Phosphoenolpyruvate-Dependent Sucrose Phosphotransferase Activity in Five Serotypes of \textit{Streptococcus mutans}

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An inducible phosphoenolpyruvate-dependent sucrose phosphotransferase system has been demonstrated in decrypcted cell suspensions of the various common serotypes of the cariogenic microorganism \textit{Streptococcus mutans}.

The group of oral microorganisms currently described as \textit{Streptococcus mutans} represents a genetically (2), biochemically (9), and antigenically (1, 5) heterogeneous group. However, with respect to the ability of these organisms to colonize teeth and induce carious lesions, the disaccharide sucrose appears to play a pivotal role (7). Previous studies in our laboratories demonstrated that one mode of sucrose transport in the serotype c representative strain \textit{S. mutans} NCTC 10449 is that mediated via an inducible phosphoenolpyruvate (PEP)-dependent sucrose phosphotransferase system (PTS) (6). Thus, it became of interest to survey other common serotypes for sucrose PTS activity because one may consider this to represent a key enzyme system in the virulence-associated sucrose metabolism of this group of cariogenic microorganisms.

All strains studied were grown, after adaptation, to early stationary phase in a defined chemical medium (FMC) (10) supplemented with 5 mM carbohydrate. A modification (6) of the scheme of Kornberg and Reeves (4) was employed to detect PTS activity in tolune-ace-tone-decrypctied cells. Suspensions of the various serotypes of \textit{S. mutans} incubated in the presence of exogenous PEP and sucrose and enzyme-couple reagents (4, 6) all exhibited a decrease in optical density at 340 nm which was linear both with time and with the number of cocci incubated. In the absence of exogenous PEP or of sucrose or after heat treatment (80°C for 15 min), no decrease in optical density was observed.

The primary energy source for this presumptive sucrose PTS was PEP. Of other potential phosphate donors (6) examined, only 2-phosphoglyceric acid, the immediate glycolytic precursor of PEP, could stimulate sucrose PTS activity and then only in the absence of NaF. Such a result is predicted because of the well-known inhibition of enolase of \textit{S. mutans} by NaF (3).

The absolute requirement for PEP, the ability of 2-phosphoglyceric acid to partially substitute for PEP in the absence of NaF, and the absence of a fluoride effect on the observed decrease in the optical density at 340 nm in the presence of exogenous PEP are all consistent with the hypothesis that at least one mode of sucrose permeation of diverse \textit{S. mutans} serotypes is group tranlocation mediated by a PEP-dependent PTS (4, 6).

Table 1 shows that sucrose-adapted strains exhibited strong sucrose PTS activity but no glucose PTS activity; by contrast, glucose-adapted strains exhibited glucose PTS activity but no sucrose PTS activity. Therefore, these two transport systems appear to be separate and under separate genetic control. These data cannot be explained as due to the action of invertase (EC 3.2.1.26; \(\beta\)-fructofuranoside fructohydrolase), resulting in the conversion of sucrose to glucose and fructose and subsequent transport by PEP-dependent PTSs. The invertase activity of \textit{S. mutans} is not classically inducible, thus being produced by glucose-adapted and sucrose-adapted cocci (8). Were invertase activity responsible for the generation of fructose and glucose for subsequent hexose PTS activity, then sucrose-challenged, glucose-adapted cocci would demonstrate PTS activity for sucrose, and they do not.

Kinetic studies for each strain indicated that the sucrose PTS in sucrose-adapted, sucrose-challenged cells exhibited classical saturation kinetics. Arrangement of such data into the Lineweaver-Burk format permitted determinations of apparent \(K_m\)'s for each serotype (Table 2). In general, for each substrate, each serotype representative possessed similar specific activities and apparent \(K_m\)'s, but these values were markedly different between the substrates. These data do not preclude the possibility that there may be differences in the regulatory mech-
### Table 1. Sucrose PTS and glucose PTS specific activities for sucrose-adapted and glucose-adapted serotypes of S. mutans

<table>
<thead>
<tr>
<th>Growth condition and carbohydrate challenge</th>
<th>Sucrose PTS activity</th>
<th>Glucose PTS activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a (E-49) b (FA-1) c (Ing britt-1600) c (NCTC 10449) d/g (6715-13) E (LM-7) d/SL (SL-1)</td>
<td>a (E-49) b (FA-1) c (Ing britt-1600) c (NCTC 10449) d/g (6715-13) E (LM-7) d/SL (SL-1)</td>
</tr>
<tr>
<td>Sucrose adapted, sucrose challenged</td>
<td>94.4 57.8 70.4 78.9 60.0 21.3 44.8</td>
<td>&lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0</td>
</tr>
<tr>
<td>Sucrose adapted, glucose challenged</td>
<td>&lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0 &lt;1.0</td>
<td></td>
</tr>
<tr>
<td>Glucose adapted, sucrose challenged</td>
<td>9.9 8.8 9.3 7.6 8.5 4.2 12.8</td>
<td></td>
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<tr>
<td>Glucose adapted, glucose challenged</td>
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</table>

* Decryptified cell suspensions were prepared from late-exponential-growth phase sucrose- or glucose-grown cells. Experimental conditions were as described in the text. Each reaction mixture contained 0.2 mg of bacterial protein per ml. Serotype notations are represented as described by Brathall (1) and Perch et al. (5). Thus, a, b, c, and E are consistent between Brathall and Perch et al. However, for those strains in dispute, the designations are given as, for example, d/g, Brathall/Perch et al. Strain designations are given within parentheses.

* NADH, Reduced nicotinamide adenine dinucleotide.
**Table 2. Apparent $K_m$'s for sucrose PTS and glucose PTS activities of sucrose-adapted and glucose-adapted cells of selected serotypes of S. mutans**

<table>
<thead>
<tr>
<th>Growth condition and carbohydrate challenge</th>
<th>Sucrose PTS $\times 10^{-3}$ M</th>
<th>Glucose PTS $\times 10^{-3}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a$ (E-49)</td>
<td>$b$ (FA-1)</td>
</tr>
<tr>
<td>Sucrose adapted, sucrose challenged</td>
<td>7.1</td>
<td>6.9</td>
</tr>
<tr>
<td>Glucose adapted, glucose challenged</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Decrypted cell suspensions were prepared from late-exponential-growth phase sucrose- or glucose-grown cells. Experimental conditions were as described in the text. Each reaction mixture contained 0.2 mg of bacterial protein per ml. Serotype notations are represented as described by Bratthall (1) and Perch et al. (6). Strain designations are given within parentheses.
cells in their peculiar ecological niche which is characterized by fluctuations of exogenous carbohydrate supply.

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**LITERATURE CITED**


