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D-Galactose-Specific Sea Urchin Lectin Sugar-Specifically Inhibited Histamine Release Induced by *Datura stramonium* Agglutinin: Differences Between Sugar-Specific Effects of Sea Urchin Lectin and Those of D-Galactose- or L-Fucose-Specific Plant Lectins

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ABSTRACT—A new sea urchin lectin from *Toxopneustes pileolus*, is D(+)galactose (Gal)-, D(+)fucose (Fuc)-specific. Incubation of rat peritoneal mast cells with the lectin in the presence of 0.3 mM $CaCl_2$ for 10 min significantly and dose-dependently inhibited the histamine release induced by *N*-acetyl glucosamine (GlcNAc)-specific *Datura stramonium* agglutinin (DSA), an activator of the G_i-protein-dependent pathway in mast cells. This inhibition by the sea urchin lectin was sugar-specifically reversed in the presence of D(+)Gal or D(+)Fuc but not L(−)Fuc. The sea urchin lectin had no effect on the histamine release induced by compound 48/80, slightly inhibited the histamine release induced by substance P and mastoparan, and slightly enhanced the histamine release induced by melittin, but these effects were not dose-dependent. Compound 48/80, substance P, mastoparan and melittin are mast cell activators without sugar residues. It is suggested that the lectin binds to D(+)Gal residues of DSA to interfere with mast cell activation induced by DSA, a glycoprotein with arabinose and Gal residues. The effects of plant lectins with affinity to D(+)Gal, *N*-acetyl galactosamine and/or sialic acid and L(−)Fuc on the histamine release induced by DSA, compound 48/80 and substance P were also examined.

Keywords: Mast cell, Histamine release, *Datura stramonium* agglutinin, D(+)Galactose-specific lectin, Compound 48/80, Substance P

The toxopneustid sea urchins, *Toxopneustes pileolus* and *Tripneustes gratilla*, have external appendages (pedicellariae and/or spines) that contain various bioactive proteins, such as venoms and lectins (1). Bioactive proteins from the large globiferous pedicellariae of *T. pileolus* induced contraction in isolated longitudinal muscles of guinea pigs in a dose-dependent manner, induced hemagglutination of horse and mouse erythrocytes without hemolysis, and showed chemotactic properties for guinea pig neutrophils (1, 2). Three fractions, named the P-I, P-II and P-III fraction, were obtained from *T. pileolus*. The P-I fraction was not bioactive and the P-II fraction contained contractin A. Our recent study has revealed that sea urchin lectin-I (SUL-I), a monomeric protein (32 kDa) with 294 amino acid residues from the large *globiferous peidcellariae* of *T. pileolus*, is D(+)galactose (Gal)-, D(+)fucose (Fuc)- and D(+)galacturonic acid-specific, but not L(−)Fuc-specific. SUL-I is a novel lectin, a major component of the P-III fraction, and different from contractin A, a 18-kDa glycoprotein from *T. pileolus* (1) and the toxin of 25 kDa from *T. gratilla* reported by Mebs (3). The P-III fraction containing SUL-I exhibits mitogenic stimulation in murine splenocytes and mesenteric lymphocytes (2, 4).

Rodent peritoneal mast cells have two activation pathways, one IgE and tyrosine kinase-dependent, the other IgE-independent and G_i-protein-coupled. The latter pathway differs from the former pathway in that it is pertussis toxin-sensitive. Matsuda et al. (5) showed that *Datura stramonium* agglutinin (DSA) *N*-acetyl glucosamine (GlcNAc)-specifically and dose-dependently released histamine from rat peritoneal mast cells. The histamine release was extracellular calcium-independent and pertussis toxin-sensitive and not enhanced by phosphatidylserine. Suzuki-Nishimura et al.

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(6) observed an increase in intracellular calcium ion and cytoskeletal assembly after the stimulation of mast cells with DSA as well as compound 48/80. Compound 48/80, substance P, mastoparan and melittin are activators of the IgE-independent pathway of mast cells; compound 48/80 is a synthetic polyamine and substance P, mastoparan and melittin are amphiphilic peptides. As they directly activated G_i protein from bovine brain and rat mast cells in vitro, they seemed to evoke mast cell activation via a G_i-protein-coupled pathway (7 – 9). In the interaction of compound 48/80 and substance P with G_i proteins of the mast cells, neuraminidase-sensitive sugar residues on the cell surface may play helpful roles (7, 10). Neuraminidase-treated rat peritoneal mast cells lost their capacity to respond to DSA (5, 10). Among the activators of the IgE-independent pathway, DSA is a lectin having affinity to GlcNAc oligomers. Moreover, our group observed that lectins with affinity to GlcNAc and/or sialic acid (Sia) inhibited histamine release induced by compound 48/80, substance P and DSA (5, 11, 12). On the other hand, the effects of D(+)Gal-, *N*-acetyl galactosamine (GalNAc)- and L(−)Fuc-specific lectins on the activation of mast cells induced by compound 48/80, substance P and DSA are unknown.

Takei et al. (13, 14) reported that the P-II fraction containing contractin A from *T. pileolus.* released histamine from rat peritoneal mast cells as a consequence of phosphoinositide-specific phospholipase C (PI-PLC) activation. The P-III fraction containing SUL-I, however, did not release histamine from rat peritoneal mast cells in the presence of 0.3 mM $CaCl_2$.

In this study, we examined the effect of D(+)Gal-specific sea urchin lectin on the histamine release induced by DSA in the presence and absence of haptenic sugar D(+)Gal. Moreover, the effects of D(+)Gal-, GalNAc- and L(−)Fuc-specific lectins on the histamine release induced by DSA were examined. As DSA is a glycoprotein with arabinose and Gal residues, there seemed to be interaction between the Gal-residue of DSA and D(+)Gal-specific sea urchin lectin. The effects of these D(+)Gal-, GalNAc- and L(−)Fuc-specific lectins on the histamine release induced by compound 48/80 and substance P having no sugar residues were also estimated. In some experiments, bee venom mastoparan and melittin, activators of G_i protein, were used.

MATERIALS AND METHODS

Isolation of sea urchin lectin

Thirty large globiferous pedicellariae per sea urchin specimen (8 – 10 cm in diameter) were removed with fine forceps. They were then extracted with 20 ml of a 0.15 M NaCl solution at 4°C for 24 h (1). Aliquots (20 ml) of the extract were centrifuged at 12,000 × *g* for 20 min, and the supernatant was collected (total protein was about 10 mg) (2). Briefly, the crude lectin was applied to a Sephadex G-200 column (2.6 × 80 cm) equilibrated with 0.15 M NaCl solution and eluted with the same solution at a flow rate of 15 ml/h. Fractions of 10 ml each were collected and analyzed for absorption at 280 nm (Fig. 1A). The pooled P-III fraction (from tube No. 19 to No. 28) was checked for agglutinating activity. The P-III fraction was lyophilized and stored at −20°C until use. The P-III fraction was used for experiments on the histamine release from rat peritoneal mast cells. For the second step of purification, the P-III fraction was dissolved in 0.15 M phosphate buffer and then applied to an immobilized D(+)Gal column (1.0 × 2.0 cm). The sample was washed with the same buffer and eluted with 100 mM D(+)Gal in this buffer. Elution fractions (2 ml) were collected and analyzed for absorption at 280 nm. The IDG-2 fractions were pooled and checked for agglutinating activity (Fig. 1B). A D(+)Gal-specific lectin of 32 kDa with agglutinating activity was found in the IDG-2 fraction. To determine the amino acid sequence of the new lectin, purification was achieved by HPLC using a reverse-phase C_8 column. Two solvents, 0.1% trifluroacetic acid (TFA) and acetonitrile in 0.08% TFA, were used. The fractions were monitored at 230 nm. The main peaks were pooled and analyzed by SDS-PAGE (Fig. 1C). The main peak (SUL-1) showed agglutinating activity. The amount of protein in each fraction was determined by the Bradford method (15) using bovine serum albumin as a standard.

Assay of agglutinating activity

The agglutinating activity was assayed by using rabbit erythrocytes in microtiter plates. Twenty five microliters of a 2.5% (v/v) suspension of erythrocytes in 6.4 mM phosphate-buffered saline (PBS) was added to 50 μl of serial twofold dilutions of the lectin fractions in PBS. The plates were incubated at room temperature for 1 h. The results were expressed as the minimum concentration of lectin fractions (μg/ml) required for positive agglutination.

Preparation of rat peritoneal mast cells

Peritoneal mast cells purified from male Sprague-Dawley rats (300 – 350 g) using Percoll (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) were suspended in HEPES-buffered Tyrode solution containing 0.3 mM Ca^{2+} (137 mM NaCl, 2.7 mM KCl, 12 mM HEPES, 1 mM $MgCl_2$, 0.3 mM $CaCl_2$, 5.6 mM dextrose and 0.03% (w/v) bovine serum albumin, pH 7.40) (6). The purity of the mast cells in the final preparation was greater than 90% as assessed by toluidine blue staining, and the viability of the mast cells was greater than 90%, as assessed by trypan blue extrusion.

Histamine release

A 0.5-ml sample of cell suspension (2×10^4 cells) in

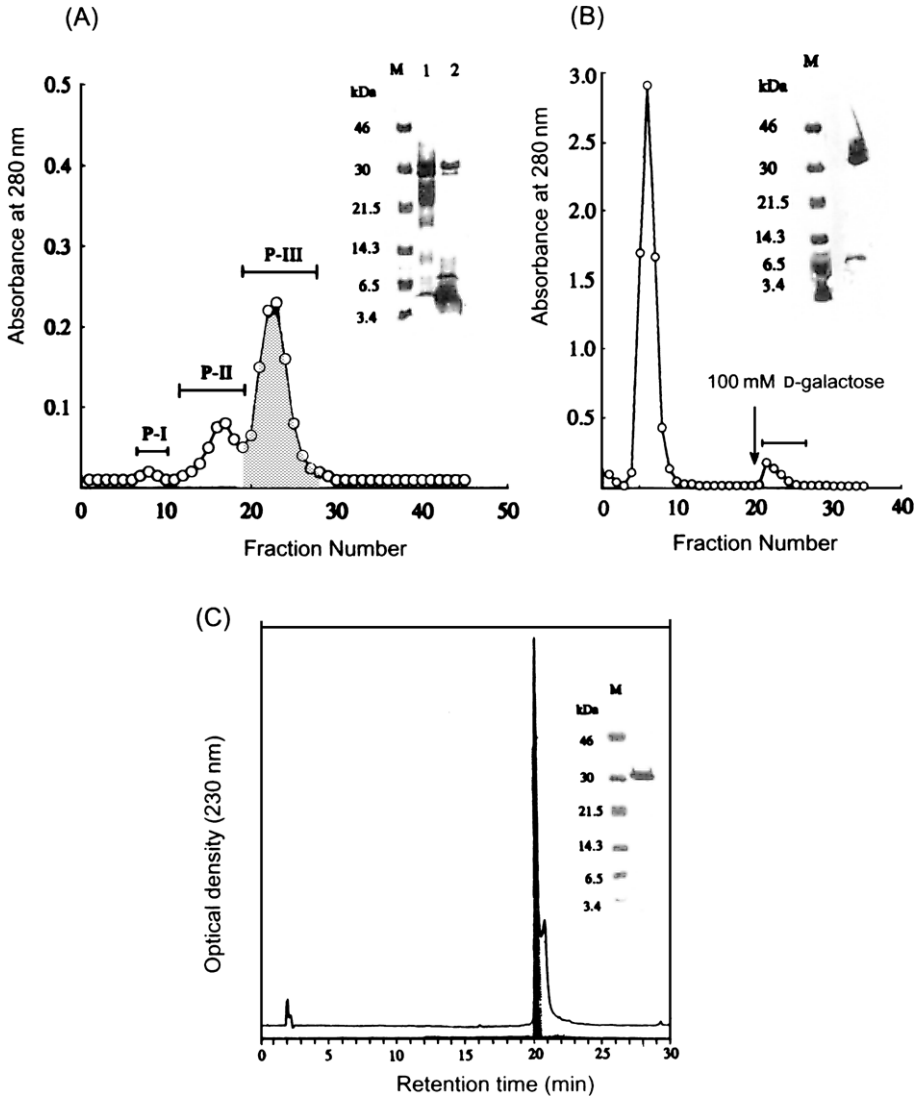

Fig. 1. Purification of sea urchin lectin. A: Sephadex G-200 column chromatography of the crude lectin from large globiferous pedicellariae of *T. pileolus*. Agglutinating activity was localized in the dotted peak (the P-III fraction). Inset, SDS-PAGE of the P-II fraction (1) and P-III fraction (2). B: Affinity chromatography on an immobilized D-galactose (IDG) column of the P-III fraction from Sephadex G-200 gel chromatography. Inset, SDS-PAGE of the IDG-2 fraction. C: HPLC separation of the IDG-2 fraction from the P-III fraction. The sample was resolved on a reverse-phase HPLC column (C8, 2 × 150 mm) monitored at 230 nm, using a linear gradient of aceonitrile (0 – 80%) TFA at flow rate of 1 ml per min. Inset, SDS-PAGE of the purified lectin (the dotted peak). M, mol. wt. markers.

HEPES-buffered Tyrode solution containing 0.3 mM Ca^{2+}, pH 7.40, was incubated in the presence or absence of 0.3 – 30 μg protein/ml of the P-III fraction, 100 μg/ml of lectin (*Agaricus bispours* agglutinin, *Aleuria aurantia* agglutinin, *Lotus tetragonolobus* agglutinin, *Arachis hypogaea* agglutinin and *Sambucus sieboldiana* agglutinin) and 10 mM haptenic sugars for 10 min and then was activated using DSA (5 μg/ml), compound 48/80 (0.3 μg/ml), substance P (30 μM), mastoparan (10 μM) and melittin (10 μg/ml) for a further 10 min. The concentrations of DSA, compound 48/80, substance P, mastoparan, melittin, plant lectins and haptenic sugars were similar to those in previous studies by our group (5, 6, 10 – 12).

After incubation, the sample was centrifuged at 1,600 × *g*, 4°C for 10 min. Histamine in the supernatant was determined using the fluorometric assay of Shore et al. (16). The percentage of histamine release and the inhibitory effects of tested lectins were calculated using the following equations:

Histamine release % = (histamine content in supernatant) / (total histamine content) × 100

% Inhibition = 100 − (histamine release % with inhibitor − spontaneous release %) / (histamine release % without inhibitor − spontaneous release %) × 100

Spontaneous release was 5.5 ± 0.7% (n = 11). Under our conditions in the presence of 0.3 mM Ca^{2+} and absence of phosphatidylserine, we observed no appreciable release of histamine in the presence of P-III, AAL, ABA, Lotus, PNA, SSA or tested sugars.

Statistical analysis

Statistical significance was evaluated by using the unpaired Student's *t*-test with $P = 0.05$ taken as the upper limit of significance.

Chemicals

Datura stramonium agglutinin (DSA: GlcNAc oligomer-specific), *Arachis hypogaea* agglutinin (PNA: Gal<Galβ1-3GalNAc-specific, mutin-type), *Agaricus bispours* agglutinin (ABA: Galβ1-3GalNAc-specific, mutin-type), *Aleuria aurantia* agglutinin (AAL: L(−)Fuc-specific), *Lotus tetragonolobus* agglutinin (Lotus: L(−)Fuc-specific), *Sambucus sieboldiana* agglutinin (SSA: Siaα2-6GalNAc>Siaα2-6GalNAc-specific) and chitooligosaccharide (a mixture of haptenic sugars of DSA) were purchased from Honen Corp. (Tokyo). D(+)Gal (a haptenic sugar of SUL-I, PNA and ABA), L(−)Fuc (a haptenic sugar of AAL and Lotus), GalNAc and *N*-acetyl lactosamine (a weak haptenic sugar of DSA), polycation compound 48/80, bee venom mastoparan, melittin, and neuropeptide substance P were purchased from Sigma Chemical Co. (St. Louis, MO, USA). GlcNAc (a haptenic sugar of WGA) and D(+)Fuc (a haptenic sugar of SUL-I) were from Wako Pure Chemical Co. (Osaka). Alpha-methyl mannoside (αMM: a haptenic sugar of concanavalin A) was from Tokyo Kasei Kogyo Co. (Tokyo).

RESULTS

Purification of sea urchin lectin

The lectins isolated from large globiferous pedicellariae of *T. pileolus* by gel chromatography, affinity chromatography and reverse-phase high performance liquid chromatography are shown in Fig. 1. The gel chromatographic fraction (P-III fraction) and the affinity chromatographic fraction (IDG-2 fraction) had relatively strong activity for agglutination with rabbit erythrocytes at concentrations up to 0.8 μg protein/ml and 0.16 μg protein/ml, respectively. The purified lectin, a 32-kDa monomeric protein shown in Fig. 1C, had intense agglutination activity at concentrations up to 0.13 μg protein/ml (data not shown). The P-III fraction appeared to have several proteins with molecular weights of 5 kDa to 32 kDa. As shown in Fig. 1, B and C, D(+)Gal-specific protein in the P-III fraction was a protein of 32 kDa. We obtained about 4 mg of the P-III fraction. We used the P-III fraction for experiments on histamine release. The P-III fraction used was not contaminated with a toxin of 25 kDa or contractin A of 18 kDa as checked by SDS-PAGE.

Inhibitory effects of sea urchin lectin on the histamine release induced by DSA

Sea urchin lectin (the P-III fraction) dose-dependently inhibited histamine release induced by 5 μg/ml DSA, but not by 0.3 μg/ml compound 48/80 (Fig. 2). At concentrations of 3 and 30 μg protein/ml, the P-III fraction containing SUL-1 slightly inhibited the histamine release induced by 30 μM substance P and 10 μM mastoparan, but the inhibition was not dose-dependent. These inhibitory effects of the P-III fraction (30 μg protein/ml) on histamine release induced by DSA, substance P and mastoparan were reduced in the presence of 10 mM D(+)Gal, suggesting that D(+)Gal-specific lectin SUL-1 in the P-III fraction reversibly and sugar-specifically inhibited the histamine release induced by DSA, substance P and mastoparan. The P-III fraction slightly enhanced the histamine release induced by melittin.

Effects of haptenic sugars

The effects of haptenic sugars on histamine release induced by 5 μg/ml DSA in the absence of sea urchin lectin were examined. At a concentration of 10 mM, GalNAc, D(+)Gal, D(+)Fuc, L(−)Fuc and αMM had no significant effect on the histamine release (Fig. 3), suggesting that the sugar-specific interaction between DSA and the mast cells was not interfered with by these sugars. On the other hand, haptenic sugars of DSA, chitooligosaccharide (1% w/v) and *N*-acetyl lactosamine (10 mM) significantly inhibited histamine release induced by DSA.

In the presence of 10 mM D(+)Gal and 10 mM D(+)Fuc, the inhibitory effect of the P-III fraction (30 μg protein/ml) on the histamine release induced by 5 μg/ml DSA was reversibly decreased (Fig. 4). There were no significant differences between the histamine release induced by DSA alone and by DSA in the presence of the P-III fraction and its haptenic sugars D(+)Gal and D(+)Fuc. In contrast, L(−)Fuc, GalNAc and αMM had no effect on the inhibition in the presence of the P-III fraction, because SUL-I in the P-III fraction has affinities to D(+)Gal and D(+)Fuc but not to L(−)Fuc, GalNAc or αMM. It was noteworthy that effects of D(+)Fuc and L(−)Fuc were quite different.

Effects of ABA, SSA, AAL and Lotus on histamine release induced by DSA

Effects of ABA, SSA, AAL and Lotus were examined. As ABA is Galβ1-3GalNAc (mutin-type)-specific, SSA is Siaα2-6GalNAc>Siaα2-6GalNAc-specific, AAL is L(−)Fuc-specific and Lotus is L(−)Fuc-specific, the sugar

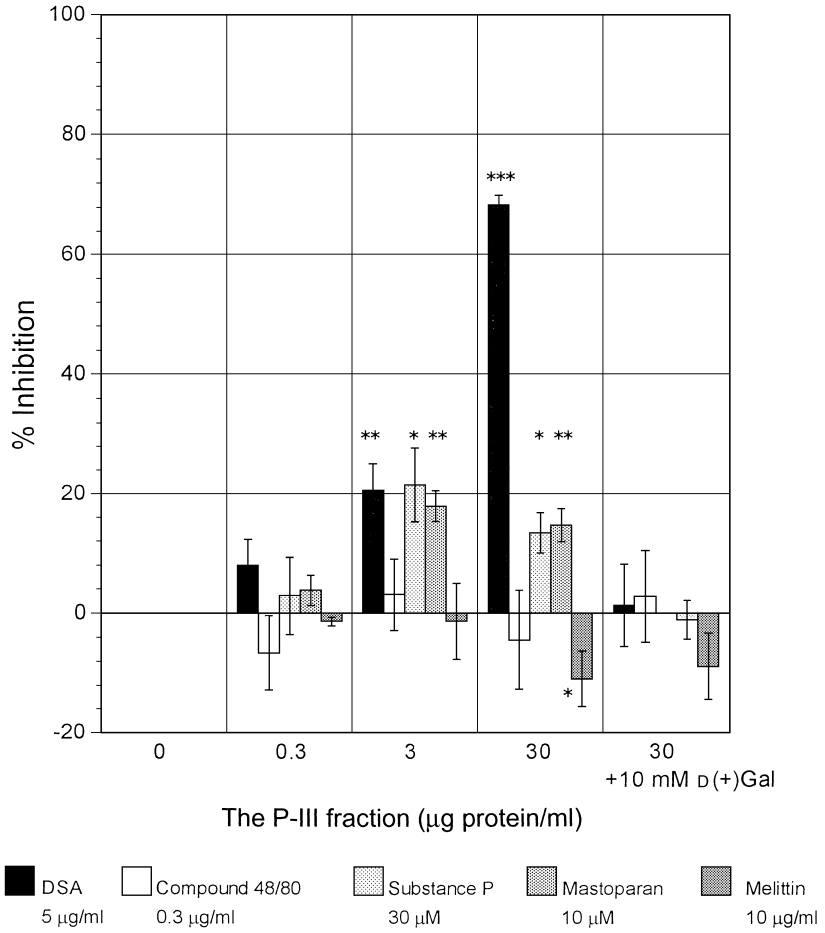

Fig. 2. Inhibitory effects of sea urchin lectin (the P-III fraction) on histamine release induced by activators of the G_i-dependent pathway in mast cells. With the addition of the haptenic sugar, 10 mM D(+)Gal, the inhibition and enhancement induced by 30 μg protein/ml of the P-III fraction were decreased. The histamine release induced by DSA (5 μg/ml), compound 48/80 (0.3 μg/ml), substance P (30 μM), mastoparan (10 μM) and melittin (10 μg/ml) in HEPES-buffered Tyrode solution containing 0.3 mM Ca^{2+}, pH 7.4, at 37°C for 10 min was 55.3 ± 3.0% (n = 6), 42.8 ± 5.0% (n = 6), 44.8 ± 2.3% (n = 12), 70.2 ± 2.5% (n = 6) and 66.1 ± 5.9% (n = 11), respectively. Values are expressed as means ± S.E.M. for 3 – 7 repeated experiments. The histamine release induced by DSA, compound 48/80, substance P, mastoparan and melittin with the P-III fraction were compared with those without the P-III fraction; **P*<0.05, ***P*<0.01, ****P*<0.001.

specificity of these plant lectins differs from that of SUL-I. ABA (Galβ1-3GalNAc (mutin-type)-specific) did not inhibit the histamine release induced by 5 μg/ml DSA. SSA (Siaα2-6GalNAc>Siaα2-6GalNAc-specific) inhibited histamine release induced by DSA in the presence and absence of D(+)Gal, suggesting that the inhibitory effect of SSA depended on Sia or GalNAc, not on D(+)Gal. In L(−)Fuc-specific lectins, AAL sugar-specifically inhibited histamine release, but Lotus had no effect (Fig. 5).

Another D(+)Gal-specific lectin, PNA, with affinity to D(+)Gal (mutin-type) was examined. PNA did not inhibit the histamine release induce by DSA. At concentrations of 1, 10 and 100 μg/ml PNA, the percent inhibition of histamine release induced by 5 μg/ml DSA was −11.6 ± 8.4%, −1.7 ± 6.3% and −0.8 ± 7.3% (n = 6 – 8), respectively. Moreover, PNA itself did not activate histamine release from rat mast cells (data not shown).

Effects of ABA, SSA, AAL and Lotus on histamine release induced by substance P and compound 48/80

ABA, SSA, AAL and Lotus did not inhibit or enhance the histamine release induced by 30 μM substance P in the presence or absence of D(+)Gal or L(−)Fuc (Fig. 6). In this study, the effects of AAL and Lotus on the histamine release induced by compound 48/80 were examined, our group having previously reported the effects of ABA and SSA on this release (12). AAL sugar-specifically inhibited and Lotus sugar-specifically enhanced the histamine release induced by 0.3 μg/ml compound 48/80 (Fig. 7). The effects were significant (P<0.05) but not large. On the other hand,

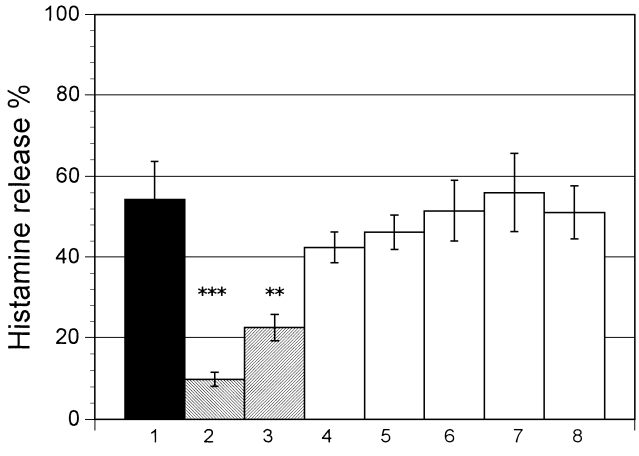

Fig. 3. The effect of haptenic sugars on histamine release induced by DSA. 1, Control histamine release induced by DSA (5 μg/ml); 2, in the presence of 1% w/v chitooligosaccharide; 3, in the presence of 10 mM *N*-acetyl lactosamine; 4, in the presence of 10 mM *N*-acetyl galactosamine; 5, in the presence of 10 mM D(+)Gal; 6, in the presence of 10 mM D(+)Fuc; 7, in the presence of 10 mM L(−)Fuc; 8, in the presence of 10 mM αMM. Values are expressed as means ± S.E.M. for 3 – 6 repeated experiments. ***P*<0.01, ****P*<0.001 vs control.

the histamine release induced by 0.3 μg/ml compound 48/80 in the presence of 100 μg/ml ABA and SSA decreased from 63.5 ± 3.0% to 54.4 ± 1.4% (*P*<0.05, n = 6) and from 49.1 ± 2.0% to 20.4 ± 1.0% (*P*<0.001, n = 6), respectively, as previously described (12).

DISCUSSION

Carbohydrates on the cell surface play a critical role in cell-cell interaction, cell recognition, cell adhesion and cell activation. Many lectins have been detected and isolated from various plants and animals, including invertebrates. Lectins, generally considered not to have enzyme activity, recognize specific carbohydrate structures and agglutinate a variety of animal cells. Cell surface receptors have a lectin-like domain (17) and furthermore, sugar residues on the cell surface act as co-receptors (18).

Animals have self-defense lectins to recognize non-self substances. SUL-I is a novel D(+)Gal-specific lectin. SUL-I was shown to have chemotactic properties for guinea pig neutrophils at a concentration of 0.625 μg/ml, suggesting a defensive role, in which mast cells are important, in processes such as histamine release and metabolism of arachidonic acid (2). SUL-I is different from a heat labile toxin of 25 kDa purified from *T. gratilla*, whose LD_{50} was 0.85 mg/kg (i.p.) in mice (3). Its toxicity did not depend on histamine shock because pretreatment of the mice with H_1-blocker did not prevent its toxic effect.

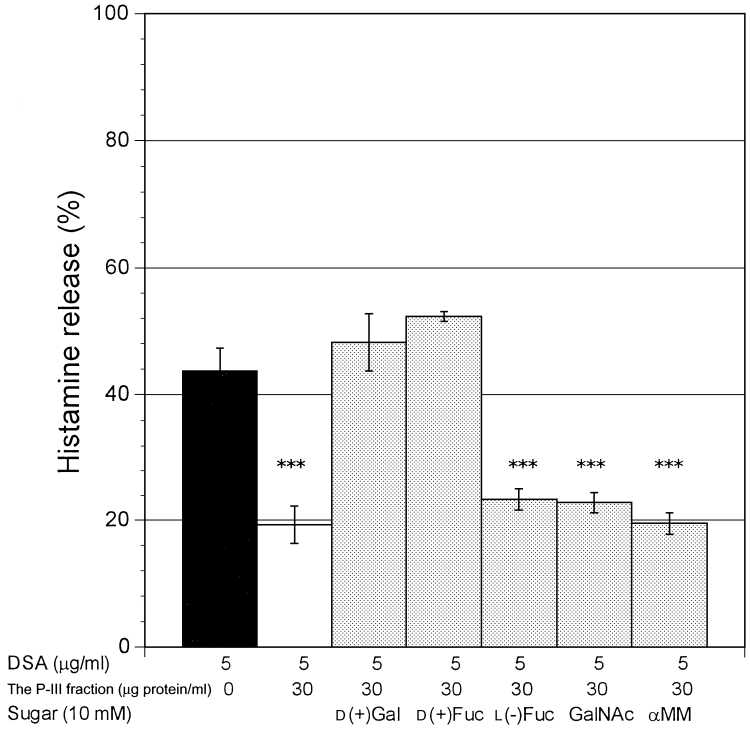

Fig. 4. The effects of D(+)Gal, D(+)Fuc, L(−)Fuc, GalNAc and αMM on inhibition induced by the P-III fraction (30 μg protein/ml) of histamine release induced by DSA (5 μg/ml). The concentration of the sugar was 10 mM. Values are expressed as means ± S.E.M. for 4 – 8 repeated experiments. ****P*<0.001 vs control histamine release induced by DSA (5 μg/ml).

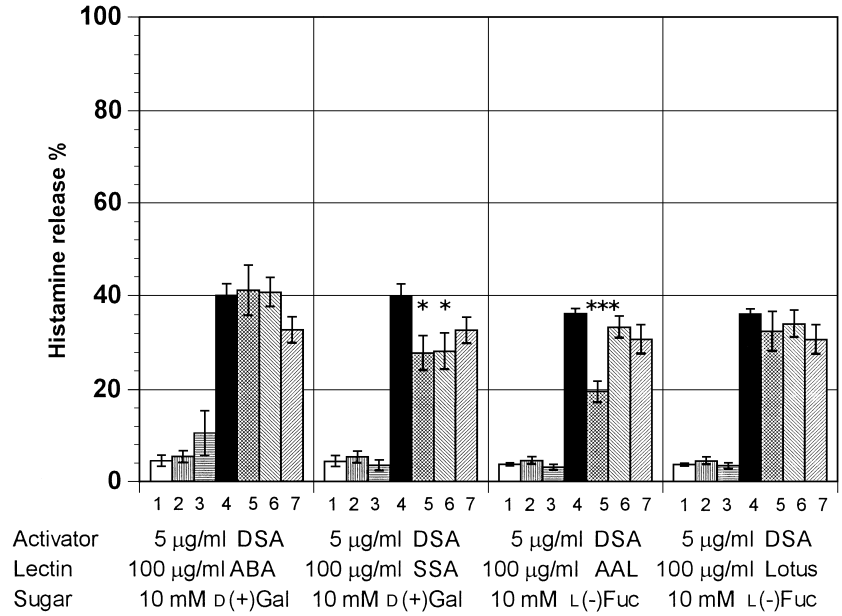

Fig. 5. The effects of ABA, SSA, AAL and Lotus on histamine release induced by DSA (5 μg/ml). 1, Spontaneous release; 2, 10 mM sugar; 3, 100 μg/ml ABA, SSA, AAL and Lotus (lectin); 4, 5 μg/ml DSA; 5, lectin + DSA; 6, lectin + sugar + DSA; 7, DSA + sugar. Values are expressed as means ± S.E.M. for 3 – 6 repeated experiments. The values shown in columns 5, 6 and 7 were compared with those in column 4; **P*<0.05, ****P*<0.001.

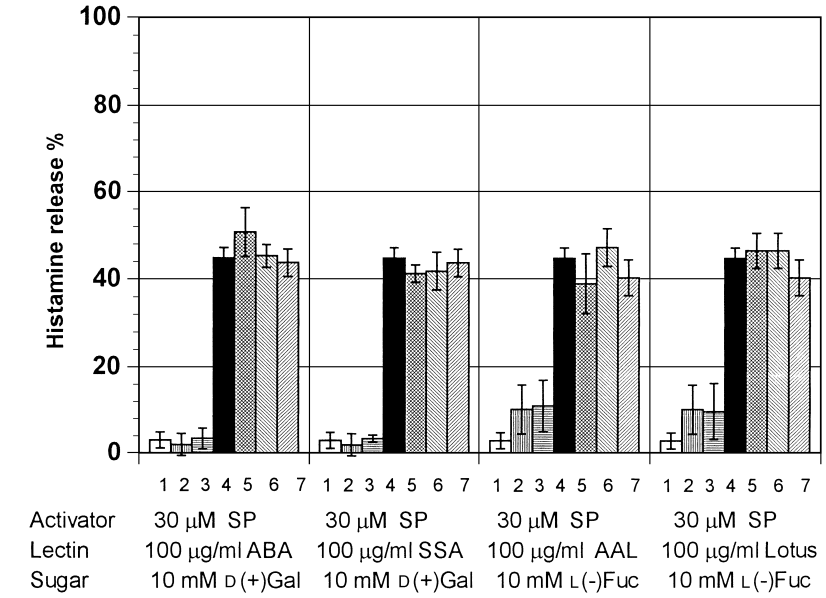

Fig. 6. The effects of ABA, SSA, AAL and Lotus on histamine release induced by substance P (SP, 30 μM). 1, Spontaneous release; 2, 10 mM sugar; 3, 100 μg/ml ABA, SSA, AAL and Lotus (lectin); 4, 30 μM SP; 5, lectin + SP; 6, lectin + sugar + SP; 7, SP + sugar. Values are expressed as means ± S.E.M. for 3 – 12 repeated experiments. The values shown in columns 5, 6 and 7 were compared with those in column 4; no significant differences.

In this study, we found that SUL-I is a novel lectin regulating mast cell functions with unique mechanisms. It seems to be a strong inhibitor of mast cell activation induced by DSA.

Datura stramonium agglutinin (DSA) is an activator of the G_i protein-dependent pathway, recognizing GlcNAc residues of mast cells (5). Compound 48/80, substance P, mastoparan and melittin are known to directly stimulate

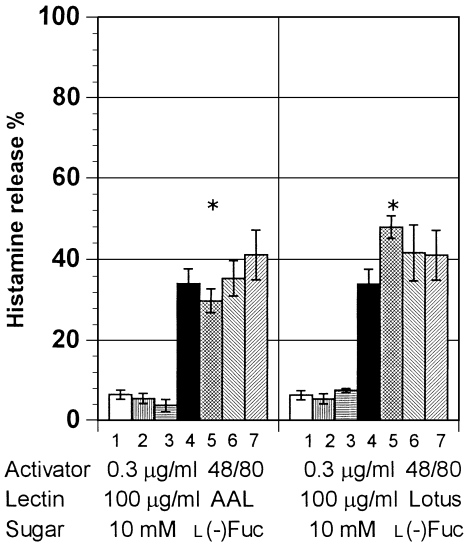

Fig. 7. The effects of AAL and Lotus on histamine release induced by compound 48/80 (48/80, 0.3 μg/ml). 1, Spontaneous release; 2, 10 mM L(−)Fuc; 3, 100 μg/ml AAL and Lotus (lectin); 4, 0.3 μg /ml 48/80; 5, lectin + 48/80; 6, lectin + L(−)Fuc + 48/80; 7, 48 /80 + L(−) Fuc. Values are expressed as the means ± S.E.M. for 3 – 8 repeated experiments. The values shown in columns 5, 6 and 7 were compared with those in column 4; *P<0.05.

G_i activities in vitro (7 – 9, 19). Recently, various effects of substance P, mastoparan and melittin were reported. Substance P-related inhibitors inhibited mastoparan stimulated GTPase activity in homogenized rat peritoneal mast cells (20). Mastoparan bound to a 97-kDa MP-binding protein to cause a transient release of Ca^{2+} from skeletal muscle sarcoplasmic reticulum (21) and inhibited the sarcoplasmic reticulum Ca^{2+}-ATPase (22). The 97-kDa MP-binding protein is not G_i, triadin or the Ca^{2+} pump. Melittin formed ion channels to modify the electrical activity of the cell membranes (23). Compound 48/80, substance P, mastoparan and melittin possibly have various effects on rat mast cells including direct activation of G_i protein. Their various activities would explain why the P-III fraction inhibited and activated the histamine release induced by substance P, mastoparan and melittin.

Galli and Weshil (24) reviewed the importance of antibody-independent, G_i protein-dependent mast cell activation in self-defense. Since DSA is a glycoprotein with 93 – 94% arabinose and 6 – 7% galactose residues (25), there seemed to be a D(+)Gal-specific interaction between SUL-1 and DSA. The P-III fraction containing SUL-I did not inhibit the histamine release dose-dependently induced by compound 48/80, substance P, mastoparan or melittin, which had no sugar residues, suggesting a D(+)Gal-specific interaction between SUL-I and DSA. According to the binding of D(+)Gal-specific sea urchin lectin to the mast cell surface, histamine release induced by substance P and mastoparan was slightly inhibited. The modification of the mast cell surface by SUL-I seemed to be so weak that the histamine release induced by substance P and mastoparan was inhibited only about 20%. It is interesting that the P-III faction did not inhibit the histamine release induced by compound 48/80, because our group reported that *Sophora japonica* agglutinin (SJA) inhibited the histamine release induced by compound 48/80, the IC_{30} being 37.8 μg/ml (10). The sugar-specificity of SJA is Gal/GalNAc (both mutin-type and Asn-type). On the other hand, the P-III fraction slightly stimulated the histamine release induced by melittin, but the mechanisms of the stimulation were not clear. This led to the conclusion that the D(+)Gal-specific lectin SUL-I inhibited the histamine release induced by DSA because the binding of SUL-I to the D(+)Gal residues in DSA possibly interfered with the GlcNAc-specific interaction between DSA and the mast cells via GlcNAc oligomers.

The inhibitory effect of SUL-I is noteworthy because the other Gal-specific plant lectins, ABA and PNA, were not inhibitory; ABA and PNA have affinities to mutin-type sugar residues of Galβ1-3GalNAc and Gal<Galβ1-3GalNAc, respectively. As these plant lectins mainly recognize the dimer, Gal-GalNAc, they seemed not to bind to DSA having Gal and arabinose residues.

It was clear that the inhibitory effect of SUL-I was D(+)Gal-specific because D(+)Gal and D(+)Fuc specifically reversed the inhibition induced by SUL-I, but not L(−)Fuc. The difference in effect between D(+)Fuc and L(−)Fuc was consistent with the effect of D(+)Gal, suggesting that SUL-I binds to the D(+)Gal residue in DSA. As described by Nakagawa et al. (2), agglutination with mouse erythrocytes by SUL-I was effectively inhibited by D(+)Gal (3.13 mM), D(+)galacturonic acid (3.13 mM) and D(+)Fuc (12.5 mM) but not by GalNAc. We, therefore, examined the effect of D(+)galacturonic acid (a haptenic sugar of SUL-I), but the solution was so acidic that it damaged the mast cells. The SUL-I molecule whose binding sites were filled with D(+)Gal or D(+)Fuc seemed to competitively lose activity bind to DSA. As SUL-I is not a glycoprotein, the interaction between DSA and SUL-I depends on the lectin activity of SUL-I and not that of DSA.

In our experiment, we used the P-III fraction. This fraction contained proteins with a molecular weight of about 5 kDa, which are almost insensitive to agglutination (2). To confirm the D(+)Gal-specific inhibitory effect of SUL-I on the histamine release induced by DSA, we first examined the effect of the IDG-2 fraction (Fig. 1B). The D(+)Gal-specific lectin in the IDG-2 fraction significantly inhibited the histamine release induced by DSA, suggesting that the D(+)Gal-specific lectin of 32 kDa from sea urchin had an inhibitory effect on mast cell activation induced by DSA. The histamine release induced by 5 μg/ml DSA decreased

from $42.3 \pm 8.6\%$ to $15.5 \pm 2.6\%$ ($P<0.05$, $n = 4$) in the presence of the IDG-2 fraction (3 μg protein/ml). The fraction obtained by HPLC using a reverse-phase C_8 column was used for analysis of amino acid sequence and SDS-PAGE (Fig. 1C). The amount of SUL-1 from HPLC was too small to examine the effect on the mast cells. Therefore, the possibility remains that the inhibitory effects of the P-III fraction depended on the protein with the molecular weight of 5 kDa shown in Fig. 1B.

From Figs. 5 and 6, it is clear that ABA did not inhibit the histamine release induced by DSA and substance P. SSA inhibited the histamine release induced by DSA, but not by substance P. AAL inhibited the histamine release induced by DSA and compound 48/80 but not by substance P, whereas Lotus slightly enhanced the release induced by compound 48/80, but showed no effect on the release induced by DSA and substance P (Figs. 5 – 7). Effects of ABA, SSA, AAL and Lotus were different from those of SUL-I. The inhibitory effect of SSA on the histamine release was similar to that of a Sia-specific lectin *Macckia amurensis* mitogen (MAM) as described previously (10 – 12). L(−)Fuc-specific lectin AAL sugar-specifically inhibited the histamine release induced by DSA and compound 48/80, whereas Lotus had no effect on the histamine release induced by DSA and slightly enhanced the release induced by compound 48/80 (Figs. 5 and 7). The effect of Lotus on the histamine release induced by compound 48/80 seemed to be L(−)Fuc-specific. Pretreatment of the mast cells with neuraminidase or GlcNAc-specific lectins led to an inhibition of the histamine release induced by compound 48/80, substance P and DSA (8 – 10). By modification of the cell surface with various lectins, the mast cells became insensitive to IgE-independent stimuli. Although compound 48/80 and substance P are activators of G_i proteins on mast cells, the effects of various lectins were different.

Shibasaki et al. (26) reported that some lectins inhibited the IgE-dependent histamine release, since they bound to IgE and consequently inhibited the binding of IgE to FcεRI. Recently, two inhibitors of the interaction between IgE and FcεRI were reported: soluble human recombinant FcεRIα subunit (27) and recombinant humanized anti-IgE monoclonal antibody (28, 29). These inhibitors and lectins were designed to empty the receptor site to prevent mast cell activation via the IgE-dependent pathway.

In the IgE-independent pathway, SUL-I was an inhibitor of DSA. This mechanism of inhibition of SUL-I seemed to be different from those of other lectins tested in this study. The inhibitory effect of SUL-I on mast cell activation induced by DSA has provided clues that will help solve the unanswered questions concerning antibody-independent mast cell activation.

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