abbreviations used: C1qR, C1q receptor; CRD, carbohydrate recognition domain; HSA, human serum albumin; MBP, mannan-binding protein; SP-A, pulmonary surfactant-associated protein A; rSP-A, recombinant SP-A; e.l.l.b.a., enzyme-linked lectin-binding assay; TBS, (10 mM Tris/HCl, 145 mM NaCl, 15 mM Na₂SO₄, pH 7.4); TBS/T, TBS containing 0.05 % (v/v) Tween 20; TBS/T/HSA/Ca, TBS containing 0.05 % Tween 20, 100 µg of HSA per ml and 1 mM CaCl₂; AP-avidin, alkaline phosphatase-conjugated avidin.

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In the present study we have examined the carbohydrate specificity of SP-A by inhibition of the binding of SP-A to mannan from *Saccharomyces cerevisiae* (baker's yeast) with various carbohydrates. Moreover, the specificity was compared with those of MBP and conglutinin. We also studied the ionic requirements for the carbohydrate-binding activity of SP-A, MBP, and conglutinin, and we compared SP-A obtained from various sources and by different purification procedures.

MATERIALS AND METHODS

Materials

Unless otherwise stated all chemicals were obtained from Sigma (St. Louis, MO, U.S.A.).

Antibodies

Chicken antibodies raised against SP-A were produced by intramuscular immunization in the breast muscle with recombinant human SP-A (rSP-A), which was kindly provided by Dr. K. P. Schäfer (Byk Gulden Pharmazeutika, Konstanz, Germany). Both primary immunization and boost (after 24 days) were performed with 40 µg of rSP-A emulsified in complete Freund's adjuvant (Difco). The antibodies were purified from the yolks as described by Jensenius et al. [14]. The purified IgG was passed through mannan coupled to a cyanogen bromide-activated Sepharose 4B column (Pharmacia Biosystems, Uppsala, Sweden) to remove anti-carbohydrate antibodies.

Rabbit antibodies raised against rSP-A, MBP [15] and conglutinin [16] were produced by subcutaneous immunization and boost with the antigens emulsified in complete and incomplete Freund's adjuvant respectively. IgG was purified from the rabbit sera by sodium sulphate precipitation and ion-exchange chromatography. The antibodies were biotinylated with 0.166 mg of biotin-*N*-hydroxysuccinimide ester per mg of IgG.

The reactivity of the chicken antibody was examined on Western blots. SDS/PAGE of normal human SP-A or alveolar-proteinosis SP-A, either purified by ion-exchange chromatography or *n*-butanol extraction, was carried out on a 4–20% (w/v) polyacrylamide gradient gel and the proteins were transferred to nitrocellulose. The blots were incubated with 4 µg of biotinylated chicken anti-(SP-A) IgG per ml of TBS (10 mM Tris/HCl, 145 mM NaCl, 15 mM NaN₃, pH 7.4) containing 0.05% (v/v) Tween 20 (TBS/T), and bound chicken antibody was detected with alkaline phosphatase-conjugated avidin (AP-avidin) at a concentration of 0.25 µg per ml of the same buffer followed by incubation with substrate (0.12 mM bromochloroindolyl phosphate, 0.12 mM Nitroblue Tetrazolium, and 4 mM MgCl₂ in 0.1 M ethanolamine, pH 9.0). Some blots were stained for proteins with colloidal gold [17]. The incubations were carried out with 1 ml of reagent in disposable polypropylene trays (Pateof, Hellerup, Denmark).

Purification of SP-A

Normal human SP-A was purified by ion-exchange chromatography from bronchoalveolar lavage fluid obtained from healthy volunteers after informed consent. The lavage fluid was filtered through nylon mesh, centrifuged (250 g, 10 min) and the supernatant stored at –70 °C until required for use. The lavage fluid was thawed and diluted 5-fold in buffer A [10 mM Tris/HCl, 10 mM EDTA, 0.1% (v/v) Emulphogene (polyoxyethylene-10-tridecyl ether), 15 mM NaN₃, pH 7.5] and applied at room temperature (since SP-A tends to aggregate in the cold [18]) to a column (12 cm × 2.6 cm) of DEAE-Sepharcel (Pharmacia) equili-

brated with buffer A containing 20 mM NaCl. The column was washed with 500 ml of buffer A containing 100 mM NaCl, and the SP-A was eluted with buffer A containing 500 mM NaCl. The eluted protein was dialysed against buffer A containing 20 mM NaCl and 0.5 mM, instead of 10 mM, EDTA and stored at –70 °C in small aliquots.

Alveolar-proteinosis SP-A was purified by organic-solvent extraction from lavage fluid obtained from patients with pulmonary alveolar proteinosis as previously described [7]. Briefly, this included precipitating the surfactant proteins by mixing surfactant with 1-butanol at room temperature followed by centrifugation. The precipitate was dried and washed twice in 20 mM octyl-β-D-glucopyranoside/100 mM NaCl/10 mM Hepes (pH 7.4) and then suspended in 5 mM Hepes, pH 7.4, and further dialysed against this buffer. Insoluble material was removed by centrifugation and the supernatant containing SP-A stored at –70 °C. To investigate the possible influence of the organic solvent on the lectin activity, alveolar-proteinosis SP-A was also purified from pulmonary alveolar proteinosis lavage fluid by ion-exchange chromatography as described above.

The SP-A purified by ion-exchange chromatography is not pure. Further purification was deemed unnecessary for the present investigation, since the antibody detection system in the lectin-binding assay, as described below, selectively estimates SP-A binding. The molecular mass of the protein responsible for the signal in this assay was determined by gel chromatography on a Superose-6B f.p.l.c. column (Pharmacia) using a low-ionic-strength buffer (with 20 mM NaCl), since SP-A aggregates at a higher ionic strength [18]. The column was calibrated with molecular-mass standards (f.p.l.c. standards, Pharmacia).

The purification of SP-A was monitored by e.l.i.s.a. using a combination of rabbit and chicken anti-(SP-A) antibodies. Microtitre wells (Maxisorp, Nunc, Kamstrup, Denmark) were coated by incubation overnight at 4 °C with 1 µg of rabbit anti-(SP-A) immunoglobulin in 100 µl of coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, 3 mM NaN₃, pH 9.6) and blocked (1 h, room temperature) with 5 mg of gelatine (Difco) per ml of TBS. Samples were diluted in TBS containing 0.5% (v/v) Tween 20 and 10 mM EDTA and added to the wells in duplicate. After overnight incubation at room temperature bound SP-A was detected with biotinylated chicken anti-(SP-A) IgG (4 µg/ml) followed by AP-avidin (10 ng per 100 µl of TBS/T) and substrate as in the lectin-binding assay (see below).

Purification of conglutinin and MBP

Conglutinin was precipitated from 1 litre of bovine serum with 4% (w/v) poly(ethylene glycol) (PEG 6000). The precipitate was redissolved in TBS containing 5 mM CaCl₂ (column buffer) and passed through a column (100 ml) of Sepharacryl S-300 beads (Pharmacia) coupled with *N*-acetylglucosamine according to Fornstedt and Porath [19]. The column was washed with column buffer and conglutinin was eluted with 10 mM EDTA in the same buffer. The eluate was recalcified, its pH value adjusted to 7.4, and re-applied to the column. After washing the column with column buffer, conglutinin was eluted with 2 mM *N*-acetylglucosamine in the same buffer. The eluate was dialysed against TBS containing 10 mM EDTA, to remove bound *N*-acetylglucosamine, and then against TBS. MBP was purified from human serum as described by Lu et al. [15].

Enzyme-linked lectin-binding assay (e.l.l.b.a)

Microtitre wells (Polysorp, Nunc, Kamstrup, Denmark) were incubated overnight at 4 °C with 100 µl of coating buffer containing 70 ng of mannan, purified from *S. cerevisiae* [15]. The

rest of the procedure was performed at room temperature, unless otherwise stated. The wells were blocked with human serum albumin (HSA) obtained from the State Serum Institute (Copenhagen, Denmark) at 1 mg per ml of TBS (2 h) and incubated overnight with 14 ng of SP-A, 70 ng of concanavalin, or 12 ng of MBP in 100 μ l of calcium-containing buffer [TBS/T with 100 μ g of HSA per ml plus 1 mM CaCl_2 (TBS/T/HSA/Ca)]. The wells were washed with TBS/T/HSA/Ca, and bound SP-A, concanavalin and MBP were detected by incubation for 3 h with biotinylated anti-(SP-A), anti-(concanavalin) or anti-(MBP) antibodies (0.4 μ g, 0.14 μ g and 0.2 μ g respectively per 100 μ l of TBS/T/HSA/Ca) followed by exposure to AP-avidin (10 ng per 100 μ l of TBS/T/HSA/Ca). The bound enzyme was detected by incubation at 37 °C with 100 μ g of *p*-nitrophenyl phosphate in 100 μ l of 10% (w/v) diethanolamine buffer/0.5 mM MgCl_2 /3 mM NaN_3 (pH 9.8) and the absorbance at 405 nm was measured on an e.l.i.s.a. reader. The ligand-binding characteristics of SP-A, concanavalin and MBP were assessed by inhibition studies where various carbohydrates or mannan were included in serial dilutions in the lectin-containing buffer. Non-specific binding was estimated by replacing calcium with 10 mM EDTA in the lectin-containing buffer.

In another set of experiments, the lectins were diluted in buffers of different pH values or calcium concentrations, before addition to the mannan-coated microtitre wells. One buffer tested was TBS/T/HSA with CaCl_2 concentrations in the range of 0.001 μ M to 177 mM CaCl_2 or 0 mM CaCl_2 . The preparations were dialysed extensively against TBS to remove any EDTA before dilution in the calcium-containing buffers. The second buffer tested was TBS/T/Ca buffered with 50 mg of HSA per ml, with a final pH value in the range 3.9–10.2. The subsequent steps were carried out as described above.

RESULTS

Carbohydrate specificity of SP-A, concanavalin and MBP

The binding of SP-A, MBP and concanavalin to mannan was examined in an e.l.i.b.a. similar to an assay previously described for mannan-binding protein [20]. The binding of normal human SP-A to mannan-coated microtitre wells in the presence of 1 mM CaCl_2 and various carbohydrates is shown in Figure 1. The concentration of the inhibitor resulting in a 50% reduction of the signal (IC_{50}) was determined graphically from the inhibition curve (Table 1), and numeric means and S.D.s were calculated from three separate experiments. *N*-acetylmannosamine was the most efficient inhibitor, followed by L-fucose, maltose, glucose and mannose. The rest of the carbohydrates did not inhibit at the concentrations tested. Also, mannan was an efficient inhibitor (IC_{50} of approx. 0.5 μ g of mannan per ml, corresponding to a molarity of hexose units of about 3 μ M). Identical inhibition profiles were found for alveolar-proteinosis SP-A irrespective of the method of purification (results not shown).

Inhibition curves were similarly obtained with concanavalin and MBP (results not shown) and the IC_{50} values determined (Table 1). Binding of concanavalin to solid-phase mannan was very efficiently inhibited by *N*-acetylglucosamine, followed by mannosamine, L-fucose, mannose, and glucose. MBP was also most efficiently inhibited by *N*-acetylglucosamine, followed by mannose, L-fucose, and *N*-acetylmannosamine.

Specificity of the anti-(SP-A) antibody

The specificity of the chicken anti-(SP-A) antibody was examined by Western blotting as shown in Figure 2. Lanes 2, 5, and 8 show the detection of SP-A in the ion-exchange-purified normal

human SP-A, ion-exchange-purified alveolar-proteinosis SP-A, and organic-solvent-extracted alveolar-proteinosis SP-A respectively. The chicken anti-(SP-A) antibody shows a predominant specific reactivity towards a 34 kDa protein in the preparation of SP-A from normal human lavage, whereas reactivity with a 60 kDa component is also prominent in the two alveolar-proteinosis SP-A preparations. The chicken anti-(SP-A) antibody also reacts strongly with a band of about 20 kDa in the alveolar-proteinosis SP-A purified by organic solvent extraction. The presence of a non-reducible dimer has been described previously for SP-A purified from alveolar-proteinosis patients [21]. Our

Figure 1 Carbohydrate selectivity of normal human SP-A purified by ion-exchange chromatography

SP-A was mixed with the various carbohydrates in a buffer containing 1 mM calcium and then incubated in mannan-coated microtitre wells. All sugars were of D-configuration, except fucose of which both L- and D-configurations were tested. Bound SP-A was detected with biotin-chicken anti-(SP-A) IgG followed by AP-avidin and substrate. Key to symbols: ☆, GalNAc; ■, D-Fuc; ★, ManN; △, GlcNAc; ◆, Gal; ◇, GlcN; ○, GalN; ▼, Man; □, Glc; ●, L-Fuc; ▲, maltose; ▽, ManNAc.

Table 1 IC_{50} values for inhibition of collectin binding to mannan

IC_{50} values (mM) were determined graphically from inhibition curves as shown in Figure 1. The experiments were performed three times and numeric means and S.D.s (given in parentheses) calculated. Abbreviation: NI, not inhibitory (i.e. resulting in less than 50% inhibition at 50 mM).

Inhibitor	IC_{50} (mM)		
	SP-A	BK	MBP
Mannose	39 (3)	8 (1)	21 (1)
Glucose	30 (1)	10 (1)	50 (1)
Galactose	NI	23 (4)	NI
D-Fucose	NI	31 (7)	NI
L-Fucose	22 (1)	8 (0.3)	21 (0.4)
Maltose	23 (5)	15 (7)	42 (2)
Mannosamine	NI	7 (1)	NI
Glucosamine	NI	15 (3)	NI
Galactosamine	NI	20 (2)	NI
<i>N</i> -Acetylmannosamine	8 (1)	15 (5)	26 (2)
<i>N</i> -Acetylglucosamine	NI	0.6 (0.1)	16 (1)
<i>N</i> -Acetylgalactosamine	NI	NI	NI

Figure 2 Specificity of the chicken anti-(SP-A) antibody

Different preparations of SP-A were subjected to SDS/PAGE at reducing conditions and blotted on nitrocellulose. Lanes 1–3, normal human SP-A purified by ion-exchange chromatography; lanes 4–6, alveolar-proteinosis SP-A purified by ion-exchange chromatography; lanes 7–9, alveolar-proteinosis SP-A purified by organic solvent extraction. Lanes 2, 5 and 8 were developed with biotinylated chicken anti-(SP-A) antibody and lanes 3, 6 and 9 with biotinylated normal chicken Ig. Subsequent steps were incubations with AP-avidin followed by enzyme substrate. Lanes 1, 4 and 7 were stained for protein with colloidal gold.

results show, in accordance with a recent report by Voss et al. [22], that this dimer is more prominent in preparations of SP-A from alveolar-proteinosis patients than in normal human SP-A.

Analysis of crude SP-A (the DEAE-Sephacel eluate), fractionated by size-permeation chromatography, showed that more than 95% of the signal in the e.l.l.b.a. was due to proteins larger than the thyroglobulin molecular-mass standard (669 kDa), consistent with a native molecular mass of SP-A of about 700 kDa [23] (results not shown). These results indicate that at the conditions employed in the e.l.l.b.a. the chicken antibody reacts selectively with SP-A. This interpretation was corroborated by the inhibition results.

Ionic requirements for lectin activity

The binding to mannan of SP-A, MBP and conglutinin at different pH values is shown in Figure 3. Incubation with normal SP-A purified by ion-exchange chromatography results in the highest signal in the e.l.l.b.a. at pH values between 6 and 8, with a peak at pH 6.7. This same pH-dependency was found for

Figure 3 Binding of normal SP-A, alveolar-proteinosis SP-A, conglutinin and MBP to solid-phase mannan at different pH values

The collectins were incubated at different pH values in mannan-coated wells and bound protein detected as described in the text. ○, Normal human SP-A purified by ion-exchange chromatography; ●, alveolar-proteinosis SP-A purified by organic solvent extraction; △, MBP; and ▲, conglutinin.

Figure 4 Binding of SP-A and alveolar-proteinosis SP-A to solid-phase mannan at different calcium concentrations

○, Normal SP-A purified by ion-exchange chromatography; ●, alveolar-proteinosis SP-A purified by organic solvent extraction; △, MBP; and ▲, conglutinin.

alveolar-proteinosis SP-A purified by ion-exchange chromatography (results not shown), as well as for conglutinin. MBP shows high binding in a broad range, from a pH value of 5 to values above 10. Alveolar-proteinosis SP-A purified by organic solvent extraction shows an increasing signal with incubation at increasing pH values up to about pH 10.

The binding of SP-A, MBP and conglutinin to mannan at different calcium concentrations is shown in Figure 4. All three lectins lose the binding activity at calcium concentrations around 0.01 mM. A binding optimum is seen for SP-A purified by organic solvent extraction and MBP between 0.1 mM and 1 mM CaCl_2 where a plateau is exhibited by conglutinin. DEAE-purified normal human SP-A behaves similarly at calcium concentrations below 10 mM, but an increasing signal is noticed

when the calcium concentration is increased gradually from 10 mM upwards to 177 mM. This is also the case with alveolar-proteinosis SP-A purified by ion-exchange chromatography (results not shown), but not with alveolar-proteinosis SP-A purified by organic solvent extraction.

DISCUSSION

We have compared the carbohydrate selectivity of SP-A with those of conglutinin and MBP by analysing the inhibition with various carbohydrates of the binding of the lectins to mannan in an e.l.i.b.a. SP-A, conglutinin or MBP bound to solid-phase mannan was detected with biotinylated antibodies.

Haagsman et al. [7] found that SP-A binds to agarose-linked mannose, glucose, galactose and L-fucose, but only weakly to *N*-acetylglucosamine and *N*-acetylgalactosamine. However, carbohydrate-affinity chromatography only provides rough information on the selectivity. This is probably the reason for the discrepancy between their findings and the results presented in this paper. Thus, we found mannose and glucose to be efficient inhibitors, while galactose did not inhibit even at the highest concentration tested. Our results support the inclusion of SP-A in the group-III C-type mammalian lectins, as defined by Bezouska et al. [24]. On the basis of structural evidence and chromosomal organization they divided mammalian lectins into four groups with group III encompassing lectins containing CRDs connected to collagen domains. Common to the lectins examined from this group (now also including SP-A) is preferential binding to mannose as compared with galactose [25].

Drickamer [26] recently demonstrated that the preferential binding of MBP to mannose residues as compared with galactose can be reversed by replacing the amino acids Glu¹⁸⁵ and Asn¹⁸⁷ in the sequence of MBP with Gln and Asp respectively, whereafter these amino acids of the mutant MBP conform to that of the galactosyl-binding C-type lectins [26]. The corresponding sequence of human SP-A [2,27] is intriguing, since the amino acid at position 185 is glutamic acid, whereas that at position 187 is alanine. Thus, our results show that asparagine at position 187 is not absolutely necessary for preferential binding of mannose as compared with galactose.

Childs et al. [28] investigated the binding of SP-A to neoglycoproteins. They found equal binding to galactose, mannose, L-fucose, and glucose coupled to BSA, but no binding to *N*-acetylglucosamine-BSA. The discrepancy between these findings (especially with regard to galactose) and our results may reflect a difference in the properties of the binding assays employed. Thus, the high-mannose structures of mannan may act as multivalent ligands and possibly produce stronger binding of each SP-A molecule than the neoglycoproteins employed by Childs et al. This may explain a difference in the inhibition profiles. Furthermore, we suggest that the discrepancy may in part be due to their use of ¹²⁵I-labelled SP-A. We radioiodinated different SP-A preparations with a variety of labelling procedures (including solid-phase glucose oxidase/lactoperoxidase as used by Childs et al. [28]) and found all the procedures unsuitable for the labelling of SP-A, as revealed by the loss of carbohydrate-specific binding activity. In contrast, MBP and conglutinin could be successfully radioiodinated by several methods (S. Thiel, unpublished work).

In addition to galactose, mannosamine, glucosamine and *N*-acetylglucosamine also did not inhibit binding of SP-A to mannan. *N*-Acetylmannosamine, on the other hand, was more potent than mannose. The binding of SP-A to *N*-acetylmannosamine was verified by carbohydrate-affinity chromatography of the DEAE-eluate on *N*-acetylmannosamine coupled

to TSK beads activated with di(vinyl)sulphone [19] (result not shown). Hence, the binding affinity of the SP-A CRD is specifically influenced by substitutions in the sugar structure and the results indicate that a specific substitution in one monosaccharide (i.e. mannose) increases affinity but in another (i.e. glucose) decreases affinity of SP-A towards the monosaccharide. The finding that D-fucose does not inhibit binding of SP-A to mannan, whereas L-fucose does, shows that the SP-A CRD discriminates between stereoisomerically different monosaccharides. SP-A binding to solid-phase mannan was very efficiently inhibited by mannan with an IC₅₀ value corresponding to a hexose-unit molarity of 3 μM. This presumably reflects a higher affinity of SP-A for structures larger than monomeric hexose.

The present study shows a similar sugar-binding activity for normal human SP-A and SP-A purified from pulmonary alveolar proteinosis patients (alveolar-proteinosis SP-A). Purification of alveolar-proteinosis SP-A using organic solvent did not influence the binding selectivity.

When comparing the carbohydrate specificity of SP-A with MBP and conglutinin, it is seen that all three proteins could be inhibited with mannose, as well as glucose, L-fucose, maltose and *N*-acetylmannosamine. Galactose on the other hand weakly inhibited the binding of conglutinin, while it did not inhibit the binding of SP-A or MBP. Thus, the present study corroborates the assignment of MBP and conglutinin on the basis of the carbohydrate specificity to the type-III mammalian lectins [24].

The IC₅₀ values are influenced by the actual assay conditions and different values are obtained at different lectin concentrations. The sequence of inhibitory potency was not influenced by the lectin concentration, and IC₅₀ values are thus useful for comparison of lectin selectivity.

Haagsman et al. [18] showed that increasing the calcium concentration and decreasing the pH value resulted in self-aggregation of alveolar-proteinosis SP-A purified by organic solvent extraction. Increasing the calcium concentration beyond 10 mM resulted in a slight decrease in the binding of this alveolar-proteinosis SP-A, whereas the binding of normal human SP-A, as well as of alveolar-proteinosis SP-A purified by ion-exchange chromatography, increased at high calcium concentrations. This difference in response at high calcium concentrations of the SP-As purified by the different methods might be caused by the SP-A purified by organic solvent extraction having a less stable configuration, resulting in a greater tendency to aggregation. Purification-associated structural changes were also indicated by the observation that the SP-A purified by organic solvent extraction showed a pH sensitivity markedly different from that of SP-A purified by ion-exchange chromatography.

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