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 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 ± 4 kDa and the subunits of ASA 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 and ASAIII exhibit the same sequence at their amino termini. ASA and ASAIII recognize monosaccharides in mannose configuration. The potencies of the ligands for ASAs increase in the following order: mannobiase (Man1–3Man) < mannotriose (Man1–6Man1–3Man) ~ mannopentaose ≪ Man9-oligosaccharide. 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 Man9GlcNAc2Asn, 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 Man20GlcNAc, exhibited the highest binding affinity. No other manno- 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, α,β-macroglubulin, 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 manno-binding lectins have been shown to exhibit this kind of specificity.

**MATERIALS AND METHODS**

* Sugars and Glycoproteins—Methyl-α-mannose, mannosamine, N-acetylmannosamine, α- β- and methylumbelliferyl mannose, glucose, methyl-α-glucose, glucosamine, N-acetylglucosamine, galactose, N-acetylgalactosamine, lactose, melibiose, maltose, allose, talose, and Manα1–4Man were purchased from Sigma. Mαn1–2Man and Mαn1–3Man were obtained from Carbohydrate International. Manα1–6Man, Manα1–3(Manα1–3Manα1–6Man), mannotetraose, Manβ1–4GlcNAc, GlcNAc2Man2, and N-acetyllactosamine were purchased from Dextra Laboratories (London). ManGlcNAc, ManGlcNAc, and ManGlcNAc were obtained from Biocarb (Lund, Sweden). ManGlcNAcAsn was a gift from Dr. M. I. Khan (National Chemical Laboratory, Pune, India). ManGlcNAcAsn and ManGlcNAcAsn prepared from quail ovalbumin were available in the laboratory from a previous study (7). Invertase, fetuin, transferrin, IgGs, fibrogen, α-1,3-acid glycoprotein, and ovalbumin were products of Sigma. Soybean agglutinin and jacalin (*Artocarpus integrifolia* agglutinin) were prepared in the laboratory according to Swamy et al. (8) and Suresh Kumar et al. (9), respectively. Glucose oxidase was purchased from Boehringer Mannheim. All other chemicals were 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).

**Purification of *A. sativum* Agglutinins (ASAs)**—Healthy dry bulbs of *A. 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 (NH)4SO4 cut

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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, *Hippastrum hybr.* agglutinin; ConA, concanavalin A.
Electrophoretic Procedures—SDS-polyacrylamide gel electrophoresis under reducing conditions was carried out as described by Laemmli (11). Molar masses of the lectins were calculated according to the method of Weber and Osborn (12) using bovine serum albumin as the standard.

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 × 110 cm) calibrated with hen egg ovalbumin, chymotrypsinogen, myoglobin, and bovine serum albumin (68 kDa) as the standards. The proteins were visualized on the gel by Coomassie staining. A P/ACE system 2100 (Beckman Instruments) was used for capillary electrophoresis with P/ACE 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) × 20 μm inner diameter, designed for P/ACE cartridges, was obtained from Beckman Instruments. On-line detection was set at 280 nm with a 50 × 200-μm aperture in the P/ACE 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.

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% (v/v)) was added to the solution, and the results were noted after 1 h.

**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 ASAIII. The affinity-purified preparation 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.

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

Inhibition of hemagglutination

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

| Sugar/glycoprotein          | Conc for 50% inhibition |
|----------------------------|-------------------------|
| Mannose                    | 150 mM                  |
| α-D-mannopyranoside        | 12 mM                   |
| Glucose                    | Not inhibitory up to 250 mM |
| α-D-mannopyranoside        | Not inhibitory up to 200 mM |
| Mannobiose (Man1-3Man)     | 14 mM                   |
| Mannobiose (Man1-3-Man1-6Man) | 9 mM                   |
| Invertase                  | 0.70 nM                 |
| Horseradish peroxidase     | 210 nM                  |

same sequence (RNILTDEGLYAGQLSLD), 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 consider-ably weakly with human erythrocytes, irrespective of their blood groupings.

ASAs Bind Most Acidly 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 mannosne, although the latter was less potent as an inhibitor (Table I). Glucose, a C-2 epimer of mannosne, 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-glucosamine and glucosamine were inactive. Of all the glycoproteins used in this assay, invertase was the strong-est 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 cata-lytic activity.

The strong affinity of these lectins for invertase, containing high mannosne-type oligosaccharides, was instrumental in desig-nating a sensitive enzyme-based assay to study the interaction of these lectins with various sugars (as shown in Scheme I) 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, respec-tively, 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 ASAIII. ASAI also showed identical patterns. The experi-ments produced by ASAIII. ASAI also showed identical patterns. The experi-mental protocol is described under "Material and Methods."

**Inhibition of ASA-Invertase Binding by Mono- and Disaccha-rides**—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 mannosne (Tables I and II). The presence of nonpolar p-nitrophenyl aglycon in d-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-mannopyranose, indicating that β-linked mannosne was not conducive for binding. N′-Acetyl-D-mannosamine was inactive, suggesting that the axially oriented hydroxyl group of mannosne cannot be substituted with a bulky acetamido group. The monosaccharide binding propensities of ASAI and ASAIII are broadly similar to other well studied mannosne-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 mannosne. Manα1–2Man and Manα1–6Man were almost eight and six times more active, respectively, over mannosne. But the other mannobioses, including Manα1–4Man, were poor ligands. Among the other mannosne-binding lectins, GNA recognizes only terminal Manα1–3Man, whereas NPA (daffodil) and HHA (amaryllis) prefer α1–6-linked mannosne. On the other hand, ConA is known to
exhibit greater affinity for Man$_{1–2}$Man (22). Artocarpin (A. integrifolia mannose-binding lectin) shows higher specificity for Man$_{1–3}$Man (7). The $\beta$-linked disaccharides like Man$\beta$-1–4GlcNAc and GlcNAc$\beta$-1–2Man were poor inhibitors, whereas Man$\beta$-1–6GlcNAc and GlcNAc$\beta$-1–6Man were inactive toward ASAs. The lectins did not interact with disaccharides like lactose (Gal$\beta$-1–4Glc), N-acetyllactosamine (Gal$\beta$-1–4GlcNAc), and melibiose (Gal$\alpha$-1–6Glc).

**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, Man$_{1–3}$(Man$_{1–6}$Man), was 30 times stronger an inhibitor than mannose and showed four, two, and five times more potency than Man$_{1–2}$Man, Man$_{1–3}$Man, and Man$_{1–6}$Man, respectively. The relative inhibitory potencies of mannopentaose and mannolactose were identical. Man$_{1}$GlcNAc and Man$_{1–3}$Man$\beta$-1–4GlcNAc were marginally better ligands than mannopentaose and Man$_{1–3}$Man, respectively, unlike artocarpin, in which the addition of GlcNAc at the reducing end of Man$_{1–3}$Man caused a dramatic enhancement of its binding affinity (7).

When the $\alpha$-1–3- and $\alpha$-1–6-linked mannosyl residues of mannotriose were substituted with GlcNAc as in GlcNAc$_{2}$Man$_{2}$, no remarkable change in activity was noted. In this regard, ASAI and ASAIII bear similarity to ConA, which displays equal affinities for mannotriose and GlcNAc$_{2}$Man$_{2}$ (23–25), but for artocarpin, the inhibitory activity is reduced by 25-fold (7). Man$_{n}$GlcNAc$_{2}$A sn has a 5-fold higher potency relative to mannotriose. Among the manno-oligosaccharides tested, Man$_{n}$GlcNAc$_{2}$A sn is the best ligand, followed by Man$_{n}$GlcNAc$_{2}$Asn and Man$_{n}$GlcNAc$_{2}$A sn, indicating that the $\alpha$-1–2-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 $\alpha$-1–2-linked mannosyl residues as in Man$_{n}$GlcNAc$_{2}$A sn is unique among the lectins described so far.

**Interaction of ASAI and ASAIII with Glycoproteins**—The ability of several manno-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 Man$_{1–2}$Man linkages in their high manno-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 manno- 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. $\alpha_1$-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 NBA and HHA, could recognize internal $\alpha$-mannosyl residues. Alternatively, it can be said that these lectins can bind to the core pentasaccharide of N-linked glycans (Man$_{1–3}$(Man$_{1–6}$Man$\beta$-1–4GlcNAc$\beta$-1–4GlcNAc) even when both of the $\alpha$-1–3- and $\alpha$-1–6-mannosyl residues are substituted with NeuAc$\alpha$-2–3Gal$\beta$-1–4GlcNAc.

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 $\beta$-linked mannose by $\beta$-linked xylose and GlcNAc, respectively, are much weaker as compared with that of artocarpin. These results suggest that substitution with xylose in $\beta$-1–2-linkage as in horseradish peroxidase (and ja-calin) or bisection with GlcNAc in $\beta$-1–4-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 $\beta$-linked mannose with xylose in $\beta$-1–2-linkage compromises the binding potency to a greater extent than the substitution of reducing end GlcNAc with fucose in $\alpha$-1–3-linkage. Invertase carries Man$_{n}$GlcNAc in its seven accessible glycans (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 manno-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 manno-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, presenting 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 ASAIII 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 isolectins that have high propensities for interaction with alliinase (20).

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2 Ovalbumin as a glycoprotein is highly heterogeneous, and individual molecules in a population exhibit either high manno 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 alliinase-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. alliinase. 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).

| Oligosaccharide          | Conc for 50% inhibition (mM) | Conc for 50% inhibition (mM) |
|--------------------------|-------------------------------|-------------------------------|
| | ASAI | ASAIII |
| Mannose                  | 72 (1)                        | 59 (1)                        |
| α-Methylmannopyranoside  | 10.5 (6.8)                    | 12 (4.9)                      |
| 4-Methylumbelliferyl α-D-mannopyranoside | 8 (9) | 11 (5.3) |
| 4-Methylumbelliferyl β-D-mannopyranoside | NI* | NI* |
| p-Nitrophenyl-α-mannopyranoside | 97 (0.74) | 92 (0.64) |
| N-Acetylmannosamine      | NI                           | NI                           |
| Manα1-2Man               | 8.3 (8.6)                    | 8.1 (7.3)                    |
| Manα1-3Man               | 6.2 (11.6)                   | 5.1 (11.5)                   |
| Manα1-4Man               | NI                           | NI                           |
| Manα1-6Man               | 9.8 (7.3)                    | 10.2 (5.8)                   |
| Manα1-3(Manα1-6)Man      | 2.5 (29)                     | 1.9 (31)                     |
| Manα1-6Manα1-6Man        | 2.5 (28.8)                   | 2.9 (29.5)                   |
| Manα1 Manα1 (manno-oligo) | 2.5 (15)                     | 3.1 (19)                     |
| Manα1-3Manα1-4GlcNAc (Man, Gna) | 4.8 (15) | 3.1 (19) |
| Manα1-6Manα1-6Manα1-4GlcNAc (Man, Gna) | 1.07 (67.2) | 1.15 (51.3) |
| GlcNAcβ1-2 Man α 1       | 1.9 (37.8)                   | 1.2 (49.1)                   |
| GlcNAcβ1-2 Man α 1 (Gna, Manα) | 1.9 (37.8) | 1.2 (49.1) |
| Man α 1                  | 0.51 (141)                   | 0.38 (155)                   |
| Man α 1                  | Manα1-4GlcNAcβ1-4GlcNAc       | 0.51 (141)                   |
| Man α 1                  | 0.38 (155)                   | 0.38 (155)                   |
| Man α 1                  | 0.46 (157)                   | 0.37 (159)                   |
| Man α 1                  | 0.37 (159)                   | 0.37 (159)                   |
| Xy1α1-2Manα1-4GlcNAcβ1-4GlcNAc | 2.8 (25.7) | 2.1 (25) |
| Man α 1                  | 2.1 (25)                     | 2.1 (25)                     |
| Man α 1                  | 2.1 (25)                     | 2.1 (25)                     |
| Xy1α1-2Manα1-4GlcNAcβ1-4GlcNAc | 1.3 (55.4) | 1.7 (34.7) |
| Man α 1                  | 1.7 (34.7)                   | 1.7 (34.7)                   |
Compared with other mannose-binding lectins, ASAI and ASAIII bind to mannose very weakly. Methyl-α-D-mannopyranoside is six times better an inhibitor than mannose. The relative potencies of α-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 necessary for interaction with these lectins. Compared with other members of this lectin family, at least one relative potencies of D-mannose and its epimers suggest that the combining site of ASAs can access residues as in the glycopeptides of quail ovalbumin and soybean lectin leads to increased potencies. This enhancement also highlights the preference of garlic lectins for a cluster of α1→2-linked mannose residues.

The mode of interaction of ASAs with the glycoproteins studied substantiates the above findings. The ability to recognize internal mannose and the affinity for core pentasaccharide were, once again, proven through the interaction of ASAs with some glycoproteins. It appears that the binding site can withstand the terminal sialic acids as well as the penultimate galactose residues, although the removal of sialic acids enhances potency. Sheep IgG and jacalin displayed moderate binding potencies compared with other glycoproteins. Since the substitution of reducing end GlcNac with α1→6-linked fucose as in Man$_2$GlcNac$_2$Fuc is impervious to binding, the relatively weak interaction of jacalin, horseradish peroxidase, and its oligosaccharide as well as goat IgG appears to be due to the substitution of the β-linked 3,6-disubstituted mannose with xylose in α1→2-linkage (as in jacalin and horseradish peroxidase) and its substitution with GlcNac in β1→4-linkage (as in sheep IgG). Using a different protocol (surface plasmon resonance analysis), Barre et al. (33) found that immobilized fetuin and asialofetuin do not bind to garlic lectins (viz. ASAI and ASAII). But hemagglutination and the enzyme-based assay reported here show that garlic lectins (ASAI and ASAIII) can bind to both fetuin and asialofetuin. Surface plasmon resonance analysis in the concentration range used appears to have failed to detect this interaction because of their moderate affinities. Notwithstanding the identity of the polypeptide chain in the lectin preparation reported by Gupta and Sandhu (31), they had purified the garlic lectin(s) using an asialofetuin-silica affinity column, confirming that they indeed are able to bind glycoproteins such as asialofetuin.

Invertase was the strongest glycoprotein ligand. This is attributed to the presence of several α1→2-linked mannose residues. Invertase contains nine N-linked high mannose oligosaccharides, seven of which are accessible. About 85% of the mannose of the accessible oligosaccharides is in species larger than Man$_n$GlcNac (30). We believe that the availability of these high mannose oligosaccharides with several α1→2-linked mannose residues at their nonreducing end is instrumental for the observed potency of ASAs. This is also borne out by the inhibitory activities of the glycopeptide from soybean agglutinin. From molecular modeling studies, it is suggested (33) that the garlic lectins, like their counterparts in the monocot mannose-binding lectin family, possess three identical mannose-binding sites per monomer and that the mannose-binding site is part of a more extended site. As a result, these lectins are expected to accommodate a larger number of mannose residues. This explains, at least in part, (i) the comparatively low affinity for the monosaccharide (mannose), (ii) the increased affinity with increasing numbers of mannose residues, and (iii) the enhanced avidity for high mannose-containing oligosaccharides/glycoproteins. Although there is no report on the interaction of high mannose-containing glycoproteins, such as invertase, with other members of this lectin family, at least one member (bulb lectin from Allium cepa) demonstrates identical affinity for invertase. The interaction of high mannose-containing oligosaccharides/glycoproteins and other larger glycans

### Table III

| Glycoprotein    | Conc for 50% inhibition |
|-----------------|-------------------------|
|                 | ASAI        | ASAII       |
| Soybean agglutin| 0.005       | 0.003       |
| Ovalbumin       | 0.0041      | 0.0028      |
| α$_1$-Acetylglycoprotein | 0.082 | 0.069 |
| Asialo-α$_1$-acid glycoprotein | 0.011 | 0.02 |
| Fetuin          | 0.009       | 0.007      |
| Asialofetuin    | 0.012       | 0.010      |
| Transferin      | 0.058       | 0.053      |
| Asialotransferin| 0.0071      | 0.0075     |
| Fibrinogen      | 0.055       | 0.049      |
| Asialofibrinogen| 0.0063      | 0.0071     |
| IgG             | 0.073       | 0.073      |
| Sheep           | 0.061       | 0.042      |
| Goat            | 0.053       | 0.049      |
| Rabbit          | 0.096       | 0.086      |

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T. K. Dam and A. Sauria, unpublished observation.
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 α-1–3-linked mannose residues. NPA and HHA interact with both the terminal and internal mannosyl residues, but the best inhibitors of NPA and HHA are α-1–6-linked mannnotriose and oligosaccharides with α-1–3- or α-1–6-mannose residues, respectively. ConA displays high affinities for oligosaccharides containing α-1–2-linked mannose, but its binding site is most complementary to mannotriose. Artocarpin does not recognize α-1–2-linked high manno-oligosaccharides and is most complementary to Man$_3$GlcNAc$_3$Fuc containing a xylose β-1–2-linked to the 3,6-disubstituted core mannose. In conclusion, our studies illustrate that the exquisite specificity of lectin-glycoprotein enzyme interaction when 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
1. Cammue, B. P. A., Peeters, B., and Peumans, W. J. (1986) *Planta* (Heidelb.) 169, 583–588
2. Van Damme, E. J. M., Allen, A. K., and Peumans, W. J. (1987) *FEBS Lett.* 215, 140–144
3. Van Damme, E. J. M., Goldstein, I. J., and Peumans, W. J. (1991) *Phytochemistry* (Oxf.) 30, 509–514
4. Shibuya, N., Goldstein, I. J., Van Damme, E. J. M., and Peumans, W. J. (1988) *J. Biol. Chem.* 263, 728–734
5. Van Damme, E. J. M., Allen, A. K., and Peumans, W. J. (1988) *Physiol. Plant.* 73, 52–57
6. Shibuya, N., Berry, J. E., and Goldstein, I. J. (1988) *Arch. Biochem. Biophys.* 267, 676–680
7. Misquith, S., Rani, P. G., and Surolia, A. (1994) *J. Biol. Chem.* 269, 30393–30401
8. Swamy, M. J., Krishna Sastry, M. V., Khan, M. I., and Surolia, A. (1986) *Biochem. J.* 261, 14621–14627
9. Sureshkumar, G., Appakuttan, P. S., and Dehkumar, B. (1982) *J. Biosci.* 7, 257–261
10. Sundberg, L., and Forath, J. (1974) *J. Chromatogr.* 90, 87–98
11. Laemmli, U. K. (1970) *Nature* 227, 680–685
12. Weber, K., and Osborn, M. (1968) *J. Biol. Chem.* 244, 4406–4412
13. Matsudaira, P. (1987) *J. Biol. Chem.* 262, 10035–10038
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–375
15. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) *Anal. Chem.* 28, 350–356
16. Dam, T. K., Sarkar, M., Ghosal, J., and Chaudhury, A. (1992) *Mol. Cell. Biochem.* 117, 1–9
17. Nelson, N. (1944) *J. Biol. Chem.* 153, 375–380
18. Van Damme, E. J. M., Smeets, K., Torrekens, S., Van Leuven, F., Goldstein, I. J., and Peumans, W. J. (1992) *Eur. J. Biochem.* 206, 413–420
19. Smeets, K., Van Damme, E. J. M., Van Leuven, F., and Peumans, W. J. (1997) *Glycoconjugate J.* 14, 331–343
20. Rabinkov, A., Wilchek, M., and Mirelman, D. (1995) *Glycoconjugate J.* 12, 690–698
21. Kaku, H., Van Damme, E. J. M., Peumans, W. J., and Goldstein, I. J. (1990) *Arch. Biochem. Biophys.* 289, 298–304
22. Goldstein, I. J., Hollerman, C. E., and Smith, E. E. (1965) *Biochemistry* 4, 876–884
23. Bhattacharyya, L., Haraldsson, M., and Brewer, C. F. (1987) *J. Biol. Chem.* 262, 1294–1299
24. Kaku, H., and Goldstein, I. J. (1991) *Carbohydr. Res.* 213, 109–116
25. Baenziger, J. U., and Fiete, D. (1979) *J. Biol. Chem.* 254, 2400–2407
26. Derland, L., van Halbeek, H., Vliegenthart, J. F. G., Lis, H., and Sharon, N. (1981) *J. Biol. Chem.* 256, 7708–7711
27. Tai, T., Yashimata, K., Ogata-Arakawa, M., Koide, N., Muramatsu, T., Iwashita, S., Inoue, Y., and Kobata, A. (1975) *J. Biol. Chem.* 250, 8569–8575
28. Tai, T., Yamashita, K., Ito, S., and Kobata, A. (1977) *J. Biol. Chem.* 252, 6687–6694
29. Yashimata, K., Tachihi, Y., and Kobata, A. (1978) *J. Biol. Chem.* 253, 3862–3869
30. Trumb, R. B., Maley, F., and Chu, F. K. (1983) *J. Biol. Chem.* 258, 2562–2567
31. Gupta, A., and Sandhu, R. S. (1997) *Mol. Cell. Biochem.* 166, 1–9
32. Smeets, K., Van Damme, E. J. M., Verhaert, S., Barre, A., Rouge, P., Van Leuven, F., and Peumans, W. J. (1997) *Plant Mol. Biol.* 33, 223–234
33. Barre, A., Van Damme, E. J. M., Peumans, W. J., and Rouge, P. (1996) *Plant Physiol. (Bethesda)* 112, 1531–1540
34. Kaku, H., Goldstein, I. J., Van Damme, E. J. M., and Peumans, W. J. (1992) *Carbohydr. Res.* 229, 347–353