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Purification and characterization of a new mannose-specific lectin from *Sternbergia lutea* bulbs

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A new mannose-binding lectin was isolated from *Sternbergia lutea* bulbs by affinity chromatography on an α(1-2)mannobiose-Synsorb column and purified further by gel filtration. This lectin (*S. lutea* agglutinin; SLA) appeared homogeneous by native-gel electrophoresis at pH 4.3, gel filtration chromatography on a Sephadex G-75 column, and SDS-polyacrylamide gel electrophoresis, These data indicate that SLA is a dimeric protein (20 kDa) composed of two identical subunits of 10 kDa which are linked by non-covalent interactions.

The carbohydrate binding specificity of the lectin was investigated by quantitative precipitation and hapten inhibition assays. It is an α-D-mannose-specific lectin that interacts to form precipitates with various α-mannans, galactomannan and asialo-thyroglobulin, but not with α-glucans and thyroglobulin. Of the monosaccharides tested only D-mannose was a hapten inhibitor of the SLA-asialothryroglobulin precipitation system, whereas D-glucose, D-galactose and L-arabinose were not. The lectin appears to be highly specific for terminal α(1-3)-mannooligosaccharides. The primary structure of SLA appears to be quite similar to that of the snow drop (*Galanthus nivalis*) bulb lectin which is a mannose-binding lectin from the same plant family Amaryllidaceae. The N-terminal 46 amino acid sequence SLA showed 76% homology with that of GNA.

***Keywords*: *Sternbergia lutea*, mannose-binding lectin**

***Abbreviations*: AAA, *Allium ascalonicum* agglutinin (shallot lectin); ASA, *Allium sativum* agglutinin (garlic lectin); AUA, *Allium ursinum* agglutinin (ramsons lectin); DAP, 1,3-diaminopropane; GNA, *Galanthus nivalis* agglutinin (snowdrop lectin); HHA, *Hippeastrum hybr.* agglutinin (amaryllis lectin); LOA, *Listera ovata* agglutinin (orchid twayblade lectin); NPA, *Narcissus pseudonarcissus* agglutinin (daffodil lectin); PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline, SLA, *Sternbergia lutea* agglutinin; SDS, sodium dodecyl sulfate; Me, methyl; Bn, benzyl; PNP, *p*-nitrophenyl.**

Introduction

Recently, several mannose-binding lectins have been isolated and characterized from bulbs of monocotyledonous plants. We showed that several lectins obtained from the families Amaryllidaceae, Alliaceae and Orchidaceae all exhibit exclusive specificity towards D-mannose because of their high specificity for the C-2 axial hydroxyl group of D-mannose. The snow drop (*Galanthus nivalis* agglutinin; GNA) [1, 2], daffodil (*Narcissus pseudonarcissus* agglutinin; NPA), amaryllis (*Hippeastrum hybr*. agglutinin; HHA) [3, 4], garlic (*Allium sativum* agglutinin; ASA), ramsons (*Allium ursinum* agglutinin; AUA) [6], shallot (*Allium ascalonicum* agglutinin; AAA) [7] and Orchid twayblade (*Lisatera ovata* agglutinin; LOA) [8] are clearly different from the well-known mannose/glucose-binding lectins, such as Concanavalin A and the other legume lectins [9] of this group, which are shown to involve the pyranose forms of D-mannose and D-glucose containing similar hydroxyl group configurations at the C-3, -4 and -6 positions.

In this study, we report the purification and characterization of *Sternbergia lutea* lectin, which is also a member of the same family to which GNA belongs. The physicochemical properties and the detailed carbohydrate binding specificity of these lectins are also compared.

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Materials and methods

Polysaccharides, glycoproteins, enzymes and miscellaneous procedures

Several monosaccharides and mannooligosaccharides were purchased from Sigma Co. Alpha-(1-2)-linked mannobiose was a gift from Dr T. Nakajima, Tohoku University, Japan.

Yeast and bacterial mannans and galactomannans from various strains and dextran 1355-S and rabbit liver glycogen were available from previous studies. Ovalbumin, fetuin, asialo-fetuin, thyroglobulin (porcine, bovine) and asialothyroglobulin were purchased from Sigma Co. Asialoorosomucoid was prepared by Dr N. Shibuya. The low-molecular-mass α(1-3)-mannan (DP 7 and 15) were prepared by periodate oxidation and mild acid hydrolysis of the glucuronoxylomannan of *Tremella fuciformis* [10] which was available from pervious studies in our laboratory. Endoproteinases Lys-C (*Lysobacter emzymogenes*) and Asp-N (*Pseudomonos fragi*) were gifts from Dr Philip Andrews. Hemagglutination assays were carried out in microtitre plates using 3% rabbit erythrocytes. Hapten inhibition of hemagglutination was carried out by serially diluting hapten and preincubated lectin (2^4 units of hemagglutinating activity) for 1 h, after which a 3% solution of rabbit erythrocytes was added. A units of hemagglutinating activity is defined as the minimal concentration of lectin required for hemagglutination.

Periodate oxidation of α(1-3)-linked mannooligosaccharides

Glucuronoxylomannan of *T. fuciformis* which contains an α-(1-3)-linked mannan backbone was dissolved in 1 ml of 50 mM $NaIO_4$ solution and oxidized for 72 h at 4 °C. After destruction of excess periodate by the addition of ethyleneglycol, the reaction product was reduced with sodium borohydride at 4 °C overnight. The resulting polysaccharide polyalcohol was hydrolysed with 0.4 M trifluoroacetic acid at 90 °C for 5 h. The α(1-3)-linked mannooligosaccharides (DP 8–15) was purified by gel filtration chromatography using a Bio-Gel P-2 column (5 × 120 cm). These α(1-3)-mannooligosaccharides were also subjected to periodate oxidation/reduction employing the same conditions, as described above. Sugars were determined by phenol-sulfuric assay [11] using mannose as standard.

Purification of the Sternbergia lutea lectin (SLA).

SLA was isolated from an 80% $(NH_4)_2SO_4$ precipitation fraction of *Sternbergia lutea* bulbs by affinity chromatography on α(1-2)-mannobiose-Synsorb and purified by gel filtration using a Sephadex G-75 column (1.5 × 120 cm). Briefly bulbs (1 kg) were homogenized and extracted with 10 mM PBS (containing 0.15 M NaCl, 10 mM EDTA, 0.5 mM PMSF, pH 7.2). To the extracted and centrifuged supernatant solution was added $(NH_4)_2SO_4$ [0–80 sat.]. The 80% $(NH_4)_2SO_4$ precipitate was dissolved in distilled water, dialysed exhaustively against distilled water and lyophilized. The lyophilized powder was dissolved in PBS (100 ml) and a 5 ml portion was applied to an α(1-2)-mannobiose-Synsorb column (1.0 × 25 cm) equilibrated with PBS. Unbound protein was washed with PBS (100 ml) until the A_{280} fell below 0.02. The lectin was desorbed with 0.5 M methyl α-D-mannoside/PBS. Final purification was achieved by Sephadex G-75 gel filtration chromatography.

Polyacrylamide gel electrophoresis(Native-PAGE) and sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE)

Native gel electrophoresis was conducted at pH 4.3 in β-alanine/acetic acid buffer using 7.5% polyacrylamide gel, according to Reisfield *et al.* [12]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis in presence or absence of 5% 2-mercaptoethanol was performed using a 20% slab gel according to the method of Laemmli [13]. Protein bands were visualized by Coomassie brilliant blue R-250 staining. Molecular weight markers were myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), lysozome (14.4 kDa) and aprotinin (6.5 kDa).

Quantitative precipitation and hapten inhibition assays

Quantitative precipitation reactions were conducted by the method of So and Goldstein [14]. Varying amounts of glycoproteins or polysaccharides were incubated with 20 μg of SLA in a total volume of 150 μl. After incubation at 37 °C for 1 h, the reaction mixtures were stored at 4 °C for 48 h. The precipitates formed were centrifuged, washed three times with ice cold PBS and analysed for protein. For hapten inhibition tests, varying amounts of sugars were added to the reaction mixture consisting of 40 μg asialothyroglobulin in a total volume of 150 μl. Protein was determined by the method of Lowry *et al.* [15] using bovine serum albumin as standard.

Molecular weight determination by gel filtration

The molecular weight of the purified SLA was estimated by gel filtration chromatography using a Sephadex G-75 column (1.5 × 100 cm) equilibrated with PBS, containing 0.15 M NaCl and 0.3 M mannose, pH 7.2. The column was calibrated with GNA (50 kDa); egg albumin (45 kDa); chymotrypsinogen Q (25.7 kDa); cytochrome C (12.4 kDa); aprotinin (6.5 kDa).

Amino acid analysis

Purified SLA was hydrolysed for 24 h at 110 °C in 6 M HCl. Amino acid composition was analysed with an Applied Biosystems amino acid analyser Model 420A. The tryptophan content was determined spectrophotometrically [16].

N-Terminal SLA amino acid sequence analysis

Amino acid sequencing was carried out on an ABI 473 (automated sequencer) at the Protein Structure Facility of University of Michigan. Purified SLA (0.2–0.5 mg in 200 μl

of 50 mM Tris/HCl, pH 8.5) was digested with 2 μg of Asp-N or 5 μg of Lys-C for 18 h at 37 °C [16]. The endoproteinases digestion products were applied to reversed phase high performance liquid chromatography (HPLC), and elution was monitored by A_{220} nm. Amino acid sequence analysis of peptides fractionated by HPLC was carried out as described above.

Results

Purification of *S. lutea* lectin

Since preliminary experiments with crude extracts from *S. lutea* bulbs indicated that the agglutinating factor they contain exhibited specificity towards mannose, SLA was purified by affinity chromatography on immobilized mannose. Affinity-purified SLA did not appear to be homogeneous by polyacrylamide gel electrophoresis at pH 4.3. Therefore gel filtration was included in the purification protocol. Inasmuch as the final lectin preparation yielded a single polypeptide band upon native-PAGE and SDS-PAGE and, in addition, eluted as a single symmetrical peak from the Sephadex G-75 column, it appears to be homogeneous. The overall yield of SLA was approximately 0.5 mg g^{-1} bulb tissue. SLA was devoid of carbohydrate.

Molecular weight and molecular structure

Both reduced (2% β-mercaptoethanol) and unreduced SLA gave only a single polypeptide band of M_r 10 000 upon SDS-PAGE gel electrophoresis (Figure 1). Gel filtration of SLA in the presence of 0.3 M D-mannose on a Sephadex G-75 column gave approximately M_r 20 000, indicating the lectin to be a dimeric protein composed of two identical subunits with no intersubunit disulfide bonds.

Amino acid analysis

The amino acid composition of *S. lutea* lectin is given in Table 1. SLA contains a high content of asparagine/aspartic acid, glycine and leucine. The lectin does not contain sugar.

Precipitation assay

The precipitation curves of SLA with various yeast mannans, galactomannans and glucans are shown in Figure 2A. *Saccharomyces cerevisiae* and *Candida tropicalis* mannans which contain multiple D-mannosyl side chains attached to an α(1-6)-linked mannose backbone gave pronounced precipitation with SLA. Neither the linear α(1-3)-mannans (DP 8–15) nor the periodate-oxidized and $NaBH_4$-reduced linear α(1-3)-mannans prepared from *T. fuciformis* glucuronoxylomannan precipitated with *S. lutia* lectin (Figure 3). Interestingly, the SLA lectin reacted strongly with the galactomannan isolated from *Torulosis magnolia*, but not with other galactomannans from *Penicillium frequantans* and *P. charlessii*. On the other hand, SLA did not give a precipitation reaction with glycogen or dextran B-1355-S. The

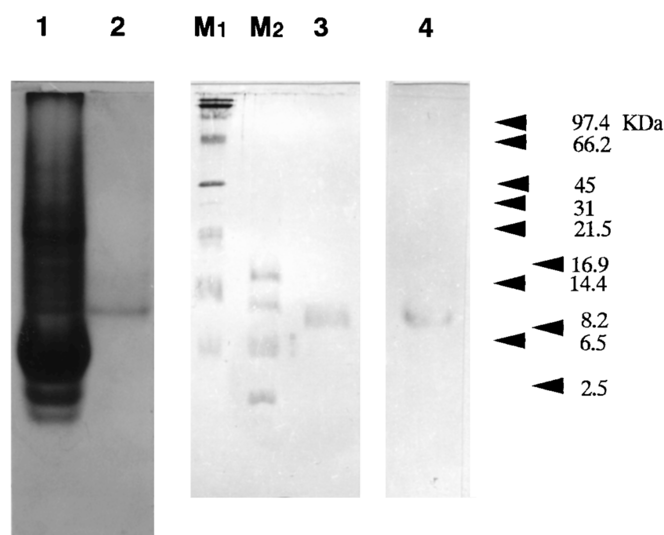

Figure 1. Polyacrylamide gel electrophoresis of the gel filtration-purified SLA. Lane 1 and 2 are native gel at pH 4.3 in β-alanine/acetic acid buffer system. Lane 1, crude extract; lane 2, purified SLA; Lane 3, 4 and M are SDS-PAGE (20% gel). Lane 3, SDS-PAGE in the presence of 5% mercaptoethanol; lane 4, SDS-PAGE in the absence of β-mercaptoethanol; lane M_1, molecular weight standards are myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), lysozome (14.4 kDa), aprotinin (6.5 kDa); lane M_2, myogloblin (16949,) myoglobin I&II (14404), myoglobin I (8159), myoglobin III (2512).

precipitation curves of several glycoproteins with SLA is shown in Figure 2B. SLA interacted most strongly with asialothyroglobulin; however, it did not interact with ovalbumin, fetuin, thyroglobulin or asialofetuin.

Hapten inhibition of precipitation reaction

The results of carbohydrate binding specificity studies of SLA, as conducted by sugar hapten inhibition of precipitation with asialothyroglobulin, are presented in Table 2. The concentrations of carbohydrate required for 50% inhibition were obtained from complete inhibition curves (Figure 4).

Of the monosaccharides tested, only D-mannose was inhibitory; epimers of D-mannose, *ie* glucose (C-2), altrose (C-3), and talose (C-4), were all noninhibitory up to 1000 mM. The 2-deoxy derivative methyl 2-deoxy α-arabinohexoside did not inhibit the interaction of SLA at 1000 mM. Methyl α-D-mannopyranoside was a somewhat better (3.3-fold) and methyl β-D-mannopyranoside poorer (0.3-fold) inhibitor than D-mannose. Other α-mannosides, *eg* benzyl α-D-mannopyranoside and *p*-nitrophenyl-α-D-mannopyranoside, showed precisely the same inhibitory potency as methyl α-D-mannopyranoside.

Among the oligosaccharides tested, those carrying terminal Man α(1-3)Man units were the most potent inhibitors and exhibited 10–40 times greater potency for SLA compared to D-mannose. As shown in Table 2 and Figure 6,

Table 1. Amino acid composition of Amaryllidaceae lectins.

		Mol %				
Amino acid	*Residue*	*INT*	*SLA*	*GNA*[a]	*NPA*[a]	*HHA*[a]
Asp	34.07	34	17.5	17.0	16.8	14.3
Gly	24.47	24	12.4	11.2	12.9	11.5
Leu	18.21	18	9.3	8.8	8.0	7.8
Ser	15.82	16	8.2	7.4	7.1	7.4
Val	15.12	15	7.7	5.6	3.9	4.8
Thr	12.47	12	6.2	9.0	7.6	7.7
Glu	12.28	12	6.1	6.8	8.0	9.7
Arg	10.49	10	5.2	2.9	3.8	4.8
Ile	10.41	10	5.2	5.5	5.9	4.5
Tyr	8.21	8	4.1	5.8	5.1	5.8
Ala	6.33	6	3.1	3.4	5.0	5.8
Trp	6.13	6	3.1	1.7	2.0	1.8
Phe	6.25	6	3.1	3.0	1.7	2.2
Lys	4.05	4	2.1	2.8	2.9	3.6
1/2 Cys	4.15	4	2.1	2.9	3.0	0.9
Pro	3.73	4	2.1	3.7	3.7	4.3
Met	3.25	3	1.5	1.7	1.8	1.9
His	1.91	2	1.0	0.9	0.9	1.3
M_r of						
SDS-PAGE (kDa)			10.0	13.0	13.0	14.0
Gel filtration (kDa)			20.0	50.0	25.0	50.0
Mol. structure			Dimer	Tetramer	Dimer	Tetramer

[a] Data are from Van Damme *et al.* [5].

Man α(1-3)Man had a significantly higher inhibitory activity than Man α(1-2)Man, Man α(1-4)Man or Man α(1-6)Man, and 14-times higher than D-mannose. Man α(1-3)Man-α-*O*-Me was a good inhibitor, being approximately two-fold better than Man α(1-3)Man. Man α(1-3)Man-α-*O*-Me also was a somewhat more potent inhibitor than the branched mannotriose, Man α(1-6)[Man α(1-3)]Man. The branching triantennary mannopentaose, [Man α(1-3)]{Man α(1-6)[Man α(1-3)]Man α(1-6)}Man, was the best inhibitor; it had a more than 300-fold greater inhibitory potency than D-mannose.

Amino acid sequencing analysis of SLA

Purification and sequencing of the peptides obtained on digestion of SLA with endoproteinase Asp-N or Lys-C provided enough overlapping sequences to obtain the partial amino acid sequence of SLA. As shown in Figure 5, the N-terminal 46 amino acid sequence of SLA, compared with that of GNA (*Galanthus nivalis* bulb lectin), showed 76% homology.

Discussion

Bulbs of *Sternbergia lutea* contain a lectin which can easily be isolated by affinity chromatography on immobilized mannose. It is somewhat different from other mannose-specific lectins from the same Amaryllidaceae family, in that a 1 M ammonium sulfate solution was not required to enhance the binding of the lectin to the affinity column. The SLA lectin is a dimer composed of two identical subunits of M_r 10 000 from SDS-PAGE (Figure 1) and gel filtration assay. In the gel filtration studies, we used GNA, a similar mannose-binding lectin, to calibrate the Sephadex G-75 column, which gave a M_r 50 000 for GNA (data not shown). The SLA lectin is a pure protein being devoid of carbohydrate.

Recently we described the carbohydrate-binding specificity of six other mannose-specific lectins isolated from the family Amaryllidaceae; GNA (*Galanthus nivalis* lectin) [1, 2], NPA (*Narcissus pseudonarcissus* lectin) and HHA (*Hippeastrum hybr*. lectin) [3, 4], Alliaceae: ASA (*Allium sativum* lectin), AUA (*Allium ursinum* lectin) [6] and AAA (*Allium ascalonicum* lectin) [7], and Orchidaceae; LOA (*Listera ovata* lectin) [8]. In the present paper we have examined the carbohydrate-binding specificity of the lectin isolated from bulbs of the *Sternbergia lutea*, one additional member of α-mannose-binding lectin from the family Amaryllidaceae. All these lectins are highly specific for α-D-mannose. *S. lutea* lectin (SLA) reacted strongly with several yeast

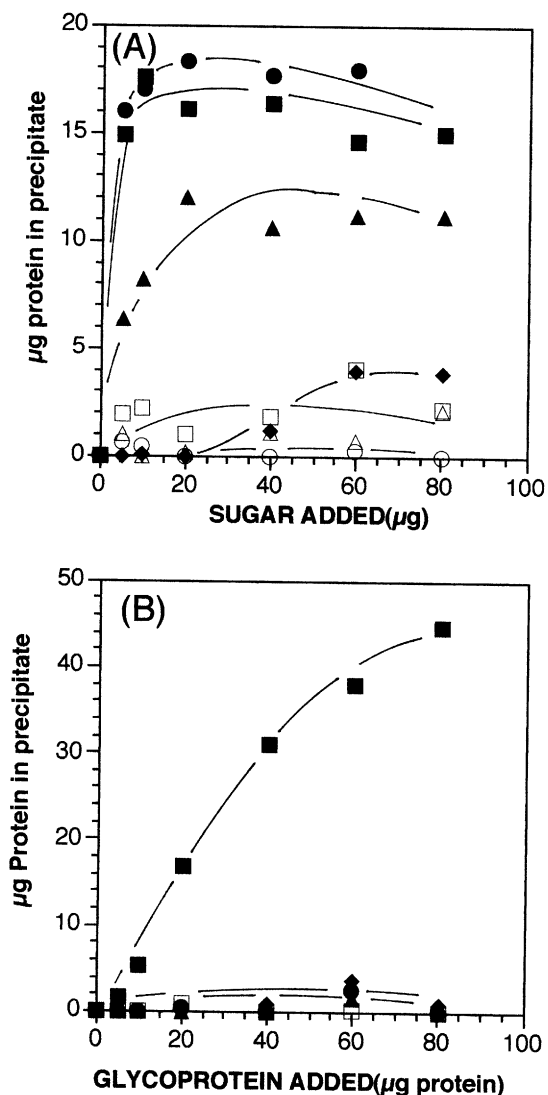

Figure 2. Quantitative precipitation curves of mannans, galactomannans and glycoproteins. Varying amount of mannan, galactomannan and glycoprotein were allowed to react with 20 μg of SLA. The amount of protein precipitated in each tube was determined by the method of Lowry *et al.* [14]. (A) ■, *S. cerevisiae* mannan; ●, *C. tropicalis* mannan; ▲, *T. magnolia* galactomannan; ◆, *P. frequantans* galactomannan; □, *P. charlessii* galactomannan; ○, glycogen (rabbit liver); △, dextran B-1355-S; (B) ■, asialothyroglobulin; ●, ovalbumin; ▲, fetuin; ◆, thyroglobulin; □, asialofetuin.

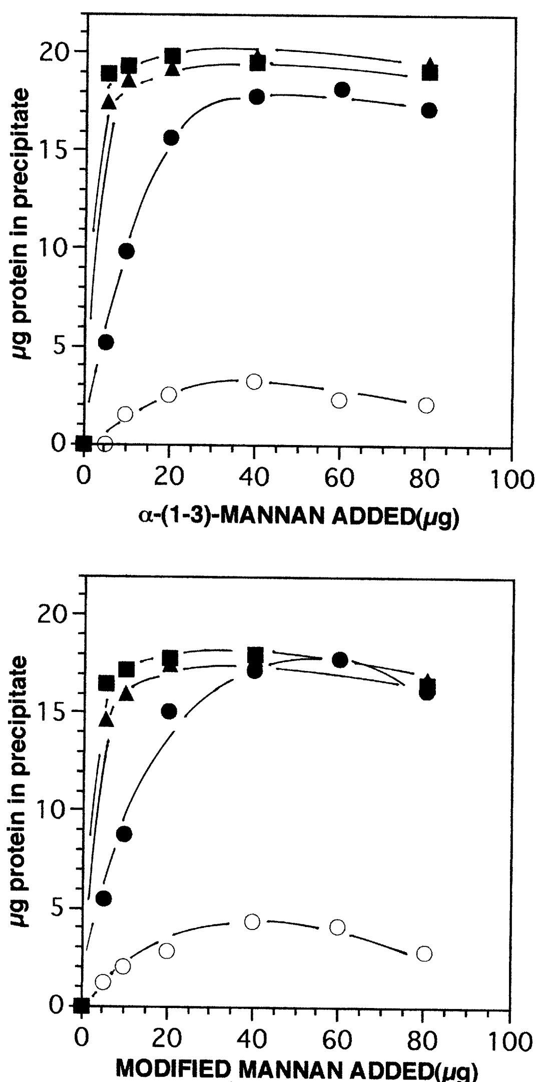

Figure 3. Quantitative precipitation curves of linear *α*(1-3)-mannan and periodate oxidized-reduced yeast mannan. Varying amount of mannan were allowed to react with 20 μg of SLA, NPA, HHA and LOA. The amount of protein precipitated in each tube was determined by the method of Lowry *et al.* [14]. ○, SLA (*Sternbergia lutia* lectin); ▲, NPA (*Narcissus pseudonarcissus* lectin); ■, HHA (*Hippeastrum hybr.* lectin); ●, LOA (*Listera ovata* lectin).

mannans (Figure 2), but only very slightly with either linear α(1-3)-mannans (DP 8–15) or periodate-oxidized and $NaBH_4$-reduced yeast mannan (Figure 3), suggesting that this lectin recognizes terminal α-D-mannosyl residues rather than exclusively internal residues. It appears to interact strongly with clustered terminal α(1-3)-linked mannosyl residues of yeast mannans. The lectin exhibits a marked preference for α(1-3)-linked mannobiose over α(1-6)-linked and α(1-2)-linked mannobiosyl units. Although, SLA recognized the branched mannotrisaccharide Man α(1-6)[Man α(1-3)]Man, the mannotriose core present in all N-linked glycans, it reacted with only one kind of N-linked asialoglycoprotein, namely asialothyroglobulin. It did not give a precipitate with thyroglobulin, ovalbumin, fetuin and asialofetuin, which contain a large number and variety of high mannose and hybrid-type glycosyl moieties. This could perhaps be due to the presence of heterogeneous sugar chains or the location of the α(1-3)-mannosyl sequences in the molecule.

The lectins derived from the bulbs of monocotyledonous plants can readily distinguish D-mannose from D-glucose and its C-3 and C-4 epimers (D-altrose and D-talose), even at

Table 2. Inhibition by various sugars of seven mannose-specific lectin precipitation systems.

Sugar	*50% inhibition (mM)*	*Inhibitory potency**							
		SLA	GNA[a]	NPA[b]	HHA[b]	ASA[c]	AUA[c]	AAA[d]	LOA[e]
D-mannose	96	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Me α-D-mannoside	29	3.3	1.6	1.2	1.5	1.5	1.2	2.9	2.0
Me β-D-mannoside	340	0.3	0.3	0.2	0.5	≪ 0.4	0.2	1.7	0.4
Bn α-D-mannoside	28	3.4							
PNP α-D-mannoside	21.3% at 12 mM								
αMan(1-2) Man	14.6	6.6	2.1	3.3	3.2	< 1.4	< 3.6		3.5
αMan(1-3) Man	6.8	14.1	12.1	2.8	5.9	11.5	< 7.2		5.2
αMan(1-6) Man	14.4	6.7							4.4
αMan(1-2) Man-(α-*O*)-Me	9.6	10.0	3.2	3.3	3.2			3.2	
αMan(1-3) Man-(α-*O*)-Me	3.1	40.0	14.2	3.1	10.5	11.9	10.0	20.0	25.9
αMan(1-4) Man-(α-*O*)-Me	19.6% at 20 mM		1.9	< 0.7	< 0.2	< 1.4	≪ 3.2		
αMan(1-6) Man-(α-*O*)-Me	4.0	24.0	4.3	5.1	8.3	≪ 1.4	5.1	10.7	
αMan(1-6)\ Man / αMan(1-3)	3.3	29.1	28.3	3.8	13.8	≪ 3.6	11.3	50–60	14.5
αMan(1-6)\ αMan(1-6) / αMan(1-3) \ Man / αMan(1-3)	0.31	309.7							

[a] These data are from Shibuya *et al.* [2] using GNA-*H. capsulata* mannan precipitation system.
[b] These data are from Kaku *et al.* [4] using NPA-, HHA-*Pichia pastoris* yeast mannan precipitation system.
[c] These data are from Kaku *et al.* [6] using ASA-, AUA-*S. cerevisiae* mannan precipitation system.
[d] These data are from Mo *et al.* [7] using AAA-asialofetuin precipitation system.
[e] These data are from Saito *et al.* [8] using LOA-*C. tropicalis* mannan precipitation system.
The relative inhibition potency normalized to D-mannose = 1.0.

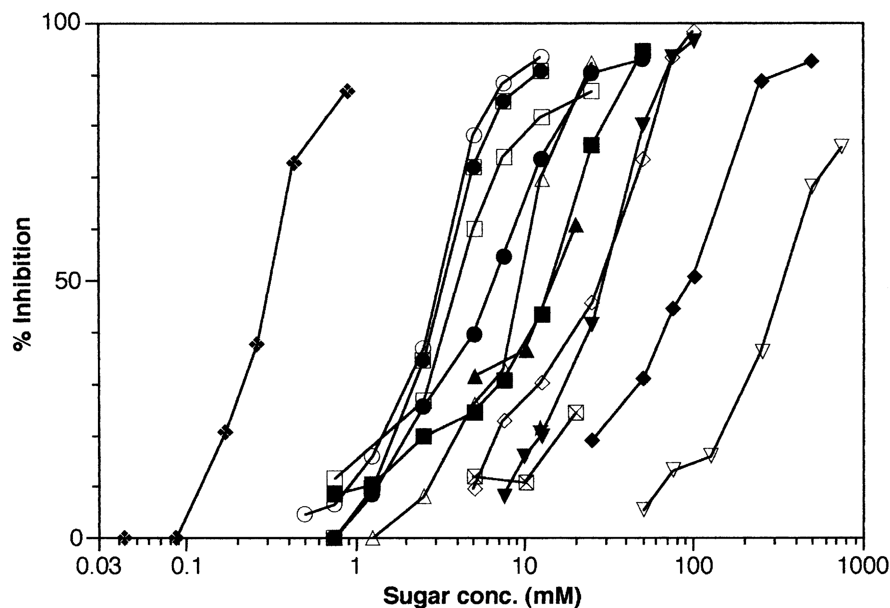

Figure 4. Inhibition of SLA-asialothyroglobulin precipitation system by various hapten saccharides. ◆, D-mannose; ▼, Me α-D-mannoside; ▽, Me β-D-mannoside; ◇, Bn α-D-mannoside; ▲, Man α(1-2) Man; ●, Man α(1-3) Man; ■, Man α(1-6) Man; △, Man α(1-2) Man α-*O*-Me; ○, Man α(1-3) Man α-*O*-Me; ■, Man α(1-4) Man α-*O*-Me; □, Man α(1-6) Man α-*O*-Me; ▣, Man α(1-6)[Man α(1-3)] Man; ❖, [Man α(1-3)] {Man α(1-6)[Man α(1-3)] Man α(1-6)}Man.

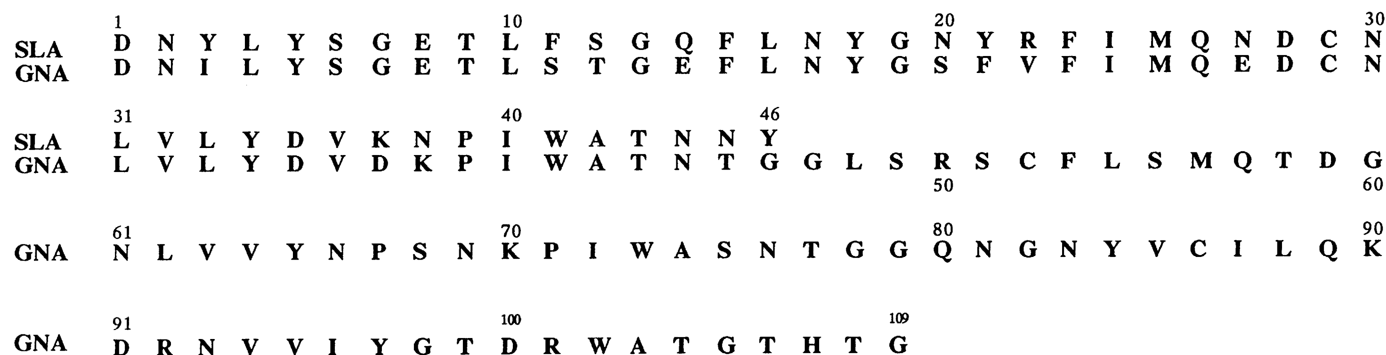

Figure 5. Comparison of composition of the N-terminal amino acid sequence between two Amaryllidaceae bulb lectins, SLA and GNA [17].

1000 mM concentrations of these sugars. The modification of the C-2 hydroxyl groups of mannose, 2-deoxy-methyl α-arabinohexoside, abolished or strongly diminished its capacity to occupy the carbohydrate-binding site of the SLA lectin. These results also indicate that the sugar binding specificity of these lectins is quite different from the previously described mannose/glucose binding lectins, *eg* Con A, lentil, pea and fava bean lectin [9]. Accordingly, the C-2 axial hydroxyl group of mannose probably plays a more important role for interaction with these mannose binding lectins than for the mannose/glucose binding lectins [8].

As indicated in Table II, a comparison of the sugar binding specificities of the seven D-mannose-binding lectins on the basis of sugar hapten inhibition of SLA and asialothyroglobulin precipitation shows that Me α-D-mannoside is 3.3 times better an inhibitor than D-mannose for SLA, but only 1.2–1.6 times more active than D-mannose for GNA, NPA, HHA, ASA and AUA. Man α(1-3)Man is 2.1 times more potent than α(1-2) and α(1-6)Man_2, and methyl α-Man α(1-3)Man is 1.7–4 times more active than Me-α(1-2)Man_2 and Me-α(1-6)Man_2 for SLA. These results strongly suggest that SLA recognizes α-linked manno oligosaccharides, in the order, α(1-3) > α(1-6) > α(1-2) > α(1-4) linkages, the same as for other mannose-binding lectins except for NPA. Interestingly, methyl α-Man α(1-3)Man is approximately three times more inhibitory than Man α(1-3)Man for SLA, and 1.4 times more active than Man α(1-6)[Man α(1-3)]Man. The branched mannotriose Man α(1-6)[Man α(1-3)]Man was the most potent inhibitor for the six other Amaryllidaceae lectins, unlike for the *S. lutea* lectin.

The results of hapten inhibition experiments indicated that the α(1-3)-mannobiose unit is most complementary to the carbohydrate binding site(s) of GNA, and the nonreducing terminal mannosyl residue is of special importance for this interaction. Perhaps, SLA has an extended binding site for α(1-3)-mannotriose, but, it does not appear to exhibit a preference for internal mannosyl residues such as NPA and HHA. This lectin may recognize longer α(1-3)-linked mannosyl units than GNA, although unlike NPA and HHA, nonreducing terminal mannosyl residues are necessary for interaction with SLA. This hypothesis is supported by the finding that the triantennary mannopentaose which has three nonreducing terminal mannosyl residues is the best inhibitor of SLA.

It has already been noted that the bulb lectins from Amaryllidaceae are related serologically and exhibit high affinity for α-D-mannose. Upon SDS-PAGE, under both reducing and nonreducing conditions, these two mannose-binding lectins, SLA and GNA, yield only a single low molecular weight polypeptide band (approximately 10 and 12.5 kDa, respectively), indicating that they are composed of a single type of low molecular weight subunit and do not contain interchain disulfide bonds. Furthermore, these lectins have very similar amino acid compositions (Table 1), featuring high contents of aspartic acid, glycine, leucine and serine [5, 18]. These lectins are similar, not only in amino acid composition, but also in the N-terminal amino acid sequence of the 46 amino acids [19]. As presented in Figure 5, there is 76% homology between the N-terminal sequences of these two mannose-binding lectins.

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