Purification and Characterization of Potato Lectin*

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Potato lectin (Solanum tuberosum agglutinin, STA), purified by affinity chromatography on tri-N-acetylchito-
triose-Sepharose 6B, has \( M_r \sim 100,000 \), as estimated by gel filtration on Sephadex G-150 and is an aggregat-
ing system with a monomer \( M_r = 54,000 \), as estimated by sedimentation equilibrium analysis. Equilibrium di-
alysis showed that STA (dimer) has two binding sites for a specific sugar per molecule. STA has a high con-
tent of sugar, most of which is L-arabinose, and is rich in Hyp and Cys. On interaction with specific sugars, STA induced a UV difference spectrum having positive peaks at 292 and 285 nm characteristic of tryc
tophyl residues. The association constants with chitin oligosaccharides, determined from the intensities of the dif-
fERENCE spectra at various concentrations of sugars, increased with increasing chain length of the sugar. Asso-
ciation constants obtained by frontal affinity chroma-
matography of chitin oligosaccharides with STA-Sepharose were in good agreement with those obtained by dif-
ference spectra, whereas the association constants obtained by frontal affinity chromatography of STA
with di- and tri-N-acetylchitotriose-Sepharose were much higher, presumably owing to the effect of multi-
valency of ligands. The CD spectra of STA in the far UV region indicate the presence of 40% of \( \beta \) and 60% of unorder
d form, and no \( \alpha \)-helix conformation, which supports the structure suggested by the amino acid com-
position and the high content of sugar.

Lectins are proteins or glycoproteins of plant and animal origin that bind specific carbohydrates and agglutinate cells of various types (2). Blood group-nonspecific potato lectin, STA, was first described by Marcusson-Begun (3) and its partial purification was first reported by Marinovich (4).

STA is specifically inhibited in its hemagglutination reac-
tion by oligosaccharides that contain \( \beta \)-linked N-acetyl-D-
glucosamine residues (5, 6). STA has a specificity similar to but not identical with WGA, which is also inhibited by oligosaccharides containing \( N \)-acetyl-D-glucosamine. WGA, but not STA, binds with free sialic acid (7) or sialyl residues in glycoproteins (8) and glycolipids (9). Recently, we have found that STA, but not WGA, binds specifically with keratan sulfate and chitin sulfate, despite the presence of sulfate groups (10).

The complete purification of STA accomplished by Allen and Neuberger (11) required time-consuming steps, including gel filtration and ion exchange chromatographies. Purified STA was found to be an unusual glycoprotein with a high content of half-cystine and hydroxyproline residues, with L-arabinose as the major sugar component. Allen et al. (12) and Muray and Northcote (13) reported that almost all hydroxy-
proline residues in STA are substituted with L-arabinose residues, that the average hydroxyproline-to-arabinose ratio is 1.3-4, and that L-arabinose is present in the \( \beta \)-linked furan-
oid form.

Delmotte et al. (14) first described an effective adsorbent for the affinity chromatography of STA by coupling 4-amino-
benzyl-2-acetamido-2-deoxy-1-thio-\( \beta \)-D-glucopyranoside to Sepharose by a long spacer group. However, the preparation of this adsorbent required a five-stage synthesis. A more convenient method (15, 16) for preparing an affinity adsorbent of STA has been described. Tri-N-acetylchitotriose, a sugar specific for STA, was coupled with epoxy-activated Sepharose prepared by treatment with epichlorohydrin (15, 16). Desai and Allen (17) reported a similar method using bis(oxirane) as the reagent for epoxy activation. Subsequently, two meth-
ods of purification of STA, by affinity chromatography on fetuin-Sepharose (18) and on glutaraldehyde-fixed erythro-
cytes (19), have been reported. The properties of STA purified by (GlcNAc)\( \tau \)-Sepharose, especially the spectroscopic prop-
erties and the specific interactions between STA and oligosaccharides, are described herein.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**DISCUSSION**

Most lectins are usually extracted with PBS at a pH of about 7. When potatoes were extracted with PBS, the color of
the extract turned rapidly to brown, and a black precipitate finally formed. Allen et al. (12) added sodium hydrogen sulfite to a PBS extract of potatoes to prevent browning. However, we found that STA is unstable under reductive or oxidative conditions, and is easily inactivated by sodium hydrogen sulfite. On the other hand, STA is very stable under acidic or alkaline conditions. When potatoes were extracted with 3 M acetic acid, the browning was prevented, and the hexosaminidase in the extract was inactivated, but not STA. The crude STA fraction obtained by ammonium sulfate fractionation of potato extract was free from browning, even if it was thereafter solubilized in PBS. When crude STA was applied to a (GlcNAc)_2-Sepharose column, it was completely adsorbed and could be eluted with 0.2 M NH_4OH. Purified STA was easily obtained when active eluted fractions were directly lyophilized. Since the total purification procedures were short and simple, the overall recovery of STA from the extract was very high, as shown in Table I, when compared with those reported earlier (11, 12, 14, 39, 40).

The purified STA was found to be homogeneous on SDS-polyacrylamide gel electrophoresis and gel filtration, and its molecular weight was estimated to be about 100,000. This value is in good agreement with the value of 120,000, previously reported by Allen and Neuberger (11) for STA purified by conventional chromatographies. Sedimentation-equilibrium centrifugation showed that STA exists in an aggregating system, and the molecular weight of the STA monomer was estimated to be 54,000, which is slightly higher than the values of 50,000 (12) and 46,000 (11) reported earlier. Equilibrium dialysis showed that STA has 2 binding sites/molecule (dimer), assuming M = 100,000. These results suggest that STA exists in a monomer-dimer system, and acts as a dimer in the hemagglutination reaction.

The STA described herein has a high content of carbohydrate (50%), most of which (89%) is L-arabinose, and a high content of hydroxyproline and half-cystine, as reported by Allen and co-workers (11, 12). The stability of STA, which is resistant to heat, base, and acid, may depend on its high content of carbohydrate residues and disulfide bonds. Inactivation of STA by sodium hydrogen sulfite may be due to the cleavage of disulfide bonds, which are essential for the conformation of STA.

Analysis of the CD spectrum gave various results, depending upon the method applied. Calculation by the method of Chen et al. (34) showed that STA has 40% β-form, 60% random coil, and no α-helix. The analysis of the CD spectrum of lysozyme by the same methods showed results similar to those obtained by x-ray crystallography (40). The content of β-form can be calculated from the value of [θ] at 195 nm, using a value of 30,000 (40) or 40,000 (41) for a model substance presumably having 100% of pleated sheets. Since STA has a [θ] value of 10,000 at 195 nm, it may be assumed that STA contains 25-35% of β-structure. The content of α-helix was also calculated by the method of Brahms et al. (42); H = \[\theta_{222}/(-40,000) \times 0.06\]. These results also showed the high content of β-structure and absence of α-helix in STA. As shown in Table II, STA has a high content of Hyp, Pro, Gly, Ser, and Cys residues, which are incompatible with α-helices. These results also support the unusual conformation suggested by the analysis of the CD spectrum of STA. Although analysis of the CD spectrum using standard data for the collagen helix given by Hayashi et al. (36) showed the existence of collagen helix, STA was not digested by the collagenase from Clostridium histolyticum. The results suggest that STA has no sequences corresponding to X-Gly-Pro-Y-Gly-Pro. (Hyp + Pro)/Gly in STA was about 3.2 which is higher than that of collagen but is close to the values for proline-rich proteins 2 and 3 (43-48). CD spectra of these proline-rich proteins were reported to be similar to those of polypeptide II (45-48). The CD spectra of many lectins have been reported. Ricinus communis agglutinin contains 13% α-helix and 5% β-form (49); Bandiera simplicifolia lectin I is 30-40% β-form (50); Pisum sativum lectin a β and unordered structure (45); and WGA has no α-helix, is 12% β-structure, and probably has some β-turns (52). From the mean residue ellipticity at 195-197 nm, the lectins of Dolichos biflorus, Helix pomatia, Lotus tetragonolobus, Phaseolus vulgaris (erythrogglutinin), Sophora japonica, and Ulex europaeus (l) were estimated to have 28-48% β-structure. Therefore, many plant lectins contain a rather large amount of β-structure, and STA is no exception in this respect.

Some lectins, such as Arachis hypogaea agglutinin (peanut lectin) (53, 54), concanavalin A (55), Lens culinaris hemagglutinin (53), S. japonica agglutinin (54), and Vicia cracca agglutinin (56), show UV difference spectra upon binding with specific sugars. Spectrophotometers having a high sensitivity have recently made it possible to obtain the association constants of lectins with specific sugars (53, 54). In the case of STA, difference spectra with two positive peaks at 292 and 285 nm and a shoulder at 275 nm were induced by chitin oligosaccharides (Fig. 4). The highest peak was at 292 nm, and only one shoulder was observed at 275 nm, in contrast with the spectra obtained with WGA, where peaks at 285 (highest), 292, and 275 nm have been observed (53); this suggested that the microenvironment of the tryptophan residues was perturbed by binding to specific sugars (57) and that the contribution of tyrosine residues is low. The value of Δε_{max} reflects the number of tryptophan residues perturbed by the binding (58). The number of perturbed tryptophan residues in STA was calculated to be two to four. The value of Δε_{max} increased with chain length of the sugar, and with the binding constant (Table III), as observed with lysozyme with chitin oligosaccharides (59).

Modification of the near-UV-CD spectra by the binding of a specific sugar has been observed for other lectins; Arachis hypogaea agglutinin (60), R. communis agglutinin (49), and WGA (52) display changes of the near-UV-CD bands upon interaction with specific sugars, which indicates alteration of the asymmetric environment at aromatic side chain chromophores on the surface of the lectin molecule. The modification of the CD spectrum of WGA at 272 and 285 nm probably involvestyroline residues and, at 290 nm, tryptophan residues. In the case of STA, (GlcNAc)_2, and (GlcNAc)_n, but not GlcNAc, induced a decrease of the CD intensity at 250-300 nm. The intensity of the CD band at 291 nm involving tryptophan residues was decreased more strongly by (GlcNAc)_n than by (GlcNAc)_2, as shown in Fig. 11. These results indicate that tryptophan residues in STA are perturbed more strongly by (GlcNAc)_2 than (GlcNAc)_n, but not by GlcNAc.

As shown in Table IV, the binding constants of STA with chitin oligosaccharides obtained by UV difference spectroscopy were in good agreement with those obtained by frontal analysis on STA-Sepharose, and much lower than those obtained by frontal analysis on (GlcNAc)_n-Sepharose. The binding capacity of STA-Sepharose was calculated to be 40-70% of that calculated from the amount of immobilized STA, while the capacities of (GlcNAc)_n-Sepharose were about 1:150 of those estimated from the amount of immobilized ligands. Since the concentration of the immobilized sugars is very high (30 μmol/ml of gel), not all ligands may interact with a large molecule of STA owing to steric hindrance, but they may contribute to the very high apparent binding constant through...
### TABLE IV

**Association constants of STA with chitin oligosaccharides**

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>(GlcNAc)_2</th>
<th>(GlcNAc)_3</th>
<th>(GlcNAc)_4</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV difference spectrum</td>
<td>2.0 × 10^5</td>
<td>4.1 × 10^4</td>
<td>5.4 × 10^3</td>
</tr>
<tr>
<td>(2200)</td>
<td>(3000)</td>
<td>(3600)</td>
<td></td>
</tr>
<tr>
<td>Frontal analysis by STA-Sepharose column</td>
<td>6.3 × 10^3</td>
<td>4.1 × 10^4</td>
<td>8.3 × 10^3</td>
</tr>
<tr>
<td>Frontal analysis by oligosaccharide-Sepharose column</td>
<td>6.5 × 10^3</td>
<td>1.5 × 10^3</td>
<td></td>
</tr>
</tbody>
</table>

*Values in parentheses are ΔE_m, the so-called multivalent effect.* The binding constant between STA and chitin oligosaccharides increased with chain length, as was observed for WGA. However, the binding constants of (GlcNAc)_2 and (GlcNAc)_3 for STA were larger than those for WGA and the binding constant of (GlcNAc)_4 for STA was smaller than that for WGA (61). This tendency was shown also by hemagglutination studies (11, 62, 63, 64). These results suggest that STA has a larger binding site than WGA.

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**REFERENCES**


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**EXPERIMENTAL PROCEDURES**

Materials—Potato lectin (PL) was purified from Aleuria aurantia by the method described in a previous report. The materials used were freshly prepared from Aleuria aurantia. PL was also purified by the method of Farrbanks et al. (15).

**Analytical Methods**—The analytical methods used include gel filtration, polyacrylamide gel electrophoresis, and chromatography.

**RESULTS**

**Purification of STX**

The purification procedure for STX was carried out as described in a previous report. The yield of STX was determined by the method of Farrbanks et al. (15).

**Analysis of Neutral Sugar Composition**

The composition of neutral sugars was determined by the method of Farrbanks et al. (15). The results are presented in Table I.

**Table I**

<table>
<thead>
<tr>
<th>Neutral Sugar</th>
<th>wt (%)</th>
<th>Carbohydrate wt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Gal</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Fuc</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Xyl</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Ara</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>GalNAc</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>FIu</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49.5</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The isolation of STX from potato tubers was carried out as described in a previous report. The isolation of STX was performed by the method of Farrbanks et al. (15). The isolation of STX was carried out as follows: 1. The potato tubers were ground to a fine powder and suspended in 0.1 M phosphate buffer (pH 7.2). The suspension was centrifuged at 10,000 g for 30 min, and the supernatant was collected. 2. The supernatant was dialyzed against 0.1 M phosphate buffer (pH 7.2) for 30 min. 3. The dialyzed supernatant was applied to a column of Sephadex G-25. The elution profile was monitored by absorbance at 280 nm. 4. The fractions containing STX were pooled and concentrated by lyophilization.

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*References and footnotes are omitted for brevity.*
Fig. 5. Effect of the concentration of (GlcNAc)_2 on the difference spectra of STA.

Fig. 6. Plot of [S]/[I] vs. (GlcNAc)_2 concentration. Theotic line was calculated from a least squares fit to the data. The dissociation constant K_d was calculated from the intercept on the abscissa.

Affinity Chromatography of Ovado STA on (GlcNAc)_2-Sepharose 4B.

A solution of ovado STA in a buffer of pH 7.4 was continuously applied to each column until STA began to pass through the column. This was reached with less than 0.05% of the solution for a (GlcNAc)_2-Sepharose column, whereas in the present case the concentration of (GlcNAc)_2 required much larger amounts of STA solution, 400 and 250 μl, respectively. The amount of STA adsorbed on the column increased with the increase of chain-length of the sugar ligand. These amounts may be used as a measure of the adsorption capacity of the column, although each column was not completely saturated with STA.

Fig. 7. Affinity chromatography of crude STA on (GlcNAc)_2-Sepharose 4B. Affinity chromatography of crude STA was performed on a column of (GlcNAc)_2-Sepharose 4B. The column was washed with 200 μl of 0.1 M GlcNAc, 0.1 M NaCl, 0.5 M NaCl, and 0.9 M NaCl, and an elution mixture was added (a) 0.1 M NaCl, (b) 0.5 M NaCl, (c) 1 M NaCl, (d) 1.5 M NaCl, (e) 2 M NaCl, (f) 3 M NaCl, and (g) water. The elution profile is shown in the figure.

Protein Affinity Chromatography—The solution of cholera oligosaccharides from a column of (GlcNAc)_2-Sepharose 4B was eluted with increasing sugar concentration. Peaks of [STA]_2 vs. [GlcNAc]_2 concentration for the frontal affinity chromatography of (GlcNAc)_2-Sepharose are shown in Fig. 1.
The association constant of STA for (GlcNAc)$_2$, (GlcNAc)$_3$, and (GlcNAc)$_4$ was calculated to be 6.3 x 10$^7$, 1.3 x 10$^7$, and 8.3 x 10$^6$, respectively. The dissociation constant of STA-Sepharose for (GlcNAc)$_2$, (GlcNAc)$_3$, and (GlcNAc)$_4$, respectively, the values of association constant calculated were 46 - 48% of that obtained from the concentration of immobilized STA, and they increased with a decrease in the size of the sugar molecule.

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