Garlic (Allium sativum) Lectins Bind to High Mannose Oligosaccharide Chains*

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Two mannose-binding lectins, Allium sativum agglutinin (ASA) I (25 kDa) and ASAIII (48 kDa), from garlic bulbs have been purified by affinity chromatography followed by gel filtration. The subunit structures of these lectins are different, but they display similar sugar specificities. Both ASA I and ASAIII are made up of 12.5- and 11.5-kDa subunits. In addition, a complex (136 kDa) comprising a polypeptide chain of 54 ± 2 kDa and the subunits of ASA I and ASAIII elutes earlier than these lectins on gel filtration. The 54-kDa subunit is proven to be alliinase, which is known to form a complex with garlic lectins. Constituent subunits of ASA I and ASAIII exhibit the same sequence at their amino termini. ASA I and ASAIII recognize monosaccharides in mannosyl configuration. The potencies of the ligands for ASAs increase in the following order: mannobiose (Manα1–3Man) < mannotriose (Manα1–6Manα1–3Man) ~ mannopentaose < Manα3-oligosaccharides. The addition of two GlcNAc residues at the reducing end of mannotriose or mannopentaose enhances their potencies significantly, whereas substitution of both α1–3- and α1–6-mannosyl residues of mannotriose with GlcNAc at the nonreducing end increases their activity only marginally. The best manno-oligosaccharide ligand is Manα1–2,3GlcNAcAsn, which bears several α1–2-linked mannose residues. Interaction with glycoproteins suggests that these lectins recognize internal mannose as well as bind to the core pentasaccharide of N-linked glycans even when it is sialylated. The strongest inhibitors are the high mannose-containing glycoproteins, which carry larger glycan chains. Indeed, invertase, which contains 85% of its mannose residues in species larger than Manα2GlcNAc, exhibited the highest binding affinity. No other mannose- or mannose/glucose-binding lectin has been shown to display such a specificity.

The majority of the well characterized plant lectins have been isolated from the seeds of dicotyledonous species. But lectins of non-seed origin from other species are also emerging as promising tools chiefly because of two reasons: (i) a good number of them might contain novel sugar-binding sites; and (ii) they can provide valuable information regarding the biological roles of plant lectins, which to a large extent still remain elusive. In the recent past, there have been several reports of non-seed lectins from monocotyledonous families (1–3), especially Amaryllidaceae. The most remarkable property of these lectins is that they show strict specificity for mannose (2, 4, 5), unlike other mannose/glucose-binding plant lectins. Hence, they are being used extensively as affinity ligands for the purification of glycoproteins, viz. IgM, α2-macroglobulin, haptoglobin, and β-lipoprotein (3, 6).

Van Damme et al. (3) examined a number of species (including Allium sativum) from the family Alliaceae (which is taxonomically close to the family Amaryllidaceae) and found them to accumulate mannose-binding lectins. They observed that lectins from both families share many common properties like their state of oligomerization, sugar specificity, amino acid composition, and serological interaction. We note that the bulbs of the species A. sativum contain an additional lectin (other than the one(s) described by them) that differs in its quaternary structure.

We found that the garlic lectins bind most avidly to invertase (which contains high mannose residues) among the glycoproteins tested. By exploiting this property, we developed a simple method to study their sugar specificities. This study reveals that the binding sites of these lectins accommodate a number of α1–2-linked mannose residues. None of the other mannose-binding lectins have been shown to exhibit this kind of specificity.

MATERIALS AND METHODS

Sugars and Glycoproteins—Methyl-a-mannosamine, mannosamine, N-acetylmannosamine, α- and β-methylumbelliferyl mannose, glucose, methyl-α-glucosamine, N-acetylglucosamine, galactose, N-acetylgalactosamine, lactose, melibiose, maltose, altrose, talose, and Manα1–3Man were purchased from Sigma. Manα1–3Man and Manα1–3Man were obtained from Carbohydrate International. Manα1–6Man, Manα1–3(Manα1–6Man)6Man, mannotriose, mannopentaose, Manβ1–4GlcNAc, GlcNAc2–Man, and N-acetyllactosamine were purchased from Dextra Laboratories (London). Manα,GlcNAc, Manα,GlcNAc, and Manα,GlcNAc were obtained from Biocarb (Lund, Sweden). Manα3GlcNAcAsn was a gift from Dr. M. I. Khan (National Chemical Laboratory, Pune, India). Manα,GlcNAcAsn and Manα,GlcNAcAsn prepared from quail ovalbumin were available in the laboratory from a previous study (7). Invertase, fetuin, transferrin, IgGs, fibrinogen, α2-acid glycoprotein, and ovalbumin were products of Sigma. Soybean agglutinin and jacalin (Artocarpus integrifolia agglutinin) were prepared in the laboratory according to Sundberg and Porath (10).

Preparation of Mannose-Sepharose Affinity Matrix—Mannose was coupled to epichlorohydrin-activated Sepharose 6B following the procedure of Sundberg and Porath (10).

Purification of A. sativum Agglutinins (ASAs)1—Healthy dry bulbs of Allium sativum were purchased from the local market. The bulbs were homogenized with a blender using 20 mM phosphate buffer (pH 7.4) containing 150 mM NaCl (PBS). The extract was filtered and centrifuged at 10,000 rpm. The supernatant was subjected to (NH4)2SO4 cut (8) and Sureshkumar et al. (9), respectively. Glucose oxidase was purchased from Boehringer Mannheim. All other chemicals used were of the highest purity available.

Preparation of Mannose-Sepharose Affinity Matrix—Mannose was coupled to epichlorohydrin-activated Sepharose 6B following the procedure of Sundberg and Porath (10).

1 The abbreviations used are: ASAs, A. sativum agglutinins; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; GNA, Galanthus nivalis agglutinin; NPA, Narcissus pseudonarcissus agglutinin; HHA, Hippeastrum hybr. agglutinin; ConA, concanavalin A.

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GARLIC BULB LECTIN BINDING TO HIGH MANNOSE OLIGOSACCHARIDES

**Scheme 1.** Enzyme adsorption assay for the binding of sugars to the garlic lectins. **OPD**, orthophenylenediamine; **HRP**, horseradish peroxidase.

(70%), and centrifuged, and the protein pellet was resuspended in PBS and dialyzed extensively against PBS. The crude protein was loaded on the mannose-Sepharose column at 4 °C. The column was then washed extensively at the same temperature with PBS until A280 nm was below 0.005. The bound protein was eluted with 0.2 M mannose in PBS at room temperature.

The affinity-purified protein was dialyzed extensively initially against 20 mM PBS and finally against 50 mM PBS and concentrated using a Centricon filtration unit. The concentrated protein was then applied to a Bio-Gel P-200 column (1.8 x 110 cm) equilibrated and eluted with 50 mM PBS.

**Electrophoretic Procedures—**SDS-polyacrylamide gel electrophoresis under reducing conditions was carried out as described by Laemmli (11). Molecular masses of the lectins were calculated according to the method of Weber and Osborn (12) using bovine serum albumin (68 kDa) as the standards. The proteins were visualized on the gel by Coomassie staining. A PAGE system 2100 (Beckman Instruments) was used for capillary electrophoresis with PAGE system software controlled by an IBM PS/2 Model 50-Hz computer. Post-run data analysis was performed on System Gold software (Beckman Instruments). A standard capillary of 27 cm length (20 cm to the detector window) x 20 μm inner diameter, designed for PAGE cartridges, was obtained from Beckman Instruments. On-line detection was carried out at 280 nm with a 50 x 200-μm aperture in the PAGE cartridge. Temperature of the capillary during electrophoresis was maintained at 25 °C. Samples were introduced by pressure injection for 5 s. Electrophoresis was performed at a constant voltage of 8 kV.

**Determination of the Amino-terminal Sequence—**Proteins after separation by 15% SDS-PAGE were electroblotted onto polyvinylidene difluoride membrane following the procedure of Matsudaira (13) using bovine serum albumin as the standard. The native protein was carried out on a Shimadzu automated gas-phase sequencer (Model PSQ-1) equipped with an on-line C-RAIA20A Chromatopac Shimadzu phenylthiohydantoin analyzer.

**Determination of Native Molecular Mass—**The native molecular masses of ASAs were determined by gel filtration on a Bio-Gel P-200 column (1.8 x 110 cm) calibrated with rabbit IgG, Vicia villosa agglutinin, soybean agglutinin, bovine serum albumin, hen egg ovalbumin, and pepsin. Void and inner volumes of the column were determined with blue dextran and myoglobin, respectively. Molecular masses of ASAI and ASAIII were also determined on a Bio-Gel P-60 column (8 x 90 cm) calibrated with hen egg ovalbumin, chymotrypsinogen, myoglobin, and bovine pancreas ribonuclease A.

**Protein Estimation—**Protein concentration was determined following the method of Lowry et al. (14) using bovine serum albumin as the standard.

**Sugar Assay—**Total neutral sugar content was determined by the phenol-sulfuric acid method of Dubois et al. (15) using mannose as the standard.

**Hemagglutination Assay—**Hemagglutination was carried out at room temperature using rabbit and human erythrocytes (16). Hemagglutination inhibition tests were done by preincubating lectin (10 hemagglutinating units) with serially diluted sugars or glycoproteins in microtiter plates. Rabbit erythrocyte suspension (25 μl of 4% (w/v)) was added to the solution, and the results were noted after 1 h.

**Estimation of the Activity of Invertase Bound to ASAs—**An appropriate amount of lectin was coated on an enzyme-linked immunosorbent assay plate and left overnight at 4 °C. The plate was washed with 20 mM PBS and then blocked with 3% bovine serum albumin PBS. After washing with the blocking buffer, invertase was added to the wells and incubated for 1 h. The plate was then washed with PBS, and 100 μl of 50 mM sucrose solution was added to each well. After incubation for 1 h, the solutions were transferred to separate test tubes. The extent of hydrolysis of sucrose was assayed by estimating the free glucose according to the method of Nelson (17).

**Enzyme-linked Lectin Absorbent Assay—**The sugar binding properties of ASAs were studied in detail using a modified enzyme-linked immunosorbent assay technique (Scheme 1). An optimum concentration of the lectin was coated on enzyme-linked immunosorbent assay plates and left overnight at 4 °C. After washing three times with 20 mM PBS, the wells were blocked with 3% bovine serum albumin for 1 h at room temperature. Subsequent to washing thrice with the blocking buffer, different sugars were added at varying concentrations and allowed to interact with the lectin for 1 h. A fixed concentration of invertase was added to each well and incubated for 30 min. The wells were then washed thrice with blocking buffer and once with PBS. An equal volume of 50 mM sucrose solution in 10 mM acetate buffer (pH 5.0) was added to each well. After incubation (for 1 h), glucose oxidase in the same buffer was then added to the solution to produce H2O2 from the liberated glucose. H2O2, thus generated, was assayed using horse-radish peroxidase and o-phenylenediamine (0.05 mg/ml in 100 mM citrate buffer). The reaction was stopped by 2 N HCl, and the absorbance was recorded on an enzyme-linked immunosorbent assay reader at 490 nm.

**RESULTS**

**Bulbs of A. sativum Contain a Group of Mannose-binding Lectins—**Earlier reports (3) have shown that dimeric proteins of 25 kDa occur in the bulbs of A. sativum. By using a modified purification procedure, we have identified an additional lectin designated as ASAII. The affinity-purification procedure revealed three peaks upon gel filtration on a Bio-Gel P-200 column with molecular masses of 136, 48, and 25 kDa, respectively (Fig. 1). Taken together, the data from SDS-PAGE (Fig. 2) and gel filtration show that peak 3 is a heterodimer of 12.5- and 11.5-kDa subunits, whereas peak 2 is most likely a heterotetramer made up of two pairs of 12.5- and 11.5-kDa polypeptide chains, although occurrence of these subunits in other proportions cannot be ruled out.
The SDS-PAGE profile (Fig. 2) of peak 1 indicates that it is composed of 54-, 12.5-, and 11.5-kDa subunits, whereas peaks 2 and 3 are made up of only 12.5- and 11.5-kDa subunits. All three peaks show distinct patterns of migration when subjected to free flow capillary electrophoresis (Fig. 3), suggesting that they are distinct proteins. Peak 1 contains 6% neutral sugar, whereas peaks 2 and 3 are devoid of neutral sugars. Sequence analysis of the individual bands of SDS-PAGE showed that the 54-kDa subunit has the amino-terminal sequence KMTWT-MKADEEA, which is different from the sequence of the 12.5- and 11.5-kDa subunits (RNILTNDEGLYAGQSLD), common to all three peaks. Van Damme et al. (18) reported the same amino-terminal sequence for the 12.5- and 11.5-kDa polypeptide chains (RNLLTNGEGLYAGQS), whereas Smeets et al. (19), from the deduced amino acid sequences of cDNA clones, showed slightly different amino-terminal sequences for the 12.5-kDa (RNLLTNGEGLYAGQS) and 11.5-kDa (RNILRNDEGLYAGQS) polypeptide chains. Altogether, these results show that the 54-kDa band corresponds to the enzyme alliinase (cysteine-sulfoxide lyase, alliin lyase, EC 4.4.1.4), which is known to form a complex with garlic lectins (20). Since the polypeptide chains corresponding to peaks 2 and 3 give the

FIG. 1. Separation of ASAs from alliinase by gel filtration. a, the concentrated affinity-purified lectin preparation was applied to a Bio-Gel P-200 column (1.8 × 110 cm) and eluted with 50 mM PBS. The Bio-Gel P-200 column was calibrated using rabbit IgG, which elutes at a molecular mass of 200 kDa on gel filtration (peak 1), V. villosa agglutinin (139 kDa; peak 2), soybean agglutinin (110 kDa; peak 3), bovine serum agglutinin (68 kDa; peak 4), hen egg ovalbumin (45 kDa; peak 5), and pepsin (34.7 kDa; peak 6). Myoglobin (17 kDa) was used for the determination of the inner volume of the column, and therefore, it was not used for constructing the calibration curve for estimating the molecular masses. b, shown is a calibration curve for the determination of the molecular masses of ASAI (peak 3) and ASAIII (peak 2) evaluated on a Bio-Gel P-60 column (2.8 × 90 cm) calibrated with hen egg ovalbumin (45 kDa; ○), chymotrypsinogen (25 kDa; △), myoglobin (17 kDa; ●) and bovine pancreas ribonuclease A (13.7 kDa; ▽).

FIG. 2. SDS-PAGE of garlic bulb lectins resolved on a gel filtration column. The conditions for SDS-PAGE (15%) are described under “Materials and Methods” (15% SDS-PAGE under reducing conditions). Lane M, molecular mass markers; lane A, peak 1; lane B, peak 2 (ASAIH); lane C, peak 3 (ASAI).

The SDS-PAGE profile (Fig. 2) of peak 1 indicates that it is composed of 54-, 12.5-, and 11.5-kDa subunits, whereas peaks 2 and 3 are made up of only 12.5- and 11.5-kDa subunits. All three peaks show distinct patterns of migration when subjected to free flow capillary electrophoresis (Fig. 3), suggesting that they are distinct proteins. Peak 1 contains 6% neutral sugar, whereas peaks 2 and 3 are devoid of neutral sugars. Sequence analysis of the individual bands of SDS-PAGE showed that the 54-kDa subunit has the amino-terminal sequence KMTWT-MKADEEA, which is different from the sequence of the 12.5- and 11.5-kDa subunits (RNILTNDEGLYAGQSLD), common to all three peaks. Van Damme et al. (18) reported the same amino-terminal sequence for the 12.5- and 11.5-kDa polypeptide chains (RNLLTNGEGLYAGQS), whereas Smeets et al. (19), from the deduced amino acid sequences of cDNA clones, showed slightly different amino-terminal sequences for the 12.5-kDa (RNLLTNGEGLYAGQS) and 11.5-kDa (RNILRNDEGLYAGQS) polypeptide chains. Altogether, these results show that the 54-kDa band corresponds to the enzyme alliinase (cysteine-sulfoxide lyase, alliin lyase, EC 4.4.1.4), which is known to form a complex with garlic lectins (20). Since the polypeptide chains corresponding to peaks 2 and 3 give the
same sequence (RNILTNDGGLYAGQSLD), the lectins ASAI and ASAIII are made up of identical polypeptide chains that apparently differ at their carboxyl termini.

Hemagglutination Properties—The agglutinins ASAI and ASAIII interact strongly with rabbit erythrocytes, but considerably weakly with human erythrocytes, irrespective of their blood groupings.

ASAs Bind Most Avidly to Invertase—Hemagglutination inhibition studies of garlic agglutinins were carried out using a series of simple sugars and several glycoproteins. Among the monosaccharides tested, only methyl-α-D-mannopyranoside was found to be inhibitory besides mannose, although the latter was less potent as an inhibitor (Table I). Glucose, a C-2 epimer of mannose, was inactive. Methyl-α-D-glucopyranoside also did not interact. Replacement of the C-2 hydroxyl group of glucose with other groups did not alter its inhibitory property as both N-acetyl-D-mannosamine and glucosamine were inactive. Of all the glycoproteins used in this assay, invertase was the strongest inhibitor, and the minimum amount of this enzyme needed for complete inhibition was 0.7 nM (Table I). The invertase bound to both ASAI and ASAIII was found to retain its catalytic activity.

The strong affinity of these lectins for invertase, containing high mannose-type oligosaccharides, was instrumental in designing a sensitive enzyme-based assay to study the interaction of these lectins with various sugars (as shown in Scheme 1) and glycoproteins. The binding of invertase to varying amounts of garlic agglutinins coated on the wells of microtiter plates was checked (Fig. 4a). In other set of experiments, the amounts of horseradish peroxidase and glucose oxidase were varied, respectively, keeping the amounts of other ingredients fixed (Fig. 4, b and c). Based on these studies, the following concentrations were considered optimal for all subsequent sugar inhibition studies: 0.35 μg of lectin, 20 ng of invertase, and 3 units of glucose oxidase for ASAI and 0.2 μg of lectin, 20 ng of invertase, and 3 units of glucose oxidase for ASAIII. The amount of horseradish peroxidase was 10 ng and the concentration of sucrose was 50 mM in all experiments.

Inhibition of ASA-Invertase Binding by Mono- and Disaccharides—Sugar inhibition assays were carried out in triplicate, and each value is an average of three experiments. The amount of sugar required for 50% inhibition was calculated from complete inhibition curves (Fig. 5), and values are listed in Tables I–III. Relative affinities of the lectins for different sugars were determined from the concentration of sugars required for 50% inhibition of the binding of invertase. Consistent with hemagglutination inhibition studies, methyl-α-D-mannopyranoside was better as an inhibitor than mannose (Tables I and II). The presence of nonpolar p-nitrophenyl aglycon in β-mannose did not improve the binding affinity, but the introduction of a nonpolar 4-methylumbelliferyl aglycon at the anomeric position in α-linkage slightly enhanced its inhibitory potency. The lectins did not bind to 4-methylumbelliferyl-β-mannopyrano-side, indicating that β-linked mannose was not conducive for binding. N-Acetyl-D-mannosamine was inactive, suggesting that the axially oriented hydroxyl group of mannose cannot be substituted with a bulky acetamido group. The monosaccharide binding propensities of ASAI and ASAIII are broadly similar to other well studied mannose-binding lectins from snowdrop (4) and daffodil and amaryllis (21). Of all the mannobioses tested, Manα1–3Man was the most potent inhibitor. Its potency was 12 times greater than that of mannose. Manα1–2Man and Manα1–6Man were almost eight and six times more active, respectively, over mannose. But the other mannobioses, including Manα1–4Man, were poor ligands. Among the other mannose-binding lectins, GNA recognizes only terminal Manα1–3Man, whereas NPA (daffodil) and HHA (amaryllis) prefer α1–6-linked mannose. On the other hand, ConA is known to

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<tr>
<th>Sugar/glycoprotein</th>
<th>Conc for 50% inhibition</th>
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<tr>
<td>Mannose</td>
<td>150 mM</td>
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<tr>
<td>α-Methylmannopyranoside</td>
<td>12 mM</td>
</tr>
<tr>
<td>Glucose</td>
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<tr>
<td>α-Methylglucopyranoside</td>
<td>Not inhibitory up to 200 mM</td>
</tr>
<tr>
<td>Mannobiose (Manα1–3Man)</td>
<td>14 mM</td>
</tr>
<tr>
<td>Mannotriose (Manα1–4(Manα1–3Man))</td>
<td>9 mM</td>
</tr>
<tr>
<td>Invertase</td>
<td>0.70 nM</td>
</tr>
<tr>
<td>Horseradish peroxidase</td>
<td>210 nm</td>
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</table>

Table I

Inhibition of Hemagglutination

Data reported are for ASAIII. ASAI gives quantitatively and qualitatively a similar pattern.

FIG. 4. Optimization of the components of the enzyme-based lectin absorbent assay. a, except for the lectin, all other parameters, viz. 20 ng of invertase, 3 units of glucose oxidase, 10 ng of horseradish peroxidase, and 50 mM sucrose, were constant. In b and c, the concentrations of horseradish peroxidase and glucose oxidase were varied, whereas the same amounts of invertase, sucrose, and lectin (0.35 μg of ASAI/0.2 μg of ASAIII) were used. The profiles presented in a–c were produced by ASAIII. ASAI also showed identical patterns. The experimental protocol is described under “Material and Methods.”
ability of several mannose-containing glycoproteins to inhibit and ASAIII bear similarity to ConA, which displays equal remarkable change in activity was noted. In this regard, ASAI chose panel of manno-oligosaccharides was then undertaken for the cluster of residues at the nonreducing end are highly preferred by the interactions of different sugars/glycoproteins for 1–2 h before the addition of invertase. The curves presented here were obtained for ASAI, III. ASAI also showed similar profiles. Other details of the assay are described under “Materials and Methods” and in the legend to Fig. 4. M, mannose; Gn, GlcNAc, SBA, soybean agglutinin.

exhibit greater affinity for Manα1–2Man (22). Artocarpin (A. integrifolia) mannose-binding lectin shows higher specificity for Manα1–3Man (7). The β-linked disaccharides like Manβ1–4GlcNAc and GlcNAcβ1–2Man were poor inhibitors, whereas Manβ1–6GlcNAc and GlcNAcβ1–6Man were inactive toward ASAs. The lectins did not interact with disaccharides like lactose (Galβ1–4Glc), N-acetyllactosamine (Galβ1–4GlcNAc), and melibiose (Galα1–6Glc).

Binding Specificities of ASAI and ASAIII for Manno-oligosaccharides—To understand the carbohydrate specificities of ASAI and ASAIII in greater detail, their binding to a carefully chosen panel of manno-oligosaccharides was then undertaken (Table II). The core structure of N-linked oligosaccharides, Manα1–3(Manα1–6)Man, was 30 times stronger an inhibitor than mannose and showed four, two, and five times more potency than Manα1–2Man, Manα1–3Man, and Manα1–6Man, respectively. The relative inhibitory potencies of mannopentaose and mannotriose were identical. Manβ,GlcNAc and Manα1–3Manβ1–4GlcNAc were marginally better ligands than mannopentaose and mannotriose, respectively, unlike artocarpin, in which the addition of GlcNAc at the reducing end of Manα1–3Man caused a dramatic enhancement of its binding ability (7).

When the α1–3- and α1–6-linked mannose residues of mannotriose were substituted with GlcNAc as in GlcNAc2Manα2, no remarkable change in activity was noted. In this regard, ASAI and ASAIII bear similarity to ConA, which displays equal affinities for mannotriose and GlcNAc2Manα2 (23–25), but for artocarpin, the inhibitory activity is reduced by 25-fold (7). Manβ,GlcNAc2 has a 5-fold higher potency relative to mannotriose. Among the manno-oligosaccharides tested, Manβ,GlcNAc2-Asn is the best ligand, followed by Manα,GlcNAc2Asn and Manα,GlcNAc2Asn, indicating that the α1–2-linked mannosyl residues at the nonreducing end are highly preferred by the binding sites of these lectins. The preference of ASAI and ASAIII for the cluster of α1–2-linked mannosyl residues as in Manα,GlcNAc2Asn is unique among the lectins described so far.

Interaction of ASAI and ASAIII with Glycoproteins—The ability of several mannose-containing glycoproteins to inhibit the binding of ASAI or ASAIII to invertase was checked to confirm their specificities. The extent of binding of these glycoproteins was qualitatively consistent with the relative affinities exhibited by manno-oligosaccharides.

The soybean lectin and ovalbumin, which bear several terminal Manα1–2Man linkages in their high mannose-containing glycan chains, emerged as the best inhibitors (26). It has been reported that the sole glycan chain of the ovalbumin molecule is occupied by high mannose- or hybrid-type chains (27–29); as expected, it displayed a fairly good binding activity, unlike GNA, which did not interact with ovalbumin. Some of the serum glycoproteins, viz. α1-acid glycoprotein, fetuin, transferrin, and fibrinogen, were active, although the binding affinities were substantially weaker than that of soybean agglutinin. Binding of these glycoproteins in their native structure suggests that the lectins, like NPA and HHA, could recognize internal α-mannosyl residues. Alternatively, it can be said that these lectins can bind to the core pentasaccharide of N-linked glycans (Manα1–3(Manα1–6)Manβ1–4GlcNAcβ1–4GlcNac) even when both of the α1–3- and α1–6-mannosyl residues are substituted with NeuAcα2–3/Galβ1–4GlcNAc. Removal of the terminal sialic acids improved the potency of these glycoproteins. The affinities of ASAI and ASAIII for ja- calin and sheep IgG, which are substituted at their C-2 and C-4 hydroxyl groups of β-linked mannose by β-linked xylose and GlcNAc, respectively, are much weaker as compared with that of artocarpin. These results suggest that substitution with xylose in β1–2-linkage as in horseradish peroxidase (and jaca- lin) or bisection with GlcNAc in β1–4-linkage as in sheep IgG reduces their binding potencies for ASAI and ASAIII. Analyses of binding of saccharides derived from horseradish peroxidase indicate that the substitution of the β-linked mannose with xylose in β1–2-linkage compromises the binding potency to a greater extent than the substitution of reducing end GlcNAc with fucose in α1–3-linkage. Invertase carries Manα2–2,GlcNAc in its seven accessible glycan chains (30). As a result, this glycoprotein binds to ASAI and ASAIII with such an avidity that its potency surpasses the activity of all the glycoproteins tested.

**DISCUSSION**

The high mannose-binding lectins (ASAI and ASAIII) from garlic bulbs were purified in two steps using affinity chromatography and gel filtration. Van Damme et al. (3) reported a mannose-binding lectin that resembles ASAI in its subunit composition. However, it differs from ASAI reported by them in displaying the same amino-terminal sequence for both of the polypeptide chains. ASAI and ASAIII are the heterodimer and heterotetramer, respectively, of similar polypeptide chains, present in equimolar proportion, that vary slightly in their molecular masses, viz. 12.5 and 11.5 kDa, yet the N-terminal sequences of their large (12.5 kDa) and smaller (11.5 kDa) subunits are identical. Despite the dissimilarities in their subunit compositions, the contours of the carbohydrate-binding sites of ASAI and ASAIII are identical. The biosynthetic and functional significance of the occurrence of lectins that differ in their subunit composition but display similar binding propensities within the same tissue is not presently understood. Our failure to isolate ASAII as a homodimer of 12-kDa subunits is perhaps related to differences in the purification methods used by us and by Van Damme et al. (3). It is also possible that our isolation procedure permits the purification of isolecitins that have high propensities for interaction with alliinase (20). To

2 Ovalbumin as a glycoprotein is highly heterogeneous, and individual molecules in a population exhibit either high mannose chains that vary in the extent of mannosylation or hybrid-type chains.
ascertain their biological function and to develop them as potential tools for research, a knowledge of the carbohydrate specificities of these lectins becomes imperative. The sugar specificities of ASAI and ASAIII were elucidated in two steps: hemagglutination inhibition and a coupled enzyme-based assay. The hemagglutination inhibition study confirmed its exclusive specificity for D-mannose, like three other Amaryllidaceae lectins (GNA, NPA, and HHA) that do not recognize D-glucose (4, 17). Our studies also show that these lectins display extraordinary avidity for invertase as compared with other glycoproteins tested. Based on this finding, a sensitive enzyme-based assay system was designed to investigate their detailed binding specificities.

The subunit molecular mass of one of the lectins isolated by Gupta and Sandhu (31) is similar to that of the larger subunit of peak 1 on the gel filtration column. However, unlike the allinase-ASA complex or ASAI and ASAIII, the former is not retained on a mannose-Sepharose matrix. In the absence of the N-terminal sequence and comprehensive sugar binding properties of the high molecular mass lectin reported in Ref. 31, it is not possible to conclude that it is derived from another isolectin rather than being a glycoprotein contaminant, viz., allinase. Garlic plant contains at least five different lectins and lectin genes (32). The processing and post-translational modifications of the primary translation products of monocot mannose-binding lectins are rather complex as evident from the report of several lectins with different molecular masses (33).

### Table II

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<th>Oligosaccharide</th>
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![Table II](http://www.jbc.org/)

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<th>Oligosaccharide</th>
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<td></td>
<td>3</td>
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<tr>
<td>Manα1</td>
<td>0.87 (82.7)</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Manα2</td>
<td>0.84 (70.2)</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

![Table II-continued](http://www.jbc.org/)
Garlic Bulb Lectin Binding to High Mannose Oligosaccharides

TABLE III

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>Conc for 50% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASAI</td>
</tr>
<tr>
<td>Soybean agglutinin</td>
<td>0.005</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>0.0041</td>
</tr>
<tr>
<td>α1-Acetylglycoprotein</td>
<td>0.082</td>
</tr>
<tr>
<td>Asialo-α1-acid glycoprotein</td>
<td>0.011</td>
</tr>
<tr>
<td>Fetuin</td>
<td>0.069</td>
</tr>
<tr>
<td>Asialofetuin</td>
<td>0.012</td>
</tr>
<tr>
<td>Transferrin</td>
<td>0.058</td>
</tr>
<tr>
<td>Asialotransferrin</td>
<td>0.0071</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.055</td>
</tr>
<tr>
<td>Asialofibrinogen IgG</td>
<td>0.0063</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.073</td>
</tr>
<tr>
<td>Goat</td>
<td>0.061</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.053</td>
</tr>
<tr>
<td>Jacalin</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Compared with other mannose-binding lectins, ASAI and ASAIII bind to mannose very weakly. Methyl-α-L-mannopyranoside is six times better an inhibitor than mannose. The relative potencies of β-mannose and its epimers suggest that the equatorial orientation of the hydroxyl groups at C-3, C-4, and C-6 and an axial hydroxyl group at C-2 as in mannose are necessary for interaction with these lectins. Compared with other mannose-binding lectins, some of the mannobioses show much higher potency than mannose. Of all the mannobioses tested, Manα1–3Man exhibited the highest affinity as found by Kaku et al. (34), but unlike the present result, they recorded a lower potency of trimannoside than Manα1–3Man. The difference might be attributed to the different techniques used. (Although assay-dependent differences in binding affinities are not common, they have, however, been seen in some instances. For example, GNA shows altered affinities for murine IgM under different assay conditions such as in a precipitation assay and in immobilized form (34).)

The observation that ASAs are complementary to α1–3-mannosyl units merits reconsideration as (i) extension of α1–3Man as in Manα1–3Manα1–3Manα1–3Manα1–2Man-ol diminishes its binding ability (34); (ii) the affinity increases in the order of Manα1–3Man < mannotriose ≈ mannopentaose ≈ Manα2-GlcNαC-oligosaccharides; and (iii) it may not be an α1–3-linked mannopyranosyl residue, but the number of residues in α1–2-linkage at the nonreducing end as discussed subsequently that determine higher affinity of manno-oligosaccharides. This is evident by the dramatic increase in the potency of oligosaccharides that carry increasing numbers of α1–2-linked mannose residues. Unlike ASAI and ASAIII, mannotriose is the most complementary inhibitor for ConA, whereas artocarpin displays only slightly higher affinity for mannopentaose over mannotriose. When the reducing end of Manα1–3Man and mannopentaose was substituted with a GlcNαC residue, no appreciable change in activity was observed. For artocarpin, the same substitution led to an enhancement in potency by severalfold (7). The reducing end GlcNαC in ASAs is probably accommodated adjacent to the primary binding site without involving any significant interactions with the lectin. When the terminal mannose residues of mannotriose are substituted with two GlcNαC residues as in GlcNαC-Manα2, it leads to some improvement in activity, suggesting that the combining site of ASAs can access the masked/internal mannose residues of the oligosaccharides.

On the other hand, the addition of two GlcNαC residues in β1–4-linkage at the reducing end of mannotriose (i.e., the core pentasaccharide of N-linked glycans) improves the affinity by five to six times. These observations are in accordance with the speculation, made by Barre et al. (33), that the mannose-bind-
with the members of the monocot mannose-binding lectin family constitutes a novel specificity among lectins studied to date. If compared with the mannose-binding (GNA, NPA, and HHA) and mannose/glucose-binding (ConA and artocarpin) lectins, the topology of the binding site(s) of ASAs would appear quite distinct. GNA recognizes only terminal α1–3-linked mannose residues. NPA and HHA interact with both the terminal and internal mannosyl residues, but the best inhibitors of NPA and HHA are α1–6-linked mannnotrioses and oligosaccharides with α1–3- or α1–6-mannose residues, respectively. ConA displays high affinities for oligosaccharides containing α1–2-linked mannose, but its binding site is most complementary to mannotriose. Artocarpin does not recognize α1–2-linked high mann-o-oligosaccharides and is most complementary to Man₃GlcNAc₂Fuc containing a xylose β1–2-linked to the 3,6-disubstituted core mannose. In conclusion, our studies illustrate that the exquisite specificity of lectin-glycoprotein enzyme interaction coupled with the catalytic power of an enzyme provides a simple and sensitive method for elucidating the carbohydrate recognition propensities of lectins. The ability of ASAs to bind high mannose-containing oligosaccharides and glycoprotein with enhanced potencies places them in a unique position among the mannose-binding lectins reported so far. This specificity of ASAs can be utilized for several biochemical studies, viz. biosynthesis and functional aspects of high mannose oligosaccharides and purification of high mannose-containing glycoproteins like invertase and carboxypeptidase Y.

REFERENCES
Garlic (Allium sativum) Lectins Bind to High Mannose Oligosaccharide Chains
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