Protoplasts derived from developing soybean cotyledons were used to study substrate recognition by a sucrose transporting protein in plant membranes. When used as alternate substrate inhibitors of \(^{14}C\) sucrose influx, five different fructosyl-substituted sucrose derivatives, phenyl-\(\alpha\)-D-glucopyranoside, and phenyl-\(\alpha\)-D-thioglycopyranoside proved to bind effectively to the sucrose carrier-active site. These results are interpreted to indicate that a large portion of substrate recognition by this carrier may arise from the interaction of a relatively hydrophobic portion of the sucrose molecule and a hydrophobic region of the carrier protein binding site. Binding of phenyl-\(\alpha\)-D-thioglycopyranosides in which various substitutions were made for the glucosyl hydroxyls at positions 3, 4, and 6 are involved in substrate recognition by the carrier protein.

Sucrose is the major form of transported carbon in many plant species and is actively transported across cell membranes in several tissue types (1, 2). The carrier protein responsible for this transport is quite specific to sucrose. Using monosaccharides or other disaccharides as competitive inhibitors, workers using several tissue types have found only very weak competition with a very limited number of disaccharides (3, 4). This specificity suggests that either a large portion of recognition lies with the fructose moiety, as few \(\beta\)-fructofuranosides have been tested for binding, or that elements of both the glucose and fructose rings are involved in binding.

Proteins that selectively bind carbohydrates must rely on the spatial arrangement of hydroxyl groups for substrate specificity. Recognition of a particular hydroxyl group can occur by hydrogen bonding between amino acid side chains or amide oxygens in the polypeptide. Alternatively, stereoisomers can be discriminated by requiring the absence of the hydroxyl at one position in a chiral center. Many sugars have surface areas that are largely free of hydroxyls or on which hydroxyl groups are intramolecularly hydrogen-bonded and are therefore hydrophobic in character. If a hydrophobic pocket in a carbohydrate binding site of a protein exists to match this hydrophobic surface, it can eliminate the opposite and incompatible stereoconfiguration.

We have used a combined chemical and enzymic synthesis approach to produce a series of singly substituted sucrose derivatives (5, 6) and have tested the binding of these alternate substrates and of several phenylglycosides to a sucrose carrier protein in protoplasts isolated from developing soybean cotyledons. We interpret the binding and, in selected cases, transport of the substrate analogs to indicate that a large portion of substrate recognition by this carrier comes from a hydrophobic interaction of sucrose with its binding site along with hydrogen bonding to a small number of hydroxyls. The type of binding observed is similar to that described for certain carbohydrate-antibody interactions (8, 9).

**EXPERIMENTAL PROCEDURES**

**Synthesis of Alternate Substrate Inhibitors**—Alcohols and thiols were purchased from Aldrich. Carbohydrates and carbohydrate derivatives were purchased either from Sigma or Pfandl Laboratory. Inc. \(^{14}C\)Sucrose and \(^{14}C\)glucose were purchased from New England Nuclear. Melting points were taken on a Haer capillary melting point apparatus and are uncorrected. 360 MHz NMR were run in CDC\(_3\) on a Nicolet NT WB 360 \(^1\)H NMR. Solvents were evaporated under vacuum at below 45 °C.

Phenyl-\(\alpha\)-D-thioglycopyranosides were synthesized by refluxing the corresponding peracetylated glycoside in CH\(_2\)Cl\(_2\) containing 5% BF\(_3\) and a 2-fold excess of thiophenol for 2-18 h. After the BF\(_3\) was destroyed by the addition of saturated sodium bicarbonate solution, the CH\(_2\)Cl\(_2\) phase was separated, washed with water, dried over Na\(_2\)SO\(_4\), and the solvent removed. The \(\alpha\)-anomer was separated from the reaction products by flash chromatography (16) on silica with 3:2 (v/v) ether/hexane as the eluant, characterized by its 360 MHz \(^1\)H NMR spectrum, and then deacetylated with approximately 10 mM sodium methoxide in 1:1 (v/v) ether/hexane.

Benzyl-, phenylethyl-, and phenylpropyl-\(\alpha\)-D-glucopyranosides and benzyl- and phenylpropyl-\(\alpha\)-D-thioglycopyranosides were synthesized as described above using penta-acytely-\(\beta\)-D-glucose and the corresponding phenol or thiophenol. Both phenylpropyl glucosides were separated from the reaction mixture by preparative high pressure liquid chromatography on a 21.2 mm \times 25-cm silica column eluted with 3:2 ether/hexane at 10 ml min\(^{-1}\). The purified \(\alpha\)-anomers were deacylated in the usual way.

Combustion analysis and melting point when available are given in Table I for all the glycosides synthesized. References to the synthesis of the mono-fluorinated glucose derivatives and to certain deoxyglucoses are given in Refs. 10 and 11.

Details of the synthesis and physical characterization of the sucrose analogs shown in Fig. 1 are given elsewhere (5, 6). In general, the substituted fructoses were prepared and coupled to UDP-glucose in a reaction catalyzed by sucrose synthase. Yield at the coupling step ranged from 90% for 1-deoxy-1'-fluorofructose to 15% for 1-deoxy-1'-azidosucrose. 1'-Deoxy-1'-aminosucrose was prepared by the catalytic hydrogenation of 1'-deoxy-1'-azidosucrose using 10% palladium on carbon in 80% ethanol at 5 ps i. H\(_2\). Cellulose thin layer chromatography indicated complete conversion to a single, ninyhdrin-positive product after 4 h at 21 °C.

The synthesis of \([\text{glucosyl-}\(^{14}C\)]\)phenyl-\(\alpha\)-D-thioglycopyranoside was carried out as follows. \(^{14}C\)Glucose (2.14 pmol at 46.6 pCi/pmol) in 10 \(\mu\)l of pyridine was acetylated with 10 \(\mu\)l of acetic anhydride at 42 °C for 3 h. The pyridine and excess acetic anhydride were removed under an \(N_2\) stream at 42 °C, and 5.6 \(\mu\)mol of thiophenol in 5 \(\mu\)l of CH\(_2\)Cl\(_2\) containing 5% BF\(_3\)/etherate were added. After 4 h at 42 °C, a small amount of finely powdered NaHCO\(_3\) was added to neutralize the BF\(_3\). The reaction mixture was diluted with an additional 20 \(\mu\)l of CH\(_2\)Cl\(_2\) and applied to a silica TLC plate. After development in 3:2 ether/hexane, the plate was autoradiographed, and the areas corresponding to the \(\alpha\)- and \(\beta\)-anomers of tetra-\(\alpha\)-acetyl-phenyl-thioglycopyranoside were scraped from the plate and eluted with CH\(_2\)Cl\(_2\),
**TABLE I**

Names, structures, analytical data, and $K_i$ for inhibition of 0.2 mM [14C]sucrose influx for alternate substrate phenylglycosides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>$k$ for inhibition of 0.2 mM sucrose influx</th>
<th>Melting point</th>
<th>Combustion analysis (d)</th>
</tr>
</thead>
</table>
| Phenyl-$
\text{g}$-D-thioglucopyranoside            | ![Structure](image) | 0.35                                        | (c)           | (c)                    |
| Phenyl-$
\text{g}$-D-2-deoxy-thioglucopyranoside | ![Structure](image) | 0.40                                        | 105-106       | (C, 56.23; H, 6.29)    |
| Phenyl-$
\text{g}$-D-thiomannopyranoside          | ![Structure](image) | 10.1                                        | 167-168       | ---                    |
| Phenyl-$
\text{g}$-D-3-deoxy-thioglucopyranoside | ![Structure](image) | 13.9                                        | 139-139.5     | (C, 56.23; H, 6.29)    |
| Phenyl-$
\text{g}$-D-3-deoxy-3-fluoro-thioglucopyranoside | ![Structure](image) | 14.3                                        | 118-121       | (C, 52.54; H, 5.5)     |
| Phenyl-$
\text{g}$-D-thioallopyranoside          | ![Structure](image) | 10.2                                        | ---           | ---                    |
| Phenyl-$
\text{g}$-D-4-deoxy-thioglucopyranoside | ![Structure](image) | 4.7                                         | ---           | C, 56.23; H, 6.29      |
| Phenyl-$
\text{g}$-D-4-deoxy-4-fluoro-thioglucopyranoside | ![Structure](image) | 1.8                                         | 82-84 (d)     | (C, 52.54; H, 5.5)     |
| Phenyl-$
\text{g}$-D-thiogalactopyranoside       | ![Structure](image) | 10.5                                        | (c)           | (c)                    |
| Phenyl-$
\text{g}$-D-6-deoxy-thioglucopyranoside | ![Structure](image) | 1.5                                         | 76.5-78 (d)   | C, 56.13; H, 5.80 (d)  |
| Phenyl-$
\text{g}$-D-6-deoxy-6-fluoro-thioglucopyranoside | ![Structure](image) | 1.6                                         | 90.5-92 (d)   | (C, 53.99; H, 5.29 (d) |
| Phenyl-$
\text{g}$-D-thio-xylopyranoside        | ![Structure](image) | 4.4                                         | ---           | ---                    |
| Benzyl-$
\text{g}$-D-thioglucopyranoside       | ![Structure](image) | 0.22                                        | 133-136       | (C, 54.33; H, 6.34)    |
| Benzyl-$
\text{g}$-D-glucopyranoside           | ![Structure](image) | 0.85                                        | 113-117       | (C, 57.77; O, 35.52)   |
| Phenylthethyl-$
\text{g}$-D-glucopyranoside    | ![Structure](image) | 0.30                                        | 78-82         | (C, 59.14; O, 33.96)   |
| Phenylpropyl-$
\text{g}$-D-thioglucopyranoside  | ![Structure](image) | 0.18                                        | 94-97         | (C, 57.10; H, 7.05)    |
| Phenylpropyl-$
\text{g}$-D-glucopyranoside     | ![Structure](image) | 0.25                                        | ---           | C, 60.19; O, 32.18     |

* Hydroxyl groups are indicated by the ends of unmodified, straight lines. Hydrogen atoms are omitted.

**Values calculated for the empirical formula are on the top line; analytical values are on the second line of each entry.**

* Analytical data were equivalent to literature values.

* Analytical data were obtained on the corresponding, peracetylated compound.
Substrate Recognition by a Sucrose Transporting Protein

**FIG. 1.** The structures of the singly substituted sucrose derivatives used as alternate substrate competitors of [14C]sucrose influx into protoplasts from developing soybean cotyledons.

The β-anomer was reduced to dryness, reanomerized in 5 μl of CH2Cl2 containing 5% BF3/etherate, and again chromatographed as above. The tetra-O-acetyl-phenyl-a-D-thioglucopyranoside from four anomersizations was pooled, decacylated with 10 mM sodium methoxide in 1:1 methanol/ether, spotted on TLC, and developed in 9:1 ethyl acetate/methanol. The [glucosyl-14C]phenyl-a-D-thioglucopyranoside was located by autoradiography, eluted from the plate, and stored in methanol.

Measurement of Sucrose Influx into Protoplasts from Developing Soybean Cotyledons—The isolation of protoplasts from the cotyledons of developing soybean seeds has been described by Lin et al. (12). [14C]Sucrose influx was measured after 30 min of incubation at 30 °C in 0.5 ml of a media consisting of 0.5 M sorbitol, 25 mM MES-KOH buffer at pH 6.0, 0.5 mM CaCl2, 0.2 mM [14C]sucrose at 5 μCi/μmol, and the desired concentration of an alternate substrate. Two samples of approximately 220 μl each were taken, and the protoplasts were separated from the media by the silicone oil centrifugation technique described previously (8). Total 14C and protein in each subsample were also determined as described (8).

Influx of [glucosyl-14C]phenyl-a-D-thioglucopyranoside—The influx of either [14C]sucrose or [14C]phenyl-a-D-thioglucopyranoside ([14C]phenylthioglucopyranoside from 0.2 mM solutions at 1 μCi/μmol) was measured at intervals over a 45-min incubation period as described above. The kinetics of [14C]phenylthioglucopyranoside influx were determined after 30 min of incubation in various concentrations of phenylthioglucopyranoside in 0.5 ml of the above assay media containing 0.08 μCi of [14C]phenylthioglucopyranoside.

**RESULTS AND DISCUSSION**

Affinity of Substituted Sucrases for the Sucrose Carrier Protein in Soybean Cotyledon Protoplasts—Sucrose influx into protoplasts derived from developing soybean cotyledons occurs by at least three pathways acting simultaneously (4). Of these pathways, the one that predominates at sucrose concentrations below about 10 mM is carrier mediated, saturable with respect to sucrose binding, and seems likely to involve sucrose-proton cotransporting protein (12). Sucrose and the sucrose analog 1'-deoxy-1'-fluorosucrose compete for the same carrier binding site and have the same Vₘₐₓ for influx; however, the Kₘ for 1'-deoxy-1'-fluorosucrose was one-half that of the natural substrate (7). The altered Kₘ for sucrose influx is thus most simply ascribed to an altered binding affinity of the substrate for the external sucrose binding site of the carrier protein.

Fig. 2 is the plot of (V/Vₘₐₓ)⁻¹ versus concentration of the sucrose analogs of Fig. 1 when used as alternate substrate inhibitors of [14C]sucrose influx. Vₘₐₓ is the influx of 0.2 mM [14C]sucrose in the absence of unlabeled alternate substrate and Vₙ is the influx in the presence of inhibitor. At substrate concentrations that are low compared to the Kₘ for sucrose (2.2 mM), the slope of the resulting line is 1/Kₘ for the alternate substrate. If Vₙ remains unaltered in each case as it did for the 1'-fluoro and 6'-fluoro substitutions (7), then Kₘ should approximate the protein/substrate dissociation constant Kₘ.

The results are interpreted to suggest that fructose hydroxyls at carbons 1 and 6 are within the binding domain of the protein. Substitution of fluorne, azide, or a hydrogen for the hydroxyl at C-1' or C-6' decreased Kₘ to about 1 mM. Substitution of an amine at C-1' resulted in a doubling of the Kₘ for the resulting sucrose. The hydroxyl at fructose C-4 is either not within the binding surface or the hydroxyl in fluorine substitution was neutral with respect to binding at this position. Fluorine is capable of accepting hydrogen bonds (13), but neither the 6'-deoxy nor the 1'-azido substitutions can participate in hydrogen bonding. Hydrogen bonding is not involved at these positions, although both hydroxyls appear to be on a topographical region of the sugar that associates with the protein. The common feature in the four substitutions that result in increased binding affinity is an increase in hydrophobicity when compared to the hydroxyl group they
replaced. In contrast, the substitution in 1'-deoxy-1'-amino-sucrose is potentially more hydrophilic at the assay pH and results in decreased binding affinity.

Space filling models of sucrose in two views generated from the neutron diffraction spectrum of crystalline sucrose are shown in Fig. 3. Sucrose is quite rigidly held in a wedge-shaped configuration (Fig. 3B). The 1' and 6' hydroxyls (Fig. 3A) are positioned at the vertices of the wedge and define the outer edges of a relatively hydrophobic surface on the molecule (shaded area in Fig. 3A), which is formed by the stereoconfiguration of the hydroxyl groups oriented toward the inside surface. Hydroxyls 2 and 1' are intramolecularly hydrogen-bonded in solution (14) and so normally extend this surface in sucrose. Substitutions at 1' and 6' that increased binding affinity were substitutions that tend to increase the size or hydrophobic nature of this surface. The 4'-hydroxyl lies at the extreme outer surface of the fructose ring and is oriented away from the relatively hydrophobic surface of the molecule.

With the possible exception of the OH-3', none of the hydroxyl groups of fructose appear to participate in intermolecular bonding with the carrier protein. Rather, the fructose moiety of sucrose represents a large portion of the hydrophobic surface that interacts with the binding site.

Binding of Glucopyranosides and Thioglucopyranosides---
From the above observations, it seemed possible to mimic the fructose portion of sucrose by a nonhydroxylated, hydrophobic surface that can be correctly oriented in the molecule with respect to glucose. Two possible substrates with these traits are phenyl-α-D-glucopyranoside and phenyl-α-D-thiogluco-

Plots of (V/V0)-1 for 0.2 mM sucrose influx in the presence of various concentrations of the two glucosides is shown in Fig. 4. Both were apparently competitive inhibitors with Ki values of 2.1 and 0.3 mM for the O- and S-glucoside, respectively. To recheck the competitive nature of binding by phenylthioglucopyranoside, the influx characteristics of [14C]phenylthioglucopyranoside were determined. [14C]Phenylthioglucopyranoside was accumulated by the soybean cotyledon protoplast in a manner that was linear with time for at least 40 min, and that influx was greatly reduced by addition of 15 μM carboxyl cyanide p-trifluoromethoxyphenylhydrazone to the incubation medium (data not shown). The concentration dependence of [14C]phenylthioglucopyranoside influx is shown in Fig. 5 both with and without the inclusion of 10 mM sucrose in the incubation medium. Double-reciprocal transformation of the data of Fig. 5 yields a Km for phenylthioglucopyranoside of 0.32 mM, essentially the Ki calculated when that substrate was used as an inhibitor of [14C]sucrose influx. The Ki calculated for sucrose inhibition of phenylthioglucopyranoside influx was also equal to the Km of the carrier for sucrose influx. From these observations, we conclude that phenylthioglucopyranoside is transported at the same site as sucrose in these protoplasts. One further observation from the data of Fig. 5 is of note. The Vmax observed for this substrate (35 nmol 10^-6 protoplasts h^-1) was nearly the same as that usually observed for sucrose. Therefore, a 7-8-fold change in Km occurred without a change in Vmax, and the difference in Km between the two substrates is most likely due to an increased binding affinity.

Although it seems clear from these results that phenylthiogluco-
pyranoside acts as an analog for sucrose in recognition by the carrier protein, we cannot state that the phenyl moiety specifically mimics portions of the fructose ring. The phenyl ring of either the O- or S-glucoside can rotate with respect to the glucose ring, and that allows the possibility that binding occurs due to glucose binding at its usual site in the disaccharide binding pocket, whereas the phenyl ring occupies a hydrophobic region near that site but not necessarily related to

**Fig. 3.** A, a computer-generated space filling model of crystalline sucrose as viewed toward the β-face of the glucose ring and the α-face of the fructose ring. The shaded area indicates the relatively hydrophobic region, which may allow binding to a reciprocal surface on the carrier protein. B, the view of the space filling model toward the C-2 and C-1' regions showing the wedge shape of the overall structure.
the normal fructose binding region.

Two lines of evidence support the contention that the phenyl ring of phenyl glucosides does behave as an essential, hydrophobic surface for binding, as does fructose. First, it can be seen from molecular overlap modeling of the structure of sucrose and phenylthioglucopyranoside (Fig. 6) that the C-1 to S and S to C-1' bonds of phenylthioglucopyranoside can be rotated so that good spatial alignment is obtained between the fructose and phenyl rings of the two structures. The preferred conformation in solution for hydrophobic aglycons is likely to be in a straight line away from the hydroxylated portion of the carbohydrate (15); however, the conformation in Fig. 6 is also possible. Both the larger size of the sulfur atom compared to oxygen and the larger bond angle of the C to S bond versus the C to O bond make adoption of this conformation more favorable in the S-glucoside. The much tighter binding of phenylthioglucopyranoside relative to its O analog may be a result of this conformation occurring more frequently in solution.

One test of this postulate is to provide potential substrates with additional flexibility in the connection between the glucose ring and the phenyl moiety. A series of α-glucopyranosides and α-thioglucopyranosides with methylene chains of increasing length (e.g. benzyl, phenylethyl, phenylpropyl) between the glucose moiety and the phenyl ring were synthesized and tested as inhibitors of sucrose influx. The results are summarized in Fig. 7. Although increasing the length of the methylene chain decreased $K_i$ in both the O- and S-glucoside series, the decrease was greatest with the addition of the first and to a lesser extent the second methylene into the O-glucosides. Since neither methyl-α-D-glucopyranoside nor ethyl-α-D-thioglucopyranoside were inhibitors, and isobutyl-α-D-thioglucopyranoside was only a poor inhibitor (data not shown), it is unlikely that the methylene chain itself causes the tighter binding of the substrates. As well, the increased binding affinity does not seem to be a simple function of increased length between the glucose and phenyl ring. Although that length is increasing over the series, it increases in both the S and O series, yet binding affinity increases much more in the O-glucosides. The fact that no further increase in binding affinity occurs after the third methylene is added may also suggest that more degrees of freedom in orientation of the glucose and phenyl rings is the causal factor in increasing affinity rather than the distance between the rings, although it is troublesome that poorer binding does not occur after an “optimal” chain length for flexibility is reached.

**Binding of Substituted Phenyl-α-D-thioglycosides—** The results shown in Fig. 5 suggest that the glucose moiety of phenylthioglucopyranoside represents a model for the glucose moiety of sucrose during binding to the carrier protein in this system. As such, phenylthioglycosides with modifications at the glucose hydroxyls may be used to test the interaction of the glucose hydroxyls with the carrier binding site. We chose to make three different modifications in an attempt to characterize possible interactions at each hydroxyl. Hydrogen bonding can be eliminated by formation of the deoxythioglycoside at each position, whereas substitution of a fluorine for the hydroxyl should allow hydrogen bonding if the protein donates the hydrogen to the substrate. The importance of hydrophobic interactions with the three-dimensional structure can also be assessed by testing binding of the opposite stereoconfiguration at chiral carbons bearing a free hydroxyl.

The $K_i$ values for 11 phenylthioglycosides when used as competitive alternate substrates for the inhibition of $[^{14}C]$ sucrose influx into soybean cotyledon protoplasts are summarized in Table I. Overall, it is clear that a large portion of substrate recognition by this carrier resides in the glucose moiety as only one substituted glucose (2-deoxy) retained full binding efficacy in the resulting glycoside (the $K_i$ for phenylthioglucopyranoside averaged 0.4 mM in these studies). Specifically, at the 2-position hydrogen bonding does not occur, but the face of carbon 2 which bears the hydrogen is apparently involved in the hydrophobic interaction since replacement of the hydrogen by a hydroxyl (manno-configuration) eliminates the phenylglycoside as a substrate. The interaction at 3-OH apparently occurs by donation of the hydrogen on that hydroxyl to an acceptor on the protein since neither the deoxy nor the fluorine were acceptable substitutes for the hydroxyl. Inversion of the configuration at the 3-position (allo-configuration) may remove the hydroxyl from the proximity of the accepting group, since this substitution greatly reduced binding affinity also. The hydrophobic interaction of the β-face of glucose must extend to the region of C-4 since replacement of the hydrogen at this position by an OH (galacto-configuration) also eliminated the structure as a substrate, presumably by placing an incompatible functional group into the binding surface.

Assessment of the interaction of the hydroxyls at C-4 and C-6 is more complex, since no other substitutions at these positions resulted in complete loss or complete retention of activity. It is possible that this portion of the molecule is recognized as an intramolecularly hydrogen-bonded surface.

![Fig. 6. A molecular overlap model for sucrose and phenyl-α-D-thioglycosyranoside. The glucose moieties of the two glucosides were aligned and the C-1 to O and O to C-1' bonds of sucrose were held constant as in Fig. 3. The C-1 to S and S to C-1' bonds of the phenyl ring were then rotated to give maximal spatial overlap of the fructose and phenyl rings.](image-url)
in which the 4-OH and 6-OH are hydrogen-bonded. Removal of either hydroxyl would then disrupt the recognition surface by eliminating this hydrogen bond and leaving the other hydroxyl free and presumably more hydrophilic. This idea seems unlikely inasmuch as one of the two fluorine substitutions should have filled this requirement and resulted in a normal or near-normal substrate. Alternatively, hydrogen bonding may be involved with the hydroxyls of the glycoside donating the hydrogens, but the interactions at these positions are not an absolute requirement for binding.

The C-6 methylene group is also within the hydrophobic surface required for binding since phenyl-α-D-thioglycopyranoside proved to be a poorer substrate than the 6-deoxy derivative.

CONCLUSIONS

The binding properties of the 23 glycosides tested as alternative substrates for the sucrose transporting protein in these protoplasts are consistent with substrate approach to the binding site with the shaded surface shown in Fig. 3B toward the site. Since removal of three of the four hydroxyl groups on the fructose moiety of sucrose gave substrates with apparent binding affinities equal to or better than sucrose, it seems unlikely that fructose participates in binding except as an essential and largely hydrophobic surface. This contention is greatly strengthened since some phenyl glycosides, but not others, are competitive inhibitors of sucrose uptake and since the influx of [14C]phenyl-α-D-thioglycopyranoside is competitively inhibited by sucrose. The phenyl ring of these substrates fills one requirement for binding either by filling the space normally occupied by fructose or by fitting into a non-specific, hydrophobic region of the protein and allowing glucose to properly align in its binding site.

The 3-, 4-, and 6-hydroxyls of glucose all participate in binding, apparently by donating their hydrogen to the protein in hydrogen bonding. Of these, the 3-hydroxyl is absolutely required for substrate recognition. The β-face of the glucose moiety also participates in binding as a portion of the hydrophobic interaction.

In two respects, the substrate characteristics required by the sucrose transporting protein in developing soybean cotyledons are similar to other carbohydrate-protein interactions. Study of three monoclonal antibodies and two plant lectins by the laboratory of Lemieux (8, 9) has shown that in all cases binding consists of the interaction of an essentially nonpolar surface of the carbohydrate ligand with a corresponding hydrophobic surface of the protein along with binding of a cluster of two or three hydroxyl groups. In the case of the sucrose-sucrose transporter interaction, the cluster of hydroxyl groups is the 3-, 4-, and 6-hydroxyls of the glucose moiety, and the nonpolar surface is the area formed by the β-face of the glucose ring and the α-face of the fructose ring.

REFERENCES