FEBS 19170 FEBS Letters 414 (1997) 387–392

A novel type of binding specificity to phospholipids for rat mannose-binding proteins isolated from serum and liver

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Abstract Mannose-binding protein (MBP) belongs to the collectin subgroup of C-type lectins with specificity for mannose and *N*-acetylglucosamine sugars. We investigated whether rat MBPs isolated from serum (S-MBP) and liver (L-MBP) interact with phospholipids using antibody against each MBP. Both S- and L-MBPs bound to phosphatidylinositol coated onto microtiter wells in a concentration- and a Ca^{2+}-dependent manner. L-MBP also bound to phosphatidylglycerol and weakly to phosphatidylserine. MBPs interacted with liposomes composed of these lipids. S- and L-MBPs bound to phosphatidylinositol 4-monophosphate. L-MBP also bound to cardiolipin. These results provide evidence for a novel type of ligand binding specificity for MBPs, and raise the possibility that phospholipids are ligands for collectins.

Key words: Mannose-binding protein; Collectin; C-type lectin; Phospholipid

1. Introduction

Mannose-binding protein (MBP) is a C-type lectin with specificity for mannose and *N*-acetylglucosamine sugars [1]. MBP belongs to the collectin subgroup of C-type lectins, which also includes pulmonary surfactant proteins SP-A and SP-D [2]. The collectins share a common structural domain arrangement: a cysteine-containing amino terminus; a collagen-like domain; a neck domain; and a carbohydrate recognition domain (CRD). MBP is believed to play important roles in the innate immune system, which is critical in the first line of host defense. Two rat MBPs, MBP-A and MBP-C, have been characterized and are homologous to each other with 56% sequence identity [3]. MBP-A appears to be a predominant form of serum MBP in rat. MBP-C is a hepatic lectin. Serum MBPs isolated from rat, human and rabbit activate the classical complement pathway [4], referred to as a lectin pathway, which may be associated with bactericidal activity and opsonization. C-type lectins have been shown to bind to certain glycosphingolipids; human MBP binds to *N*-acetylglucosamine-terminated glycosphingolipids [5]; rat MBP-A also binds neoglycolipids containing terminal *N*-acetylglucosamine residues [6], whereas rat MBP-C binds neoglycolipids containing the trimannosyl core of complex *N*-linked oligosaccharides [7]. SP-A binds to galactosylceramide, lactosylceramide and asialo-G_{M2} [8,9]; SP-D binds to glucosylceramide [10]. Lung surfactant lectins are also unique phospholipid-binding proteins; SP-A binds to phosphatidylcholine (PC) and sphingomyelin with the highest affinity for dipalmitoyl species of PC [11], which is an essential lipid component of surfactant to reduce surface tension in the air-liquid interface; SP-D binds to phosphatidylinositol [12]. The CRDs of C-type lectins are characterized by 14 invariant and 18 highly conserved amino acid residues [13]. The strong sequence conservation in the CRD, and the similarities in carbohydrate binding specificity and oligomeric structure among collectins [14] may suggest that these lectins are functionally homologous. In this study we investigated whether MBPs isolated from serum and liver of rats bind to certain phospholipids. We here report evidence for a novel type of binding specificity for MBPs to phospholipids.

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Abbreviations: MBP, mannose-binding protein; S-MBP, serum MBP; L-MBP, liver MBP; SP-A, surfactant protein A; SP-D, surfactant protein D; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-monophosphate; PIP_2, phosphatidylinositol 4,5-diphosphate; PG, phosphatidylglycerol; PS, phosphatidylserine

2. Materials and methods

2.1. Isolation of mannose-binding proteins from rats

Serum mannose-binding protein (S-MBP) was isolated from sera of Sprague-Dawley rats by the method of Kozutsumi et al. [15]. Briefly, the pooled rat sera were mixed with an equal volume of 40 mM Tris buffer (pH 7.4) containing 2.5 M NaCl and 40 mM $CaCl_2$. After incubation for 2 h at 4°C, the sera was centrifuged at $10000 \times g_{av}$ for 10 min. The supernatant was then applied to an affinity column of mannose-Sepharose 6B (5 ml bed volume/100 ml serum) that had been equilibrated with 20 mM Tris buffer (pH 7.4) containing 1.25 M NaCl and 20 mM $CaCl_2$ (binding buffer). After the column had been washed with the binding buffer, the proteins binding to the affinity matrix were eluted with 20 mM Tris buffer (pH 7.4) containing 1.25 M NaCl and 2 mM EDTA. The Ca^{2+} concentration of the eluate was adjusted to 20 mM by the addition of 1 M $CaCl_2$, and the eluate was applied to the second small column (2 ml bed volume/100 ml serum) of mannose-Sepharose 6B. The column was washed with 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl and 2 mM $CaCl_2$. The MBP binding to the affinity matrix was finally eluted with 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl and 2 mM EDTA. The purified S-MBP was then dialyzed against 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl.

Liver mannose-binding protein (L-MBP) was isolated from rat liver by the method of Mizuno et al. [16]. Briefly, rat liver was minced and blended with cold acetone. After drying the cake, acetone powder was suspended in 10 volumes of 20 mM imidazole (pH 7.8) containing 0.4 M KCl, 0.5 mM EDTA and 2% Triton X-100 (extracting buffer) and centrifuged at $12000 \times g_{av}$ for 15 min. The supernatant was combined with the affinity gel of mannose-Sepharose 6B (100 ml gel/100 g liver) and the suspension was adjusted to 5 mM $CaCl_2$ by adding 1 M $CaCl_2$. After stirring for 60 min at 4°C, the gel was sedimented by centrifugation at $200 \times g$ for 10 min and washed several times with 20 mM imidazole (pH 7.8) containing 1.25 M NaCl, 5 mM $CaCl_2$ and 0.5% Triton X-100 (washing buffer), and poured into a column. The proteins binding to the affinity gel were eluted with 20 mM imidazole (pH 7.8) containing 1.25 M NaCl, 2 mM EDTA and 0.5% Triton X-

PII S0014-5793(97)01022-3

100 (eluting buffer). After the eluate was adjusted to a concentration of 20 mM Ca^{2+} by the addition of 1 M $CaCl_2$, the affinity chromatography was repeated with the washing and eluting buffer containing a lower concentration of Triton X-100 (0.1%) using a smaller column (10 ml gel). The affinity chromatography was repeated once more using a column of 5 ml gel. The purified L-MBP was passed through a column of Extracti-Gel (Pierce) to remove detergent. The protein was finally dialyzed against 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl.

Lung surfactant lectins SP-A and SP-D were purified from rat lung lavage by the method described previously [11,12].

2.2. Preparation of polyclonal antibodies and enzyme-linked immunosorbent assay

Purified S-MBP and L-MBP (100 μg protein each) were emulsified with Freund's complete adjuvant and injected into New Zealand White rabbits intramuscularly. For boost immunization, MBP with Freund's incomplete adjuvant and MBP alone were injected on day 14 and day 28, respectively. Eight days after the last immunization, the rabbits were bled and antisera were obtained. The IgG fraction of antiserum against each protein was purified by an affinity column of protein A-Sepharose CL 4B (Pharmacia Fine Chemicals).

A sandwich enzyme-linked immunosorbent assay (ELISA) for each MBP was developed. IgG was conjugated with horseradish peroxidase (HRP) using periodate oxidation by the method of Ishikawa et al. [17]. The microtiter wells were coated with anti-MBP IgG (20 μg/ml in 0.1 M $NaHCO_3$, 100 μl/well) at 4°C overnight and the wells were incubated with phosphate buffered saline (PBS) containing 3% (w/v) skim milk and 0.1% (v/v) Triton X-100 (buffer A) to block the nonspecific binding. After blocking, 50 μl of samples and purified MBP as standards were incubated at 37°C for 60 min, and then incubated with anti-MBP IgG-HRP conjugate. *o*-Phenylenediamine was used as the substrate for the peroxidase reaction. After stopping the reaction by the addition of 2 M sulfuric acid, absorbance at 490 nm was measured in a immunoreader. The ELISA was able to detect MBP at levels ranging from 3 to 200 ng/ml.

2.3. Phospholipids

Phosphatidylinositol (PI) from bovine liver, phosphatidylinositol 4-monophosphate (PIP) from bovine brain, phosphatidylinositol 4,5-diphosphate (PIP_2) from bovine brain, phosphatidylglycerol from egg yolk, sphingomyelin (SM) from egg yolk, phosphatidylserine (PS) from bovine brain, phosphatidylethanolamine (PE) from bovine liver, cardiolipin from bovine heart, phosphatidylcholine (PC) from egg yolk and dipalmitoylphosphatidylcholine (DPPC) were purchased from Sigma.

2.4. Binding of MBPs to phospholipids coated on microtiter wells

Phospholipids (1 μg in 20 μl ethanol/well) were put into microtiter wells (Dynatech Laboratories, Inc.) and air-dried. The wells were incubated with 300 μl of 50 mM Tris buffer (pH 7.4) containing 0.1 M NaCl, 2 mM $CaCl_2$ and 20 mg/ml bovine serum albumin (buffer B) for 30 min to block nonspecific binding. 50 μl of L-MBP or S-MBP (1–10 μg/ml) in buffer B was added to the wells and incubated for 60 min at room temperature. The wells were then washed with 300 μl of the buffer B three times. The MBP binding to the lipids was detected using antibody (20 μg/ml in buffer A) against each protein, followed by incubation with HRP-labeled anti-rabbit IgG (Bio-Rad). After washing the wells with PBS containing 0.1% (v/v) Triton X-100, the substrate reaction was performed using *o*-phenylenediamine and absorbance at 490 nm was measured. In some experiments, various concentrations of Ca^{2+} or Mg^{2+}, monosaccharide (10 or 100 mM), phospholipid liposomes (500 μM), or anti-MBP antibody (10 or 100 μg/ml) were included when MBPs were incubated with solid phase phospholipids.

2.5. Binding of MBPs to phospholipid liposomes

The binding of MBPs to multilamellar liposomes composed of PI, PG, PS or PC was performed by the sedimentation method with a minor modification, as described previously [18]. To prepare multilamellar liposomes, the lipid was dried under nitrogen and hydrated in 20 mM Tris buffer (pH 7.4) containing 0.1 M NaCl at 37°C for 1 h and then vortexed vigorously for 5 min. The multilamellar liposomes (100 μg) and the protein solution (0.2 μg protein/tube) in 20 mM Tris buffer (pH 7.4) containing 0.15 M NaCl, 5 mM $CaCl_2$ and 20 mg/ml bovine serum albumin (buffer C) were separately centrifuged at $10\,000 \times g_{av}$ for 10 min. Each liposome pellet was then suspended in 50 μl from the supernatant of the protein solution. The lipid-protein mixture was incubated for 1 h at room temperature, and then put on ice and incubated for 15 min. The mixture was centrifuged at $10\,000 \times g_{av}$ for 10 min at 4°C. The supernatant was stored (unbound fraction) and the precipitate was washed once with 50 μl of ice-cold buffer C. After centrifugation, the supernatant was combined and the pellet was suspended with 100 μl of the buffer C. The amount of protein in each fraction was determined by sandwich ELISA for L-MBP or S-MBP using polyclonal antibodies against each protein as described above. Liposome binding was defined as $(MBP_{pellet}/MBP_{pellet+supernatant}) \times 100$. Control experiments where liposomes were deleted from the incubation mixture were also performed.

2.6. Other methods

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli [19]. Immunoblotting analysis of the proteins was carried out using PVDF membranes by a method based on that described by Towbin et al. [20]. Amino acid sequences of MBPs transferred onto PVDF membranes were determined by an Applied Biosystem Model 477A protein sequencer equipped with an on-line phenylthiohydantoin (PTH) analyzer (model 120A). Dot blot analysis was performed using MBPs and surfactant proteins spotted onto the nitrocellulose membranes (2 μl of 10 μg/ml protein) to examine the specificity of anti-MBP antibody. Protein concentrations were estimated by the method of Lowry et al. using bovine serum albumin as the standard [21].

3. Results

3.1. Protein analysis and antibody specificity

MBPs isolated from serum and liver of rats were analyzed by electrophoresis. Serum MBP (S-MBP) and liver MBP (L-MBP) showed protein bands at approximately 30 kDa under reducing condition (Fig. 1A). S-MBP migrated very slightly faster than L-MBP. Both S- and L-MBPs form oligomers under non-reducing conditions. Under these conditions, a significant amount of S-MBP oligomer did not even enter the dissolving gel, which is consistent with a previous report that S-MBP forms a macromolecule with larger molecular mass than L-MBP [4]. The amino-terminal sequence of S-MBP was SGSQTXEE (X: not determined), which was identical to Ser^1–Glu^8 of rat MBP-A described by Drickamer et al. [3] and Ikeda et al. [4]. The amino-terminal sequence of L-

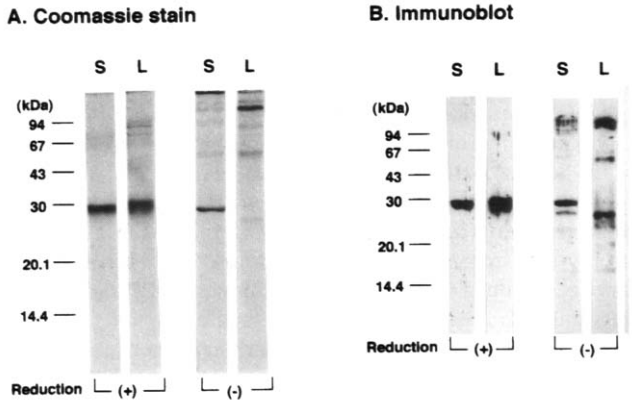

Fig. 1. Analysis of MBPs by electrophoresis and immunoblotting. Serum MBP (S) and liver MBP (L) were separated on 13% polyacrylamide gel electrophoresis under reducing (+) and under non-reducing (−) conditions, and stained with Coomassie brilliant blue (A). Each protein was also transferred onto PVDF membrane and immunostained with antibody against each protein using horseradish peroxidase-labeled anti-rabbit IgG as the second antibody and diaminobenzidine as the substrate (B).

MBP was AETLTEG, which was also identical to Ala^1–Gly^7 of rat MBP-C described by Drickamer et al. [3].

Antiserum was prepared against each MBP. Each antibody recognized denatured protein by immunoblotting analysis (Fig. 1B). Anti-MBP antibodies were also tested to see whether they bound to native forms of MBP and other C-type lectins by dot blot analysis. Each antibody recognized each MBP and crossreacted with each other, but failed to bind surfactant proteins homologous to MBPs (data not shown). The results indicate that the anti-MBP antibodies obtained are specific for the MBP molecules.

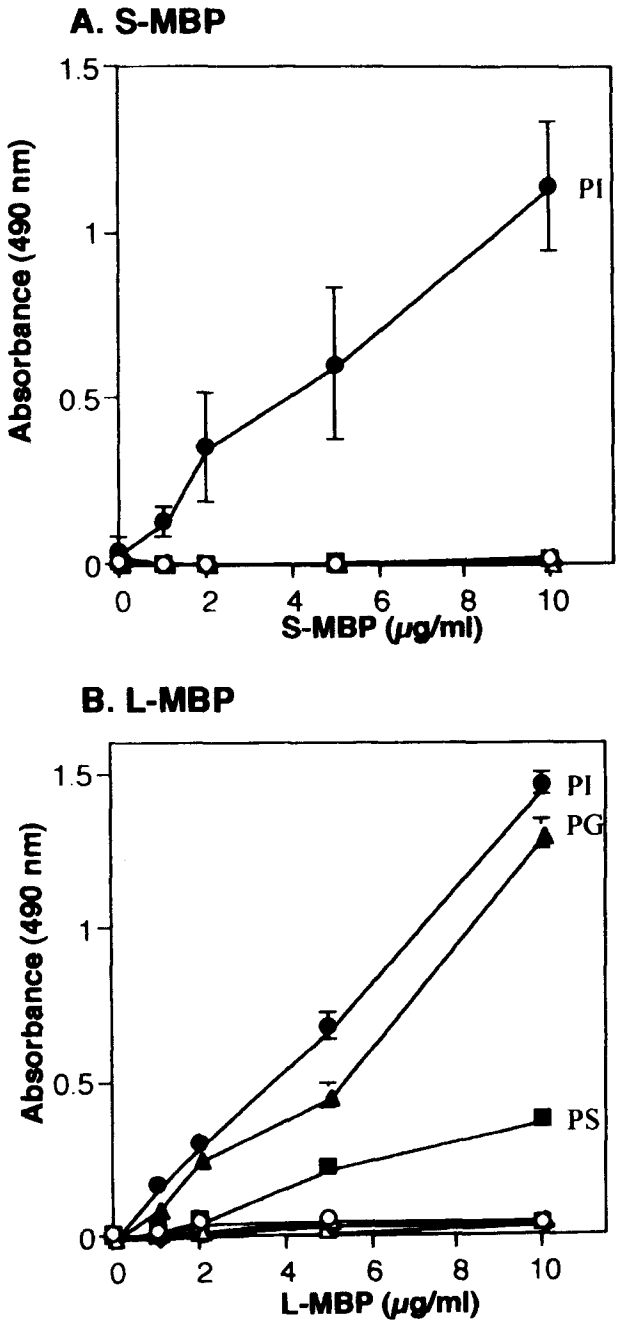

Fig. 2. Binding of MBPs to various phospholipids coated onto microtiter wells. Phosphatidylinositol (PI, ●), phosphatidylglycerol (PG, ▲), phosphatidylserine (PS, ■) phosphatidylethanolamine (□), egg phosphatidylcholine (△), dipalmitoylphosphatidylcholine (○), and sphingomyelin (◇) were coated onto microtiter wells (1 μg each lipid/well). The wells were incubated with 0–10 μg/ml of serum MBP (S-MBP) (A) or liver MBP (L-MBP) (B) at room temperature for 60 min. The MBP binding to the lipids was detected using antibody (20 μg/ml) against each MBP as described in Section 2. The data presented are mean ± S.D. ($n = 3$).

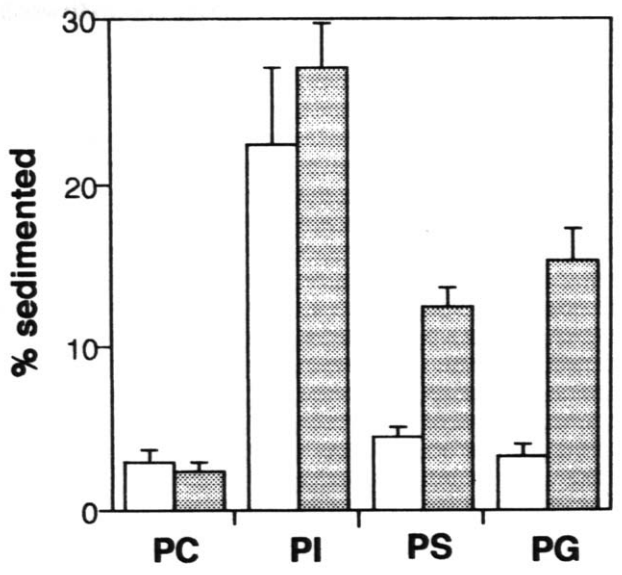

Fig. 3. Binding of MBPs to phospholipid liposomes. Multilamellar liposomes (100 μg/tube) containing phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylglycerol (PG) were mixed with 0.2 μg of serum MBP (S-MBP, white bars) or liver MBP (L-MBP, hatched bars), and incubated at room temperature for 60 min. The MBPs which bound to liposomes were sedimented at $10000 \times g_{av}$ and the amounts of proteins in the supernatant and the pellet were determined by ELISA as described in Section 2. The results show specific sedimentation that was determined by subtracting values obtained when liposomes were omitted (non-specific binding) from total sedimentation. The data shown are mean ± S.D. ($n = 3$).

3.2. Phospholipid binding specificity

The binding of rat MBPs isolated from serum and liver to various phospholipids was examined. S-MBP bound to PI coated onto microtiter wells in a concentration-dependent manner (Fig. 2A). S-MBP did not bind to phospholipids other than PI. L-MBP also bound to PI in a concentration-dependent manner (Fig. 2B). Unlike S-MBP, L-MBP bound to solid phase PG at nearly the level of PI binding. It also bound to PS but its binding was approximately one-fourth of PI binding at 10 μg/ml of L-MBP. Co-incubation of anti-MBP antibody with MBPs attenuated the binding of these collectins to PI to the level of 5–12% of the control binding at 100 μg/ml of antibody concentration. The inclusion of 10 mM EDTA almost completely diminished the binding of MBPs to lipids. When the binding of MBPs to lipids was examined in the presence of various concentrations of Ca^{2+}, the proteins exhibited maximal binding at 2–4 mM Ca^{2+}. Mg^{2+} failed to replace Ca^{2+} for the binding of MBPs to phospholipids.

3.3. Binding of MBPs to phospholipid liposomes

We next examined if MBP bound to phospholipid liposomes, by liposome sedimentation. Twenty-two and 27% of S- and L-MBPs, respectively, were co-sedimented with PI liposomes (Fig. 3). Fifteen or 12% of L-MBP was also co-sedimented with PG and PS liposomes, respectively. In contrast,

the percentages of S-MBPs that co-sedimented with PG and PS liposomes were at almost the same level as PC liposomes.

We also investigated whether excess liposomes competed with solid phase phospholipid for MBP binding. 500 μM of PI liposome competed well with solid phase PI for S-MBP binding (Fig. 4A). PI liposome reduced the binding of S-MBP to solid phase PI to the level of 22% of the control binding. Liposomes composed of PS, PG or PC failed to block the S-MBP binding to solid phase PI. PI liposomes attenuated the binding of L-MBP to all three phospholipids coated onto microtiter wells by 80–86% (Fig. 4B). The binding of L-MBP to solid phase PS and PG was blocked when the competitor liposome and the solid phase lipid were identical. Neither PS liposome nor PG liposome blocked the binding of L-MBP to solid phase PG and PS, respectively. These results demonstrate that MBPs are able to bind to phospholipid liposomes as well as to lipids coated onto microtiter wells.

3.4. Binding of MBPs to phosphoinositides and diphosphatidylglycerol

Since we had shown that MBPs bind to PI and that L-MBP binds to PG, we next examined whether MBPs bound to the derivatives of these lipids. Both S- and L-MBPs bound to PIP, albeit their bindings were at a level of 40–41% of PI binding (Fig. 5). However, the MBPs exhibited nearly negligible binding to PIP_2. L-MBP bound to diphosphatidylglycerol, cardiolipin. Its binding showed 42% of PG binding.

3.5. Effect of excess monosaccharides

We examined whether excess monosaccharides affect the binding of MBPs to phospholipids, because MBP belongs to C-type lectins. The binding study was performed in the presence of 10 or 100 mM of glucose, mannose, inositol, *N*-acetylglucosamine or galactose. The binding of S-MBP to PI was decreased by the co-incubation with sugars (Table 1). *N*-Acetylglucosamine, glucose, mannose and inositol almost equally attenuated the binding of S-MBP to PI, whereas galactose was clearly less effective in inhibiting the PI binding. In contrast, the inhibitory effects of sugars on the binding of L-MBP to three phospholipids were generally less potent than those on the binding of S-MBP to PI. Galactose was the most potent inhibitor in the monosaccharides tested for L-MBP binding to lipids.

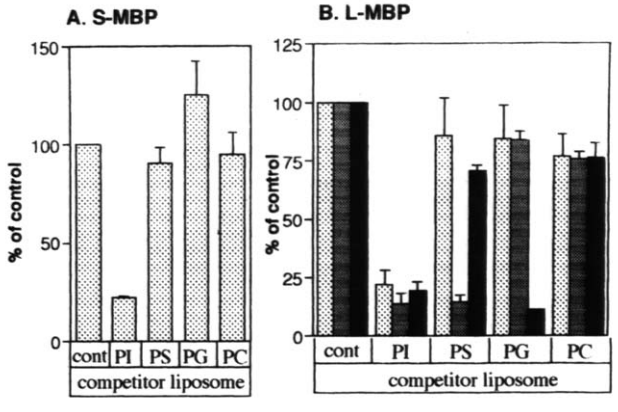

Fig. 4. Competition of phospholipid liposomes with solid phase lipids for the binding of MBPs. PI (stippled bars), PS (hatched bars) and PG (black bars) coated onto microtiter wells (1 μg lipid/well) were incubated with 10 μg/ml of S-MBP (A) or L-MBP (B) in the absence (cont) or the presence of 500 μM phospholipid liposomes composed of PI, PS, PG or PC (competitor liposome) at room temperature for 60 min. The MBP binding to the solid phase lipids was detected using antibody against each MBP as described in Section 2. The results are expressed as percent of control binding. The absorbances of the control binding were 1.076 ± 0.15 (mean ± S.D., $n = 3$) for the S-MBP binding to PI, and 1.067 ± 0.24 for the L-MBP binding to PI, 0.24 ± 0.039 for the L-MBP binding to PS and 0.62 ± 0.10 for the L-MBP binding to PG. The data presented are mean ± S.D. ($n = 3$).

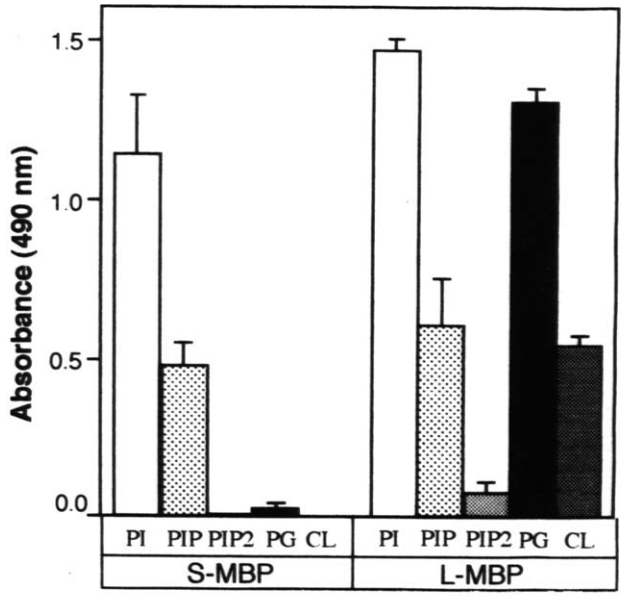

Fig. 5. Binding of MBPs to phosphoinositides and cardiolipin. Phosphatidylinositol (PI, white bars), phosphatidylinositol 4-monophosphate (PIP, stippled bars), phosphatidylinositol 4,5-diphosphate (PIP_2, light hatched bars), PG (black bars) and cardiolipin (CL, dark hatched bars) coated onto microtiter wells (1 μg/well) were incubated with 10 μg/ml serum MBP (S-MBP) or liver MBP (L-MBP) at room temperature for 60 min. The binding of MBP to the solid phase lipids was detected using antibody against each MBP as described in Section 2. The data presented are mean ± S.D. ($n = 3$).

4. Discussion

This study provides evidence that phospholipids are novel types of ligands for MBPs. Both S- and L-MBPs bound to PI and PIP. L-MBP also bound to acidic phospholipids, PG, PS and cardiolipin. We have previously shown that the lung surfactant lectins SP-A and SP-D bind to PC and PI, respectively [11,12]. The present results raise the possibility that phospholipids are ligands for collectins.

The main difficulty in lipid binding studies using microtiter wells is that some of the lipids may be washed away through the procedures. When [^{3}H]DPPC or various phospholipids that had been adsorbed onto microtiter wells were analyzed for radioactivity or phosphorus assay, 10–15% of lipids were firmly attached to the wells after the washing procedures. Previous reports also showed that 10–15% of glycolipids and approximately 30% of phospholipids were firmly attached to the wells after the washing procedures [26,27]. The use of detergent or its concentration in the washing buffer may affect the attachment of the lipids to the wells. In our previous studies [9,12], the results obtained from the binding study of surfactant proteins to glycolipids and phospholipids coated onto microtiter wells corresponded to those done on thin layer

Table 1
Inhibitory activity of various sugars on the binding of MBPs to phospholipids

Compound	Inhibition of binding (%)							
	S-MBP		L-MBP					
(phospholipids)	PI		PI		PG		PS	
(sugar concentration, mM)	10	100	10	100	10	100	10	100
Glucose	18	73	9	40	11	30	32	39
Mannose	28	70	29	37	40	36	24	35
Inositol	31	78	21	58	26	62	17	48
N-Acetylglucosamine	15	78	24	27	19	36	11	35
Galactose	2	21	24	73	48	79	43	66

The binding of MBPs to phospholipids was carried out in the presence of 10 or 100 mM of various monosaccharides as described in Section 2. The results are expressed as % inhibition of binding. The data presented are means of duplicate determinations in two separate experiments.

chromatograms. In this study the phospholipid binding specificity of MBPs in the solid phase assay was essentially the same as that obtained in the liposome binding assay. Thus, the method used in this study, at least qualitatively, reflects the binding specificity of MBPs to phospholipids.

Our results showed that the binding of MBPs to PI is Ca^{2+}-dependent. SP-D also binds to PI in a Ca^{2+}-dependent manner [12]. The possibility is raised that SP-D contaminating the MBP preparation may react with PI, since a significant level of SP-D is present in the serum of humans with certain lung diseases [22]. However, the anti-MBP antibody used in this study did not recognize native SP-D. The previous study from this laboratory indicated that SP-D failed to bind PIP [12], while both S- and L-MBPs bound to PIP in this study. Taken together, we conclude that rat MBPs from serum and liver bind to PI, and suggest that the binding mechanism of MBPs to PI may be different from that of SP-D to PI.

L-MBP bound to acidic phospholipids (PS, PG and cardiolipin) in addition to PI, whereas S-MBP bound exclusively to PI. Although the overall structure of the CRD is very similar in all C-type lectins, these MBPs exhibit significant differences in amino acid sequence despite having 56% identity [3]. Serum MBPs such as rat MBP-A and human MBP consist of hexamers of trimeric subunits ($M_r \sim 650\,000$) [15]. Liver MBP (MBP-C) shows a smaller oligomer ($M_r \sim 200\,000$) [16]. The differences of binding specificity between S- and L-MBPs may be due to the differences in the primary and oligomeric structures.

L-MBP was found to bind PG, cardiolipin and PS, which do not contain sugars. PI liposomes attenuated the binding of L-MBP to solid phase PI, PG and PS, while liposomes composed of PS and PG specifically blocked the binding of L-MBP to solid phase PS and PG, respectively, and PS or PG liposomes failed to attenuate the binding of L-MBP to solid phase PI. One simple explanation for this result is that the binding sites for PS and PG in the L-MBP molecule may be different from each other, and the binding sites for PI may straddle the sites for PG and PS when the binding affinity for PI is higher than that for PS or PG. However, it remains unknown, at present, how L-MBP interacts with these phospholipids.

Kozutsumi et al. [15] reported that sugar specificity for the inhibitory effects on the binding of S-MBP to [^{125}I]mannan was similar to that observed on the binding of L-MBP. In this study the specificity of monosaccharides for the inhibitory effects on S-MBP binding to PI appears different from that on L-MBP binding to lipids. Galactose at 100 mM does not well inhibit the S-MBP binding to PI but it is the most potent inhibitor for L-MBP binding to phospholipids. Thus, these results may suggest that the mechanism by which S-MBP binds to PI is different from that of L-MBP binding to phospholipids. Although *N*-acetylglucosamine at 100 mM blocked the binding of S-MBP to mannan [15] and to PI as shown in this study, the inhibitory activity by this sugar on the binding to PI is less effective than that on the binding to mannan. This difference may be because not only the polar group but also the non-polar group of the PI molecule is involved in the binding of S-MBP to PI.

The annexin family showed binding specificity for acidic phospholipids such as PS and PI [23]. Annexin IV also interacts with PG [24]. The binding of annexins to phospholipids is Ca^{2+}-dependent. They bind phospholipids at the micromolar range of Ca^{2+}. In contrast, millimolar Ca^{2+} is required for MBP to bind to lipids as shown in this study. MBP also binds PIP or cardiolipin, which have not been reported to have phospholipid-binding specificity with the annexins. Thus MBPs, like lung surfactant lectins, are differentiated from the annexins, a family of calcium-dependent, phospholipid- and membrane-binding proteins.

S-MBP activates complements through the classical pathway to lyse mannan-coated erythrocytes [4]. Human MBP also activates the alternative complement pathway and enhances serum bactericidal activity on virulent strains of *Salmonella* [25]. In addition to the carbohydrate binding, the phospholipid binding property of MBP may also be involved in the interaction of this protein with cell membranes, although no evidence is available at the moment. The physiological significance of MBP binding to phospholipids remains to be clarified. The current findings emphasize a novel type of ligand binding specificity for MBP.

Acknowledgements: This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan, and from the ONO Medical Research Foundation.

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