Xanthosoma sagittifolium Tubers Contain a Lectin with Two Different Types of Carbohydrate-binding Sites*

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An unusual lectin possessing two distinctly different types of carbohydrate-combining sites was purified from tubers of Xanthosoma sagittifolium L. by consecutive passage through two affinity columns, i.e. asialofetuin-Sepharose and invertase-Sepharose. SDS-polyacrylamide gel electrophoresis, N-terminal amino acid sequencing, and gel filtration chromatography of the purified lectin showed that the X. sagittifolium lectin is a heterotetrameric protein composed of four 12-kDa subunits (α2β2) linked by noncovalent bonds. The results obtained by quantitative precipitation and hapten inhibition assays revealed that the lectin has two different types of carbohydrate-combining sites: one type for oligomannoses, which preferentially binds to a cluster of nonreducing terminal α1,3,1,6-linked mannose residues, and the other type for complex N-linked carbohydrates, which best accommodates a non-sialylated, triantennary oligosaccharide with \( \text{Galβ1,4GlcNAc} \) groups at its three nonreducing termini.

Lectins are (glyco)proteins of nonimmune origin that agglutinate cells and/or precipitate complex carbohydrates (1). It is their unique ability to recognize and bind reversibly to specific carbohydrate ligands without chemically modifying them that distinguishes lectins from other carbohydrate-binding proteins and enzymes and that makes lectins invaluable tools in biomedical and glycoconjugate research.

Lectins are ubiquitous in the biosphere. In the plant kingdom, they are traditionally found in the dicotyledons, especially in seeds (2–4). However, during the last decade, lectins from monocotyledonous families such as Alliaceae (5–7), Amaryllidaceae (8–10), Araceae (11–14), Liliaceae (15, 16), and Orchidaceae (17) have been isolated and characterized. Interestingly, they all belong to a single monocot mannose-binding lectin superfamily with respect to their molecular structures, sequence homologies, and exclusive specificity for \( \text{D-mannose} \) (18).

Recently, Van Damme et al. (11) described the isolation of lectins from several Araceae species, viz. Arum maculatum, Colocasia esculenta, Xanthosoma sagittifolium, and Diefenbachia sequina. Based on their molecular structure, taxonomic relationship, and high sequence homology to the aforementioned mannose-binding lectins, they clearly belong to the monocot mannose-binding lectin superfamily. However, compared with the other members of the superfamily, these lectins exhibit either very weak or no affinity for \( \text{D-mannose} \), whereas they bind with great avidity to asialoglycoproteins. Therefore, a detailed investigation of their carbohydrate-binding specificities has been undertaken.

The results presented in this study reveal that one of these lectins, i.e. the lectin from \( X. \) sagittifolium (XSL), possesses two distinctly different types of binding sites: one type for oligomannoses and the other for complex carbohydrates, which best accommodates an asialo triantennary N-linked glycan. The knowledge obtained from this study might also shed light on the nature of the binding sites of other lectins with “complex” carbohydrate-binding specificity.

EXPERIMENTAL PROCEDURES

Materials—Tubers of \( X. \) sagittifolium L. Schott were purchased from a local store in Leuven, Belgium. Yeast mannan from \( \text{Saccharomyces cerevisiae} \), glycogens (from both rabbit liver and oyster), invertase (from bakers’ yeast), mucins, and various glycoproteins such as fetuin, \( \alpha_1 \)-acid glycoprotein, human transferrin, and thyroglobulin (bovine) were obtained from Sigma. The glycoproteins were desialylated by heating in 0.1 M hydrochloric acid at 80 °C for 1 h, followed by dialysis and lyophilization; removal of sialic acid was confirmed by the thiobarbituric acid assay (19). All monosaccharides and their methyl or \( p \)-nitrophenyl glycosides were purchased from Sigma. \( \text{Man}_1,3\text{Man}-\text{OMe}, \text{Man}_1,6\text{Man}-\text{OMe}, \text{Man}_1,6(\text{Man}_1,3\text{Man})-\text{OMe} \) and \( \text{Man}_1,6(\text{Man}_1,3\text{Man})-\text{OMe} \) were also available from Sigma; \( \text{Man}_1,3\text{Man}, \text{Man}_1,6\text{Man}, \text{Man}_1,2\text{Man}, \text{Man}_1,4\text{GlcNAc}, \text{Man}_1,6(\text{Man}_1,3\text{Man}) \) and branched mannopentaose were obtained from Dextra Laboratories Ltd. (Reading, United Kingdom). The mannan of \( S. \) cerevisiae mutant 1B4 and \( \text{Kloeckera brevis} \) mannan were generous gifts of Dr. Akira Misaki (Kanon Women’s University, Kobe, Japan).

 Purification of \( X. \) sagittifolium Lectin—Asialofetuin and invertase were coupled to cyanogen bromide-activated Sepharose 4B according to the procedure described by March et al. (20). Affinity absorbents containing 3.1 mg of asialofetuin/ml and 5.2 mg of invertase/ml of settled gel were obtained.

The \( X. \) sagittifolium lectin was isolated from tuber extracts by affinity chromatography on an asialofetuin-Sepharose 4B column essentially as described previously (11). The final purification of the lectin was accomplished by a second affinity chromatography step on an invertase-Sepharose 4B column. The affinity-adsorbed lectin was desorbed with

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1 The abbreviations used are: XSL, X. sagittifolium lectin; -OMe, -acetyl; RP-HPLC, reverse-phase high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; LacNAc, N-acetyllactosamine.
0.1 M acetic acid (pH 3.0), collected, dialyzed immediately against distilled water, and lyophilized.

Preparation of Complex Oligosaccharides—The N-linked bi-, tri-, and tetraantennary oligosaccharides were isolated from various sources as described under “Experimental Procedures” by peptide N-glycosidase treatment of the tryptic glycopeptides. The reducing ends were converted into glycosylamines and then derivatized with N-tert-butoxycarbonyl-L-tyrosine N-hydroxy succinimide ester. The tyrosinylated oligosaccharides were purified by RP-HPLC and characterized by proton NMR and fast atom bombardment mass spectrometry (21–27).

RESULTS

Purification of the Xanthosoma Lectin—After two successive affinity chromatography steps, first on an asialofetuin-Sepharose 4B column and then on an immobilized invertase column, an electrophoretically homogeneous Xanthosoma lectin preparation was obtained with a yield of 4 mg/g (wet weight). The purified lectin gave a single protein band upon native PAGE (Fig. 2A) and a single symmetric protein peak on gel filtration chromatography (data not shown).

Molecular Mass and Molecular Structure—The molecular mass of the purified Xanthosoma lectin was estimated by gel filtration chromatography on a precalibrated Superose 12 column. The lectin eluted as a single symmetric peak in the exclusion volume corresponding to an apparent molecular mass of 50 kDa (Fig. 2B). However, N-terminal sequencing of the purified Xanthosoma lectin (Table I) gave two...
Table I

N-terminal amino acid sequences of the two subunits of the purified Xanthosoma lectin

The 12-kDa band from SDS-PAGE containing α- and β-subunits was blotted onto polyvinylidene difluoride membrane and sequenced by automated Edman degradation. The sequences of the individual subunits were deduced from the double sequence using the method previously described (11).

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Subunit</td>
<td>LGTVLLSGQ</td>
</tr>
<tr>
<td>β-Subunit</td>
<td>NIPFTNLFLFS</td>
</tr>
</tbody>
</table>

a-amino acids in each Edman degradation cycle in equimolar amounts, revealing that this 12-kDa band was an equimolar mixture of two different types of 12-kDa subunits. Taken together, these data suggest that at neutral pH, the native Xanthosoma lectin exists as a heterotrimer composed of four 12-kDa subunits (α2β2) joined by noncovalent bonds.

Precipitation Assays—Yeast mannan (S. cerevisiae), which contains clusters of α1,3-linked mannosyl residues attached to an α1,6-linked mannose backbone (32), precipitated the Xanthosoma lectin at an appropriate stoichiometric ratio (Fig. 3A), whereas the mannan obtained from K. brevis mutant 1B4 and the K. brevis mannan (32), both of which contain α1,2- and α1,6-linked mannosyl residues, but lack α1,3-linked mannosyl residues, failed to precipitate the lectin. Neither glycogens nor dextrans interacted with the Xanthosoma lectin. In this regard, the Xanthosoma lectin not only exhibits exclusive specificity for mannos, but more definitively has a linkage preference for terminal α1,3-linked mannosyl residues.

Among various glycoproteins and mucins tested, the Xanthosoma lectin readily formed precipitins with various asialglycoproteins such as asialo-α1-acid glycoprotein (i.e. asialoorosomucoid), asialofetuin, asialotransferrin, and asialothyroglobulin (Fig. 3B), whereas the corresponding sialylated glycoproteins were inert. On the other hand, neither the native nor desialylated mucins tested precipitated the lectin. Taken together, it is apparent that the Xanthosoma lectin binds specifically to N-linked glycans, and the presence of terminal sialic acid groups abolishes the binding.

Inhibition of Precipitation by Haptenic Carbohydrates—To investigate the carbohydrate-binding properties of the Xanthosoma lectin, detailed precipitation inhibition assays were conducted using either yeast mannan (S. cerevisiae) or asialoorosomucoid as the precipitants. The results are shown in Fig. 4 and Table II and in Fig. 5 and Table III, respectively.

For the XSL/yeast mannan precipitation system, only α-mannose, of the monosaccharides tested, had a very weak inhibitory activity (5% inhibition at 200 mM); methyl-α-D-mannopyranoside was slightly more active than mannose (14% inhibition at 200 mM), whereas methyl-β-D-mannopyranoside and epimers of α-mannose, i.e. D-glucose (C-2 epimer), D-altrose (C-3 epimer), and D-talose (C-4 epimer), were inactive up to 200 mM. Of the mannosides tested, α1,3-mannobiose was the only inhibitor; α1,2- and α1,6-mannobiosides and Manβ1,4GlcNac all failed to show inhibitory activity at the highest concentration tested (20 mM). The branched mannopentaose, i.e. Manα1,6-(Manα1,3/Manα1,6)(Manα1,3)Man, was by far the best inhibitor, followed by the branched mannotriose, i.e. Manα1,6-(Manα1,3)Man, being 10- and 2-fold more active, respectively, than α1,3-mannobiose.

On the other hand, the precipitation reaction between the Xanthosoma lectin and asialoorosomucoid was not inhibited by any mono-, di-, or trisaccharide tested. Of the various oligosaccharides examined (Fig. 1), Tri 4 (a triantennary complex-type oligosaccharide with two LacNAc- residues and one 1,2-branch) was the best inhibitors. Tri 5 (same as Tri 4, except that the penultimate GlcNAc residue of the Galβ1,4GlcNAcβ1,4- branch was substituted with an α2,6-linked sialic acid residue) was non-inhibitory, suggesting that this complex carbohydrate-
combining site can tolerate a neutral sugar substituted at the 3-OH of the GlcNAcβ1,4- branch, but not a negatively charged sialic acid in the same vicinity. The facts that both the biantenary oligosaccharide (Bi 2) and the tetraantennary oligosaccharide (Tetra) were inferior to their triantennary counterparts (Tri 2 and Tri 4) and that the longer chain analogs (Tri 2, Tri 4, and Bi 2, all with LacNAc at their nonreducing termini) were superior to the shorter chain galactoses (in which the nonreducing terminal galactoses were absent) indicated that this binding site best accommodated triantennary complex carbohydrates with LacNAc or lacto-N-biose residues at their nonreducing termini. A bisecting GlcNAcβ1,4- at the β1,4-linked mannose residue abolished the binding (as evident by the fact that Bis 2 was non-inhibitory).

**DISCUSSION**

As noted above, during the last decade, an increasing number of lectins have been isolated from various monocotyledonous families such as Alliaceae, Amaryllidaceae, Araceae, Liliaceae, and Orchidaceae. Data accumulated from physicochemical characterization and molecular cloning of these lectins reveal that, with the major exception of the Gramineae family, they all belong to a superfamily of monocot mannose-binding lectins with respect to their sequence homology, conserved domains, tissue occurrence, and exclusive specificity for mannose. However, recently, we isolated lectins from the tubers of several Araceae species, viz. A. maculatum, C. esculenta, X. sagittifolium, and D. sequina, and observed that in contrast to our previous findings, these monocot lectins are barely inhibited by mannan, whereas they strongly react with asialoglycoproteins (11). Simultaneously, Shangary et al. (13) reported the isolation of four monocot lectins from the family Araceae, viz. Arisaema consanguineum, Arisaema curvatum, Sauroratum guttatum, and Gonanathus pumilus; they made the same

**FIG. 3.** Quantitative precipitation of the Xanthosoma lectin by yeast mannan (S. cerevisiae) and glycoproteins. Varying amounts of polysaccharides or glycoproteins, ranging from 0 to 100 µg, were incubated with 17 µg of lectin in a total volume of 120 µl of PBS (pH 7.2). After 48 h at 4 °C, the amounts of protein precipitated were quantified. A, quantitative precipitation of the Xanthosoma lectin by yeast mannan (S. cerevisiae) (■) and invertase (●); B, quantitative precipitation of the purified Xanthosoma lectin by several asialoglycoproteins: asialothyroglobulin (■), asialofetuin (●), asialoorosomucoid (▲), and asialotransferrin (♦).

**FIG. 4.** Hapten inhibition of XSL/yeast mannan precipitation. The inhibition assays were conducted under maximum precipitating conditions (i.e. 17 µg of lectin and 10 µg of mannan). Varying amounts of haptenic saccharides were added to the reaction mixture containing 17 µg of lectin and 10 µg of S. cerevisiae mannan in a total volume of 120 µl of PBS. After 48 h, the precipitated protein was determined, and the percentage of inhibition was calculated. ■, branched mannotriose; ●, branched mannotriose; ▲, α1,3-mannobiase; ♦, α1,2- and α1,6-mannobiases; □, methyl-α-D-mannopyranoside.

**Table II**

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>I&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;°&lt;/sup&gt;</th>
<th>Inhibitory potency&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>None at 200</td>
<td></td>
</tr>
<tr>
<td>D-Galactose</td>
<td>None at 200</td>
<td></td>
</tr>
<tr>
<td>D-Altrrose</td>
<td>None at 200</td>
<td></td>
</tr>
<tr>
<td>D-Talose</td>
<td>None at 200</td>
<td></td>
</tr>
<tr>
<td>D-Mannose</td>
<td>5% at 200</td>
<td></td>
</tr>
<tr>
<td>Methyl-α-D-mannoside</td>
<td>14% at 200</td>
<td></td>
</tr>
<tr>
<td>Methyl-β-D-mannoside</td>
<td>None at 200</td>
<td></td>
</tr>
<tr>
<td>Man0,1,2Man-OB</td>
<td>None at 20</td>
<td></td>
</tr>
<tr>
<td>Man0,1,6Man-OB</td>
<td>None at 20</td>
<td></td>
</tr>
<tr>
<td>Man0,1,6Man-α-Me</td>
<td>None at 20</td>
<td></td>
</tr>
<tr>
<td>Man0,1,4GlcNAC</td>
<td>None at 20</td>
<td></td>
</tr>
<tr>
<td>Man1,3Man-α-Me</td>
<td>15.4</td>
<td>1</td>
</tr>
<tr>
<td>Man1,3Man-OB</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>p-Nitrophenyl α-trimannoside</td>
<td>None at 10</td>
<td></td>
</tr>
<tr>
<td>Man0,1,6/β-O-Me</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td>Man0,1,3/β-O-Me</td>
<td>8.5</td>
<td>2</td>
</tr>
<tr>
<td>Man0,1,6/β-O-Me</td>
<td>1.5</td>
<td>10</td>
</tr>
<tr>
<td>Man0,1,3/β-O-Me</td>
<td>None at 1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentration (±15%) required for 50% inhibition, interpolated from the plot of percentage inhibition versus concentration (cf. Fig. 4), unless indicated otherwise.

<sup>°</sup> Relative to Man0,1,3Man.
reaction by various complex oligosaccharides. For structures, see Fig. 1. Varying amounts of oligosaccharides were added to the reaction mixture containing 17 μg of lectin and 40 μg of asialoorosomucoid in a final volume of 120 μl of PBS. After 48 h at 4 °C, the precipitated protein was measured, and the percentage of inhibition was calculated.

observations. This discrepancy with other monocot lectins prompted us to make a detailed investigation into the carbohydrate-binding properties of these lectins. X. sagittifolium (also known as tannia, taro, cocoyam, and yautia), an edible member of the Araceae family, was chosen to pursue a detailed investigation.

Inasmuch as the hemagglutination activity of the tuber extracts of X. sagittifolium was inhibited by asialofetuin, affinity chromatography of the crude extract was performed on an asialofetuin-Sepharose column. Preliminary experiments showed that this lectin preparation gave a single low molecular mass band migrating close to the bromphenol blue dye front upon conventional SDS-PAGE (33), which had insufficient resolution in the low molecular mass range. Therefore, the Tricine/SDS-PAGE system of Schagger and von Jagow (29), which allowed an excellent separation of small proteins, was used instead, revealing a minor impurity in this lectin preparation (data not shown). By introducing a second affinity chromatography step on immobilized invertase, taking advantage of its nine N-linked high mannose-type glycans (34), a final purification of the lectin was achieved.

The data presented in this paper indicate that the Xanthosoma lectin possesses two distinctly different types of carbohydrate-combining sites: one type for oligomannoses and the other for N-linked complex carbohydrates. Since the lectin is precipitated by both mannans and asialoglycoproteins, it presumably contains at least two binding sites of each type per molecule.

The oligomannose-binding site not only is exclusively specific for mannose, but also has very strict linkage specificity for terminal α1,3-linked mannosyl residues. As revealed by precipitation and inhibition assays, it reacts only with mannans and oligomannoses bearing nonreducing terminal α1,3-mannosyl groups, such as yeast mannan (S. cerevisiae) and branched tri- and pentamannoses, but not with glucans (e.g., glycogens, dextrans, etc.) and other types of mannans (such as the mutant 184 mannan and K. brevis mannan, both of which lack terminal α1,3-linked mannosyl residues). The oligomannose-binding site does not react with Manα1,2GlcNAc2, which has internal α1,3-linked mannosyl residues substituted by a number of α1,2-linked mannoses. The branched mannopentaose is 5-fold more potent as an inhibitor than the branched mannotriose, suggesting there is a clustering effect, which is quite common for lectin/carbohydrate interactions.

On the other hand, of a variety of native and desialylated glycoproteins and mucins tested, all those that precipitate the Xanthosoma lectin bear asialo N-linked glycans, and the precipitation reactions are not inhibited by any mono-, di-, or trimannoses, suggesting that the lectin might possess yet another type of binding sites for complex N-glycans. In an effort to elucidate the structural requirements of this type of binding site, a group of oligosaccharides that represent the major components of the asparagine-linked (N-linked) glycans was prepared. By studying the effects of these complex oligosaccharides on both XSL/yeast mannan and XSL/asialoorosomucoid precipitation reactions, the following was revealed.

1) The Xanthosoma lectin does possess a distinct type of extended binding site for complex N-glycans, which best accommodates a non-sialylated, triantennary carbohydrate with LacNAc (i.e. Galβ1,4GlcNAc-, such as Tri 2) or lacto-N-biose (i.e. Galβ1,4GlcNAcα1,4-branch by an L-fucose group α1,3-linked to the penultimate GlcNAc residue (as in Fuc-Tri), but not substitution by a sialic acid group (as in Tri 5) or a bisecting GlcNAc (as in Bis 2).

2) The oligomannose-binding sites and the complex carbohydrate-combining sites are most likely non-overlapping, i.e. the oligomannose-binding site is not part of the complex carbohydrate-combining site. This was indicated by the fact that none of the complex carbohydrates tested is inhibitory in the XSL/yeast mannan precipitation system. On the other hand, the branched mannopentaose, which is by far the best inhibitor of the XSL/mannan precipitation system, is an extremely weak inhibitor of XSL/asialoorosomucoid precipitation, being 3 orders of magnitude less effective compared with the complex carbohydrates; it is also an order of magnitude less effective than it is against mannan precipitation.

Allen (12) characterized the lectin from A. maculatum as being inhibited (hemagglutination activity) primarily by N-acetyllactosamine, but not by lacto-N-biose. Our data (Table III) clearly indicate that in contrast to the A. maculatum lectin, glycoprotein precipitation by the Xanthosoma lectin is inhib-
peated by N-acetyllactosamine and lacto-N-biose only when they occur at the nonreducing termini of complex N-linked glycans, but not by the free disaccharides (up to 100 mM).

Although the gene(s) for the *Xanthosoma* lectin has not yet been cloned, the conclusion that the lectin is a heterotetramer of two different subunits of nearly identical size, but with distinct N-terminal amino acid sequences, is supported by cloning and sequencing of the genes of closely related species, especially *C. esculenta* (11). The sequence contains an open reading frame comprising a 24-amino acid leader sequence followed by an 11-amino acid sequence identical to the X. sagittifolium lectin α-subunit N-terminal sequence; 140 residues from the initiation site is found a sequence identical to the N-terminal sequence of the β-subunit. Two clones from *A. maculatum* show highly homologous, but not identical sequences similarly placed. Thus, these aroid lectins are most likely synthesized as a single 27-kDa peptide that undergoes post-translational cleavage of the 24-residue leader sequence and a second cleavage near the center into α- and β-subunits of nearly identical size, which probably remain associated and assemble into the (αβ)₂-heterotetramer before or after the cleavage.

We have observed that other aroid lectins, including those from *A. maculatum* and *C. esculenta*, are also precipitated by both asialoglycoproteins and yeast mannan,² suggesting that the presence of two distinct types of binding sites might be a general characteristic of the Araceae lectins. It is likely that the two types of sites reside specifically in the α- or β-subunits (or their juxtaposition) of the heterotetramer, but the relationship between the different subunits and the distinct binding sites has yet to be resolved.

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