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Phosphoenolpyruvate-Dependent Sucrose Phosphotransferase Activity in Five Serotypes of *Streptococcus mutans*

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An inducible phosphoenolpyruvate-dependent sucrose phosphotransferase system has been demonstrated in decryptified cell suspensions of the various common serotypes of the cariogenic microorganism *Streptococcus mutans*.

The group of oral microorganisms currently described as *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 *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 toluene-acetone-decryptified cells. Suspensions of the various serotypes of *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 *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 *S. mutans* serotypes is group translocation 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; β-D-fructofuranoside fructohydrolase), resulting in the conversion of sucrose to glucose and fructose and subsequent transport by PEP-dependent PTSs. The invertase activity of *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*[a]

Growth condition and carbohydrate challenge	μmol of NADH[b] consumed per min per mg of protein													
	Sucrose PTS activity							Glucose PTS activity						
	a (E-49)	*b* (FA-1)	*c* (Ingbritt-1600)	*c* (NCTC 10449)	*d/g* (6715-13)	*E* (LM-7)	*d/SL* (SL-1)	*a* (E-49)	*b* (FA-1)	*c* (Ingbritt-1600)	*c* (NCTC 10449)	*d/g* (6715-13)	*E* (LM-7)	*d/SL* (SL-1)
Sucrose adapted, sucrose challenged	94.4	57.8	70.4	78.9	60.0	21.3	44.8							
Sucrose adapted, glucose challenged								<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Glucose adapted, sucrose challenged	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0							
Glucose adapted, glucose challenged								9.9	8.8	9.3	7.6	8.5	4.2	12.8

[a] 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 Bratthall (1) and Perch et al. (5). Thus, *a*, *b*, *c*, and *E* are consistent between Bratthall and Perch et al. However, for those strains in dispute, the designations are given as, for example, *d/g*, Bratthall/Perch et al. Strain designations are given within parentheses.

[b] 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*[a]

Growth condition and carbohydrate challenge	Apparent K_m											
	Sucrose PTS × 10^{-5} M						Glucose PTS × 10^{-3} M					
	a (E-49)	*b* (FA-1)	*c* (Ingbritt-1600)	*c* (NCTC 10449)	*d/g* (6715-13)	*E* (LM-7)	*d/SL* (SL-1)	*a* (E-49)	*b* (FA-1)	*c* (Ingbritt-1600)	*c* (NCTC 10449)	*d/g* (6715-13)	*E* (LM-7)	*d/SL* (SL-1)
Sucrose adapted, sucrose challenged	7.1	6.9	7.1	7.1	6.2	3.4	5.5							
Glucose adapted, glucose challenged								4.0	3.0	3.0	3.3	5.0	4.0	8.0

[a] 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 Bratthall (1) and Perch et al. (5). Strain designations are given within parentheses.

anisms operating among the glucose- and sucrose-specific enzyme systems.

Only the trisaccharide raffinose and the disaccharide lactose inhibited sucrose PTS activity, whereas cellobiose, gentiobiose, melibiose, turanose, trehalose, and melezitose failed to inhibit the sucrose PTS activity of all serotype representatives examined. No monosaccharides or monosaccharide alcohols tested inhibited or stimulated the sucrose PTS reaction. The observed inhibition by raffinose and lactose appeared to be competitive as judged by the Lineweaver-Burk plots of the data (Fig. 1). It should be noted that lactose and raffinose failed to stimulate the conversion of PEP to pyruvate in sucrose-adapted cells in the absence of exogenous sucrose. Fructose was the only other carbohydrate examined that elicited any significant generation of PEP-dependent oxidized nicotinamide adenine dinucleotide by sucrose-adapted cells, but it did not inhibit the generation of oxidized nicotinamide adenine dinucleotide in sucrose-adapted, sucrose-challenged cells. By contrast, fructose, raffinose, sucrose, and lactose (all at 0.5 mM) failed to inhibit the glucose PTS of glucose-adapted cells, nor could they substitute as the transported solute. These data further confirm the distinctiveness of the sucrose PTS and glucose PTS activities found in the various serotype representatives.

The sucrose transport product for strain 6715-13 (serotype *d/g*) by this vectorial phosphorylation system was isolated and identified by previously detailed methods (6). Thus, a sucrose monophosphate ester was produced as a result of the sucrose PTS activity. (i) Dowex-formate anion-exchange column chromatography of the ethanolic extract of sucrose-adapted, [*U*-^{14}C]sucrose-challenged cells yielded a radioactive peak which eluted in a position consistent with its monophosphate nature and which, upon rechromatography by liquid-liquid partition on paper, showed the presence of a moiety which did not cochromatograph with authentic glucose or fructose monophosphates. (ii) Upon alkaline phosphatase treatment of this peak and rechromatography on the Dowex column, most of its radioactivity was eluted as though it were a sugar which upon rechromatography on paper had the same mobility as authentic sucrose. (iii) After mild acid hydrolysis of this uncharged material, it was seen to contain approximately equal quantities of moieties with the same mobilities on paper as authentic glucose and fructose.

The prevalence of this sucrose transport system among the various serotypes of *S. mutans* may be energetically advantageous (6) to these

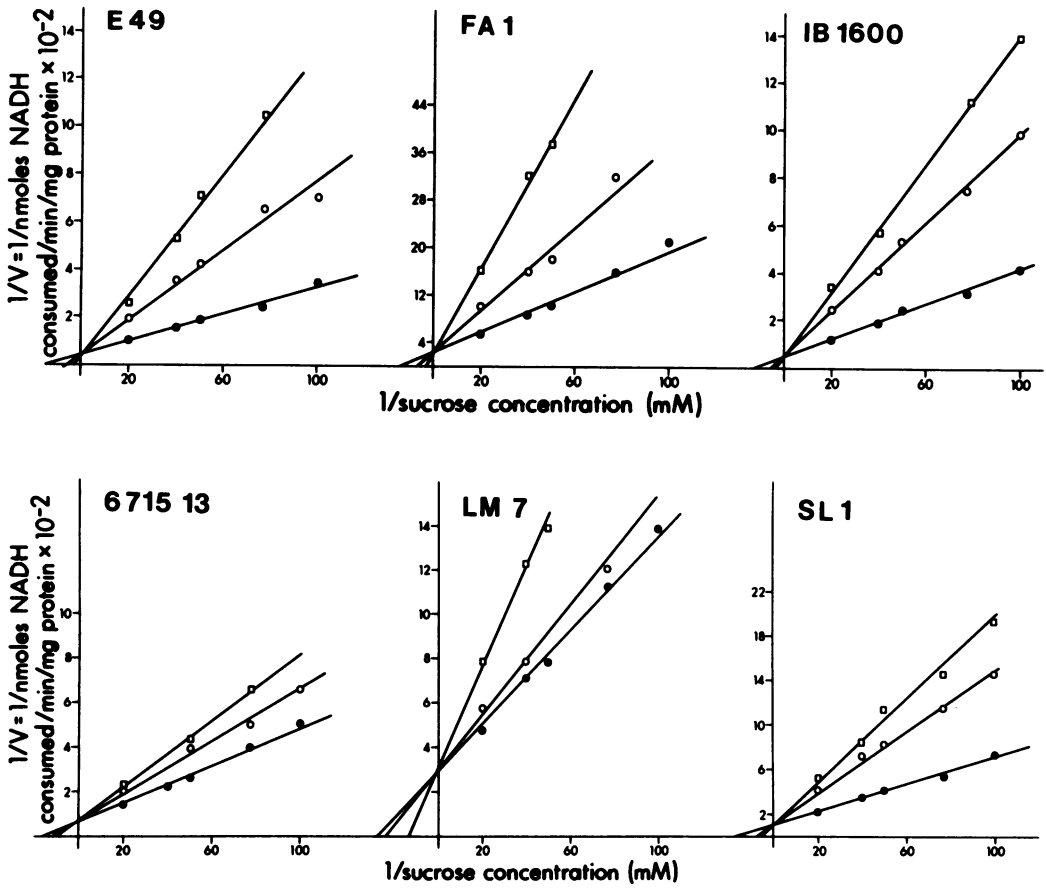

FIG. 1. *Lineweaver-Burk plots of sucrose PTS activities for sucrose-grown serotypes of S. mutans challenged with either sucrose, sucrose plus raffinose, or sucrose plus lactose. Curves were plotted by linear-regression analysis. Symbols: ●, sucrose challenged; ○, sucrose plus 0.5 mM raffinose challenged; □, sucrose plus 0.5 mM lactose challenged. NADH, Reduced nicotinamide adenine dinucleotide.*

cells in their peculiar ecological niche which is characterized by fluctuations of exogenous carbohydrate supply.

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