Identification and Properties of Distinct Sucrose and Glucose Phosphotransferase Enzyme II Activities in *Streptococcus mutans* 6715g

GARY R. JACOBSON,* CAROL S. MIMURA, PETER J. SCOTT, AND PETER W. THOMPSON

Department of Biology, Boston University, Boston, Massachusetts 02215

Received 25 July 1984/Accepted 13 September 1984

We investigated phosphoenolpyruvate-dependent phosphotransferase system enzyme II activities for sucrose and glucose in *Streptococcus mutans* 6715g. Two integral membrane proteins, enzyme II<sub>1</sub> and enzyme II<sub>2</sub>, each specific for its sugar substrate, sucrose or glucose, were identified by their abilities to catalyze specific sugar:sugar-phosphate exchange reactions. Some of the properties of these two transport proteins are also presented.

Both glucose (16, 17) and sucrose (20, 21) are taken up and phosphorylated in *Streptococcus mutans* by a phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS). PTS activities for mannitol and glucitol (2, 8), lactose (3), and fructose and mannose (22) also have been described in *S. mutans*. The reactions catalyzed by all PTSs are (11, 12):

\[
\text{PEP} + \text{HPr} \xrightarrow{\text{enzyme I}} \text{phospho-HPr} + \text{pyruvate (cytoplasm)} \quad (1)
\]

\[
\text{sugar}_{\text{out}} + \text{phospho-HPr}_{\text{in}} \xrightarrow{\text{enzyme II}} \text{enzyme III} \quad \text{sugar-P}_{\text{in}} + \text{HPr}_{\text{out}} \text{ (membrane)} \quad (2)
\]

Enzyme I and HPr participate in the transport of all PTS sugars of a particular organism, whereas enzymes II are integral membrane permeases and are sugar specific. The uptake of some sugars may also require the participation of a sugar-specific enzyme III. Recently, we reported the purification of HPr and a complex between HPr and a putative sucrose-specific enzyme III from *S. mutans* (9). In this report, we provide evidence for distinct enzymes II for sucrose and glucose in *S. mutans* and describe some of their properties.

Radiochemicals were purchased from New England Nuclear Corp., Boston, Mass. Sucrose-6-phosphate was the generous gift of S. Dills. Other chemicals used were purchased from Sigma Chemical Co., St. Louis, Mo. *S. mutans* 6715g (provided by S. Dills) was grown, washed, stored, and broken as described previously (9). Cell-free extracts were separated into membrane and cytoplasmic fractions also as described previously (9). *Escherichia coli* K-12, strain DS409 (provided by J. Lengeler), contains a plasmid which encodes a sucrose PTS (18). It was grown on medium 63 (15) containing 0.5% sucrose, with 10 μg of tetracycline and 50 μg of thiamine per ml at 37°C to midexponential phase. Cells were broken and harvested as described previously (5). Before enzyme assays, *S. mutans* membranes were treated with 1% toluene (vol/vol) to render any vesicular structures permeable to charged substrates. Whole-cell PTS activities were also measured by pretreatment of unbroken cell suspensions with toluene in the same manner. PEP-dependent sugar phosphorylation was estimated as previously described (5, 9). Exchange reactions between sugar-phosphates and 14C-labeled sugars (transphosphorylation [12]) were determined in assay mixtures (0.1 ml) containing 0.1 M potassium phosphate (pH 7.0), 1 mM dithiothreitol, 5 mM EDTA, 10 mM KF, 0.5 μM 14C-labeled sugar (300 to 400 μCi/μmol), 1 mM sugar-phosphate, and the sample to be assayed. Mixtures were incubated for 1 h at 37°C, and the 14C-labeled sugar-phosphate formed was determined as described previously (13). Total protein was estimated by the method of Lowry et al. (7).

To obtain direct biochemical evidence that distinct enzymes II for sucrose and glucose exist in *S. mutans*, we measured the abilities of membrane fractions from sucrose-grown cells to catalyze specific transphosphorylation reactions characteristic of these enzymes (14). Activities catalyzing phosphotransfer reactions between sucrose-6-phosphate and [14C]sucrose and between glucose-6-phosphate and [14C]glucose were observed in these membrane preparations (Table 1). As is the case with other bacteria, optimal activities occurred at high ratios of sugar-phosphates to sugar (ca. 10<sup>2</sup>; see reference 14), and the data in Table 1 were obtained at [sugar-phosphate] = 1 mM and [sugar] = 0.5 μM, which was found to be optimal for *S. mutans* (data not shown). Pretreatment of membranes with toluene was necessary to reproducibly observe these activities in fractions derived from glass bead-ruptured cells. This suggests that these membranes are at least in part vesicular structures, containing enzymes II with their sugar-phosphate binding sites exposed at the intravesicular surface. Transfer of the phospho group from glucose-6-phosphate to sugar was highly specific, with glucose as the preferred acceptor. Phosphotransfer from sucrose-6-phosphate occurred only with sucrose as an acceptor. However, activity with glucose as an acceptor and sucrose-6-phosphate as a donor was observed in membrane fractions that had been washed only once (Table 1, footnote b), and it may reflect sucrose-6-phosphate hydrolyase activity peripherally associated with the membrane (1) which could give rise to glucose-6-phosphate in situ. Fructose-6-phosphate was a poor phosphodonor, and fructose was a poor phosphoacceptor, in all cases (Table 1).

These experiments provide evidence for highly specific enzymes II for sucrose and glucose in membrane fractions of *S. mutans*. Recently, glucose:glucose-6-phosphate transphosphorylation also was reported with membranes of *S. mutans* GS5 cells (6).

Separate sucrose and glucose enzymes II were further

---

* Corresponding author.
confirmed by measuring PEP-dependent activity of enzyme II<sup>cr</sup> in the presence of a 10-fold molar excess of glucose or fructose over [<sup>14</sup>C]sucrose. No inhibition of sucrose phosphorylation was observed under these conditions compared to controls lacking unlabeled sugars (data not shown).

Because <i>S. mutans</i> produces large amounts of lactic acid from sugar fermentation, we measured the pH dependence of both PEP-dependent and transphosphorylation activities for sucrose and glucose in sucrose-grown <i>S. mutans</i>. PEP-dependent activities in permeabilized cells exhibited pH maxima near pH 8 (Fig. 1A). These maxima were considerably sharper than that previously reported for PEP-dependent glucose phosphorylation in <i>S. mutans</i> (23). In contrast, transphosphorylation activities in membranes were maximal near pH 6 (Fig. 1B). Similar pH optima have been reported for the purified manninit enzyme II from <i>E. coli</i> (5). Most probably, <i>S. mutans</i>, like other bacteria (10), maintains a relatively constant intracellular pH under a wide variety of environmental conditions.

Finally, as a first step in attempting to purify enzyme II<sup>cr</sup> and enzyme II<sup>ts</sup> from <i>S. mutans</i>, it was of interest to compare these proteins with those of enteric bacteria from which two sugar-specific enzymes II have been purified to apparent homogeneity (4, 5). Cytoplasmic fractions from <i>S. mutans</i> failed to complement PEP-dependent sucrose or glucose phosphorylation catalyzed by washed membrane fractions of <i>E. coli</i> K-12 DS409, nor could cytoplasmic fractions from the latter organism complement washed <i>S. mutans</i> membranes in the same activities (data not shown).

Thus, the enzymes II of these organisms have diverged sufficiently that they no longer recognize the terminal soluble phosphotransfer proteins of one another, as has also been shown for <i>Staphylococcus aureus</i> and <i>E. coli</i> PTSs (19). Nonetheless, we have found that enzyme II<sup>ts</sup> activity can be solubilized from <i>S. mutans</i> membranes with deoxycholate under the same conditions in which the mannitol enzyme II of <i>E. coli</i> is extracted (5) and that the resulting extract is stable to chromatography on hydrophobic resins (P. Scott and G. Jacobson, unpublished data). Thus, it should be possible to obtain homogeneous PTS enzymes II from <i>S. mutans</i> in the future for further studies on the structure and mechanisms of the PTS of oral streptococci.

This research was supported by Public Health Service grant 1 R01 DE05966 from the National Institute of Dental Research and a research gift from Warner-Lambert Co., Morris Plains, N.J.

**LITERATURE CITED**


---

**TABLE 1. Sugar:sugar-phosphate transphosphorylation activities in membranes from sucrose-grown <i>S. mutans</i> 6715<sup>a</sup>**

<table>
<thead>
<tr>
<th>Sugar-phosphate donor</th>
<th>14C-labeled sugar acceptor</th>
<th>14C-labeled sugar-phosphate formed (pmol/h per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose-6-phosphate</td>
<td>Sucrose</td>
<td>39.5</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>Sucrose</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>Sucrose</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Membranes were prepared from glass bead-ruptured cells and treated with tolune as described in reference 9. Without tolune treatment, transphosphorylation activities were highly variable depending on the membrane preparation.

<sup>b</sup> This value depended on the extent to which membrane preparations were washed. Higher values were found in membrane preparations washed only once. The value shown in this table was obtained with a preparation washed 3 times. These observations may reflect the amount of sucrose-6-phosphate hydrolase peripherally associated with the membrane (see text).

**FIG. 1. pH-dependence of PTS activities in <i>S. mutans</i> 6715<sup>g</sup>. (A) PEP-dependent phosphorylation of glucose (●) and sucrose (○) was measured as a function of pH, using 10 μl per 0.1 ml of assay mixture of a suspension of sucrose-grown cells (10 mg of protein per ml) treated with 1% tolune (vol/vol). The buffers used were: pH 4 and 0.1 M sodium citrate; pH 6 and 0.1 M potassium phosphate; pH 8, 9, and 10, 0.1 M glycine-hydrochloride. (B) Sugar-phosphate-dependent transphosphorylation activities in membranes of sucrose-grown cells broken by homogenization with glass beads were measured as a function of pH. Glucose:glucose-6-phosphate (●) and sucrose:6-phosphate (○) transphosphorylation activities were determined using 20 μl of washed, toluenized membrane suspension (0.5 mg/ml) per 0.1 ml of assay mixture. The buffers used were the same as those described in (A).**


