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SUCROSE TRANSPORT BY *STREPTOCOCCUS MUTANS*

EVIDENCE FOR MULTIPLE TRANSPORT SYSTEMS

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The transport of sucrose by selected mutant and wild-type cells of *Streptococcus mutans* was studied using washed cocci harvested at appropriate phases of growth, incubated in the presence of fluoride and appropriately labelled substrates. The rapid sucrose uptake observed cannot be ascribed to possible extracellular formation of hexoses from sucrose and their subsequent transport, formation of intracellular glycogen-like polysaccharide, or binding of sucrose or extracellular glucans to the cocci. Rather, there are at least three discrete transport systems for sucrose, two of which are phospho*enol*pyruvate-dependent phosphotransferases with relatively low apparent K_m values and the other a non-phosphotransferase (non-PTS) third transport system (termed TTS) with a relatively high apparent K_m. For strain 6715-13 mutant 33, the K_m values are $6.25 \cdot 10^{-5}$ M, $2.4 \cdot 10^{-4}$ M, and $3.0 \cdot 10^{-3}$ M, respectively; for strain NCTC-10449, the K_m values are $7.1 \cdot 10^{-5}$ M, $2.5 \cdot 10^{-4}$ M and $3.3 \cdot 10^{-3}$ M, respectively. The two lower K_m systems could not be demonstrated in mid-log phase glucose-adapted cocci, a condition known to repress sucrose-specific phosphotransferase activity, but under these conditions the highest K_m system persists. Also, a mutant devoid of sucrose-specific phosphotransferase activity fails to evidence the two high affinity (low apparent K_m) systems, but still has the lowest affinity (highest K_m) system. There was essentially no uptake at 4°C indicating these processes are energy dependent. The third transport system, whose nature is unknown, appears to function under conditions of sucrose abundance and rapid growth which are known to repress phospho*enol*pyruvate-dependent sucrose-specific phosphotransferase activity in *S. mutans*. These multiple transport systems seem well-adapted to *S. mutans* which is faced with fluctuating supplies of sucrose in its natural habitat on the surfaces of teeth.

Introduction

Sucrose metabolism by *Streptococcus mutans*, a dental plaque-forming microorganism, appears central to its ability to initiate carious lesions of enamel [1–4]. Sucrose transport may be the rate-limiting step in the pathway which culminates in lactic acid production by these tooth-demineralizing homofermentative bacteria [5]. Indirect enzyme-coupled assays and growth cycle studies have demonstrated that sucrose can be transported by at least two distinct mechanisms: (i) an inducible phospho*enol*pyruvate-dependent sucrose-specific phosphotransferase system (sucrose PTS) [6–9], and (ii) an uncharacterized second transport system which, although requiring energy, is not directly dependent on phospho*enol*pyruvate for activity [9]. The sucrose-specific phosphotransferase

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system appears to function primarily under environmental conditions of sucrose paucity and slow growth [6,7,9] and the other system appears to function when sucrose is available in relative abundance and the cocci are growing rapidly [9].

The study of sucrose transport by *S. mutans* is problematic because of the multiplicity of mechanisms whereby this cell metabolizes sucrose [1,3,5,10]. *S. mutans* strains have extracellular glucosyltransferase activities (EC 2.4.1.5; α-1,6-glucan : D-fructose 2-glucosyltransferase) which form extracellular water-soluble and cell surface associated water-insoluble glucans and free fructose [5,11]. *S. mutans* strains, except for those of serotype *d/g* [12–14], have extracellular fructosyltransferase activity (EC 2.4.1.10; β-2,6-fructan : D-glucose 6-fructosyltransferase) whose products are water-soluble extracellular fructans and free glucose [1,3,5,11]. It has intracellular invertase (EC 3.2.1.26; β-D-fructofuranoside fructohydrolase) [15–17], and some authors have also reported extracellular invertase [18,19]. Furthermore, *S. mutans,* except for those of serotype *d/g*, make intracellular glycogen-like polysaccharides from a variety of sugars [1,3,10,20,21].

Fortunately, it appears possible, by a variety of ploys, to discriminate between true sucrose transport and either the binding of sucrose or water-insoluble glucans to the cell surface, the transport of glucose or fructose formed by hexosyltransferase or invertase, and the formation of intracellular glycogen. These include use of specific characterized mutants, study of cells under growth conditions which repress various aspects of sucrose metabolism, use of metabolic inhibitors and conditions inhibitory to energy-yielding metabolism, and use of appropriately labelled substrates. By such methods the present studies of sucrose uptake by intact cells confirm the previous findings with respect to sucrose transport, viz. of the existence of sucrose specific, phospho*enol*pyruvate-dependent phosphotransferase-mediated transport by *S. mutans,* and also indicate the existence of two such phosphotransferase systems. Furthermore, a third non-phosphotransferase system (TTS) has been characterized with respect to the kinetics of sucrose permeation into two strains of *S. mutans* which are representative of the most ubiquitous serotypes of *S. mutans* [12,13] found in human populations [22–24]. These observations cannot be ascribed to extracellular glucosyl- or fructosyltransferase activities or to extracellular invertase activity and subsequent hexose transport by the cells, nor can they be ascribed to sucrose or glucan binding to cell surfaces.

Materials and Methods

Microorganisms. Streptococcus mutans serotype *c* strain representative NCTC 10449, its mutant 417-8:2 which has an apparent defect of its sucrose-specific phosphotransferase, and *S. mutans* serotype *d/g* strain representative 6715-13 mutant 33, which is unable to synthesize cell surface-associated glucans [4,11,25], to make extracellular fructans, or to make appreciable intracellular glycogen-like polysaccharide [11,20] were used throughout these studies. Working stock cultures were maintained by monthly transfer in fluid thioglycollate medium (Difco) supplemented with 20% (vol/vol) beef extract and excess $CaCO_3$.

Isolation of mutant strains. The mutagenesis techniques and the procedures for isolation and characterization of mutants have been previously detailed [4,9,11]. The sucrose-specific phosphotransferase-defective mutant (417-8:2) used here is phenotypically indistinguishable from mutant 217-7:2 previously characterized [9].

Growth medium and growth conditions. Following two successive transfers in a chemically defined growth medium (FMC, [26]) supplemented with 5 mM carbohydrate, cocci for experimental use were grown for 18 h at 37°C in screw-capped 2 liter Erlenmeyer flasks with no head space. Cocci were harvested in either mid-logarithmic or early stationary phase by centrifugation (10000 × *g* for 5 min at 4°C), washed twice with 250 ml of 25 mM potassium phosphate buffer (pH 7.0) containing 10 mM magnesium sulfate, and resuspended in the same buffer such that a 10-fold dilution had an absorbance at A_{600nm} (1 cm light path) of 0.20. This final coccal suspension was maintained at 0°C until used within 2 h from harvest.

Mechanics of sucrose uptake studies. Ten ml samples of appropriate buffer-washed coccal suspension were preincubated for 2 min at 37°C after which NaF was added to a final concentration of 10 mM, a concentration known to completely block

glycolysis by *S. mutans* (Tanzer and Gibberman, unpublished data), powerfully inhibit its intracellular polysaccharide synthesis [21], yet not deplete existing intracellular pools of phospho*enol*pyruvate in *Streptococcus lactis* and *Streptococcus pyogenes* [27]. The suspension was incubated for an additional 2 min and subsequently challenged with radiolabelled sucrose: [U-^{14}C]sucrose (spec. act. 620 mCi/mmol); [*glucosyl*-U-^{14}C]sucrose, (spec. act. 201 mCi/mmol); or [*fructosyl*-U-^{14}C]sucrose, (spec. act. 201 mCi/mmol), all in various concentrations of sucrose carrier; or with [U-^{14}C]glucose (spec. act. 345 mCi/mmol) or [U-^{14}C]fructose (spec. act. 305 mCi/mmol), in various concentrations of the respective hexose carriers. Sugars were added to the suspension while it was being vigorously agitated on a vortex mixer, thereby ensuring rapid and uniform distribution of both cells and substrate. One ml samples were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90 and 120 s following the addition of the carbohydrate and rapidly filtered through membrane filters (0.45 μm pore size, Millipore type HAWP) which had been soaked in a 100 mM solution of the appropriate carbohydrate, and which were located on a 12 port filtration manifold (Millipore Corp., Bedford, MA, U.S.A.) connected to a vacuum pump. Filtration required less than 3 s. Following filtration and washing with 5 ml of the buffer at 37°C, the filters were removed and placed into scintillation vials and, subsequent to drying, a thixotropic gel scintillation fluid was added which cleared and dissolved the membrane filters while evenly resuspending the cocci. The accumulation of radiolabel by the cocci was measured with a Model 3375 liquid scintillation spectrometer (Packard Inst. Co., Downers Grove, IL, U.S.A.).

Phosphoenolpyruvate-dependent sucrose-specific phosphotransferase activity. Phospho*enol*pyruvate-dependent sucrose-specific phosphotransferase activity was analysed with glucose- and sucrose-adapted decryptified cocci, as previously described [6,7,9].

Detection of invertase, fructosyltransferase and glucosyltransferase activities. Free extracellular glucose, potentially generated by either the action of extracellular invertase or fructosyltransferase activities, was detected as previously detailed [28]. Buffer-washed cocci were incubated in 50 mM sucrose for 5 min and the appearance of free glucose assessed after the addition of glucose oxidase reagent (Worthington Biochemical, Freehold, NJ, U.S.A.). The glucose oxidase reagents were dissolved in 500 mM Tris buffer to ensure inhibition of the invertase contaminant found in commercially available glucose oxidase [28,29]. The generation of free hexoses by either extracellular invertase, fructosyltransferase, or glucosyltransferase activities was detected by analysis of the appearance of reducing sugar using the microdetection method of Nelson-Somogyi [30], as previously detailed [28]. To be assured of the ability to detect invertase activity, some cocci were initially subjected to ultrasonic energy to free intracellular invertase [16,28] prior to incubation with sucrose. The sensitivity of glucose detection by the glucose oxidase method used is less than 0.8 nmol of glucose/min per mg protein; that for reducing sugar by the micro-Nelson-Somogyi method is less than 0.3 nmol hexose equivalent/min per mg protein [28].

To further assure that a failure to detect free extracellular fructose or glucose was not the result of their rapid transport by the cocci, the direct measurement of uptake of labelled glucose and fructose was compared with those of glucosyl- or fructosyl-labelled sucrose by sucrose-adapted cocci, using the methods described above.

Protein determination. The method of Lowry et al. [31] was used to estimate the protein content of coccal suspensions, using bovine serum albumin as standard.

Chemicals and radiochemicals. Unless otherwise indicated, all biochemicals were purchased from Sigma Chemical, St. Louis, MO, U.S.A. Analytical reagent grade sucrose and glucose were obtained from J.T. Baker, Phillipsburg, NJ, U.S.A. The radiolabelled sugars and Aquasol scintillation cocktail were obtained from New England Nuclear, Boston, MA, U.S.A. The radiochemical purity of each sucrose isotope batch was checked by liquid-liquid partition chromatography on paper using the solvent system and procedures previously detailed [6]. In all instances, their radiochemical purity was $>99\%$ and, importantly, no radioactive substances with the mobilities of either fructose or glucose could be detected.

Results

Absence of detectable extracellular invertase, fructosyltransferase or glucosyltransferase activities

Production of free glucose or reducing sugars from 50 mM sucrose by sucrose-adapted washed cocci of *S. mutans* 6715-13-33 or *S. mutans* NCTC 10449 could not be detected either immediately after completion of the washing procedure or up to 120 min later, a period in excess of that used in the sucrose uptake experiments (Table I). However, that glucose and reducing sugars could be detected by these assays is illustrated by their production subsequent to sonic disruption of the cocci or by adding standard amounts of glucose and/or fructose to the assay systems. Therefore, within the low limits of detectability, the uptake of radiolabelled sucrose by intact cocci can not be explained as reflecting the uptake of hexoses generated by extracellular metabolism of sucrose. Further evidence for this conclusion is given below.

Kinetics of sucrose uptake

For washed and buffer-resuspended, sucrose-adapted cocci of the cell surface-associated glucan synthesis-defective mutant 33, a reciprocal plot of

TABLE I

DETERMINATION OF THE PRODUCTION OF FREE HEXOSES FROM 50 mM SUCROSE FOLLOWING INCUBATION WITH INTACT OR SONICATED WHOLE CELLS

	Sugar formed (μmol/min/mg protein)	
	Glucose	Total reducing sugar
S. mutans 6715-13-33		
Intact whole cells [a]		
5 min post-washing	<0.001	<0.001
60 min post-washing	<0.001	<0.001
120 min post-washing	<0.001	<0.001
Sonified whole cells	0.22	0.45
S. mutans NCTC 10449		
Intact whole cells [a]		
5min post-washing	<0.001	<0.001
60 min post-washing	<0.001	<0.001
120 min post-washing	<0.001	<0.001
Sonified whole cells	0.15	0.32

[a] The cells were separated from the incubation mixture by membrane filtration and the supernatant fluid was analyzed. Experimental conditions and limits of detectability were as described in the text.

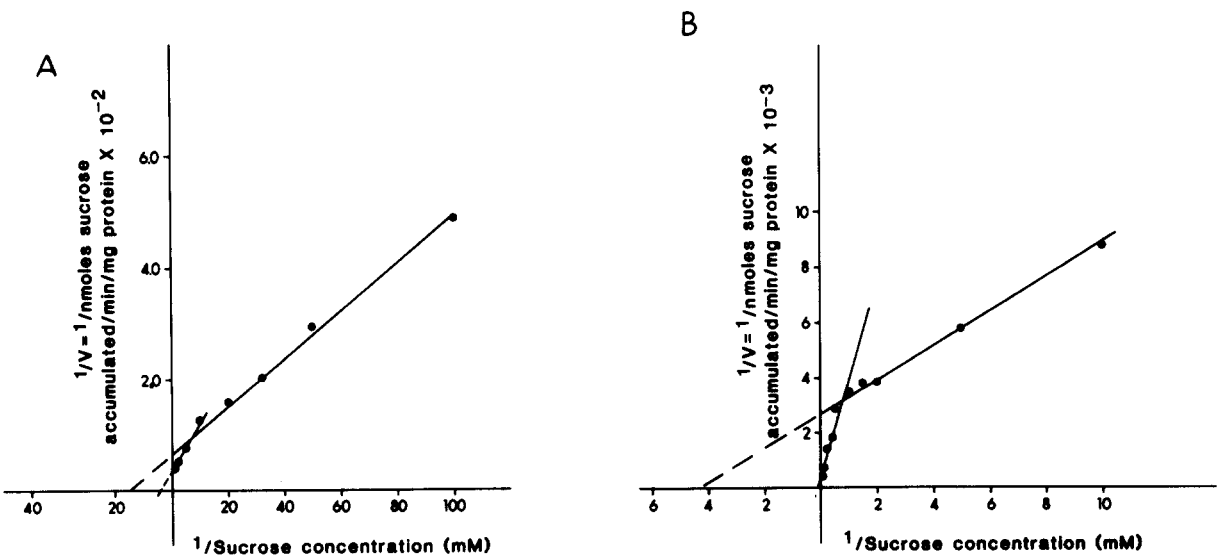

Fig. 1. Lineweaver-Burk plot of the uptake of uniformly labelled sucrose by sucrose-adapted *S. mutans* 6715-13 mutant 33. (a) Monitored with sucrose concentrations ranging from 0.01 to 1.0 mM. (b) Monitored with sucrose concentrations ranging from 0.1 to 30 mM.

the rate of incorporation of sucrose versus the substrate concentration (in a range of 0.01 to 1.0 mM sucrose) was biphasic (Fig. 1a). This suggested the presence of at least two transport systems. The apparent K_m value for the higher affinity system was calculated to be $6.25 \cdot 10^{-5}$ M. This apparent K_m is close to that determined for the sucrose-specific phosphotransferase, detected by the enzyme-couple method [6,7]. The higher K_m, lower affinity sucrose uptake system possessed an apparent K_m of $2.4 \cdot 10^{-4}$ M; while its presence was previously suggested by the Lineweaver-Burk and Eadie-Hofstee plots of sucrose-specific phosphotransferase activity using the enzyme couple assay, it could not be kinetically characterized by that method (Slee and Tanzer, unpublished data).

Subsequent direct studies of sucrose uptake at higher concentrations (in the range of 0.1 to 30 mM sucrose) also yielded biphasic plots when transformed by Lineweaver-Burk format (Fig. 1b). In this plot, the higher affinity system had an apparent K_m of $2.4 \cdot 10^{-4}$ M, corresponding to the low affinity system, demonstrated in the lower concentration range by Fig. 1a. Eadie-Hofstee plots (not shown) supported this interpretation. In addition, there was demonstrated another, yet lower-affinity system which possessed an apparent K_m of $3.0 \cdot 10^{-3}$ M. The observed patterns of uptake thus suggested that this strain possesses at least three transport systems for sucrose and that none of the kinetic processes monitored were likely to have reflected glucose or fructose transport, formation of cell surface associated glucans or fructans, or intracellular polysaccharide synthesis.

When identical sucrose uptake studies were conducted using serotype *c* representative strain *S. mutans* NCTC 10449, similar data resulted (Figs. 2a and 2b). There was a high affinity system with an apparent K_m of $7.1 \cdot 10^{-5}$ M, and two lower affinity systems with apparent K_m values of $2.5 \cdot 10^{-4}$ M and $3.3 \cdot 10^{-3}$ M, respectively. Again, the high affinity transport system possessed an apparent K_m similar to that previously described by the enzyme-couple assay for the sucrose-specific phosphotransferase [6,7]. Because free glucose or reducing sugars were not detected upon incubation of these washed intact cocci with sucrose, these data for 10449, like those for 6715-13 mutant 33, were not attributable to the transport of hexoses.

It was nonetheless conceivable that extremely rapid uptake of hexoses may have reduced their extracellular levels below the limits of detectability. To test for such an occurrence, sucrose-adapted *S. mutans* 6715-13-33 was harvested during the mid-logarithmic growth phase and used to study the uptake of ^{14}C-labelled sugar upon challenge

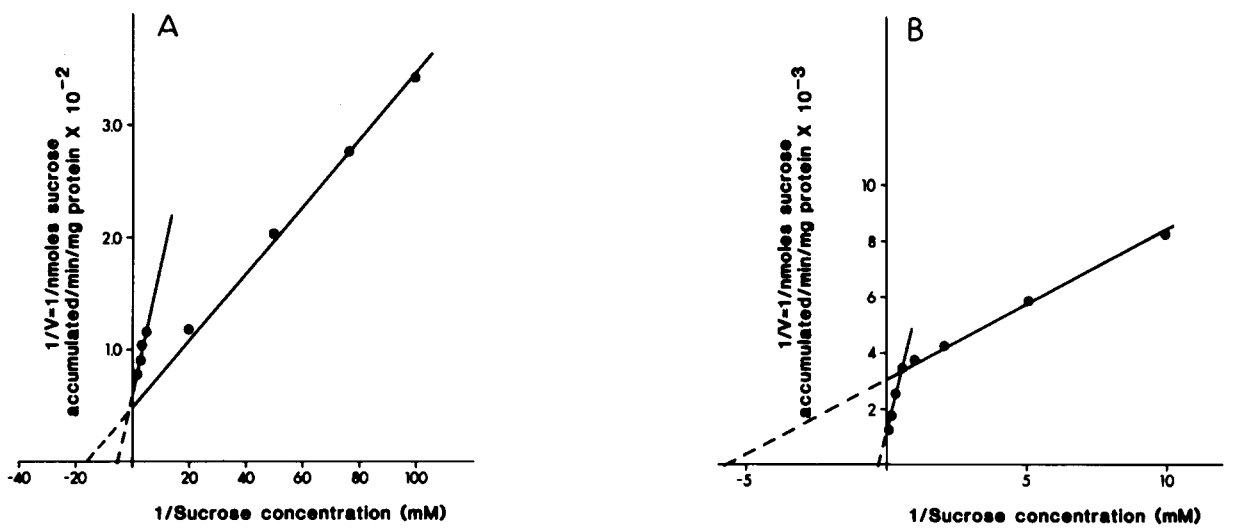

Fig. 2. Lineweaver-Burk plot of the uptake of uniformly labelled sucrose by sucrose-adapted *S. mutans* NCTC 10449. (a) Monitored with sucrose concentrations ranging from 0.01 to 1.0 mM. (b) Monitored with sucrose concentrations ranging from 0.1 to 30 mM.

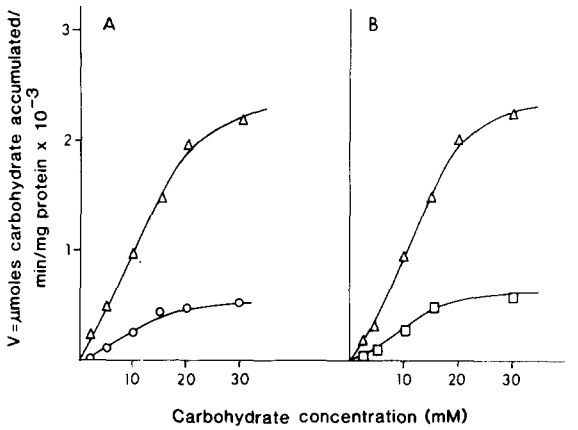

Fig. 3. Michaelis-Menten plot of the uptake of specifically labelled sucrose and uniformly labelled hexoses by sucrose-adapted *S. mutans* 6715-13 mutant 33. (a) Uptake of [*glucosyl*-U-^{14}C]sucrose (△) and [U-^{14}C]glucose (○) for concentrations ranging from 2 to 30 mM. (b) Uptake of [*fructosyl*-U-^{14}C]sucrose (△) and [U-^{14}C]fructose (□) for concentrations ranging from 2 to 30 mM.

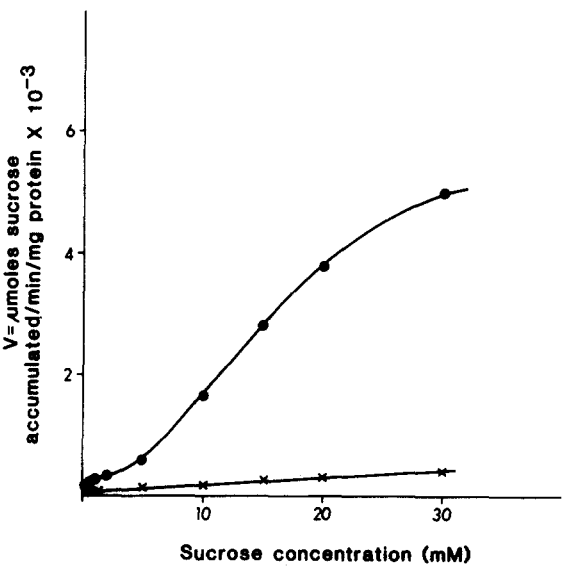

Fig. 4. Michaelis-Menten plot of the uptake of labelled sucrose by sucrose-adapted *S. mutans* NCTC 10449 at 4°C (×) and 37°C (●).

with either [*glucosyl*-U-^{14}C]sucrose or [U-^{14}C]glucose, or with [*fructosyl*-U-^{14}C]sucrose or [U-^{14}C]fructose. The rate of uptake for sucrose was several-fold higher than that for glucose (Fig. 3a) and several-fold higher than that for fructose (Fig. 3b). It should also be noted that the sum of the uptake rates of glucose and fructose was less than the rate of uptake of sucrose.

Uptake studies at 4°C and 37°C

The transport processes with respect to *S. mutans* NCTC 10449 and 6715-13 mutant 33 were sensitively temperature dependent (Fig. 4). The rate of apparent uptake of radiolabelled substrate was very low at 4°C and was linearly related to the environmental sucrose concentration. By contrast, uptake at 37°C was very rapid and approached saturation at about 30 mM sucrose.

Comparison of sucrose- and glucose-grown cocci

The initial velocity of sucrose uptake was lower in glucose-adapted cocci than in sucrose-adapted cocci (Fig. 5). In fact, the two sucrose uptake systems found in strain NCTC 10449 which had apparent K_m values of $7.1 \cdot 10^{-5}$ M and $2.5 \cdot 10^{-4}$ M could not be demonstrated in the glucose-adapted, sucrose-challenged mid-log phase cocci (Fig. 6). The remaining, evident transport system had an apparent K_m of $3.3 \cdot 10^{-3}$ M. Thus, whereas the two higher affinity systems appear classically inducible, the lower affinity one does not. Its apparent K_m is the same for

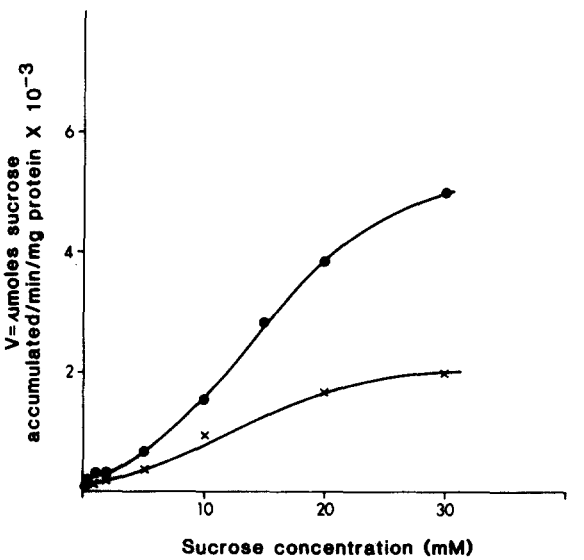

Fig. 5. Michaelis-Menten plot of the uptake of labelled sucrose by glucose-adapted (×) and sucrose-adapted (●) *S. mutans* NCTC 10449.

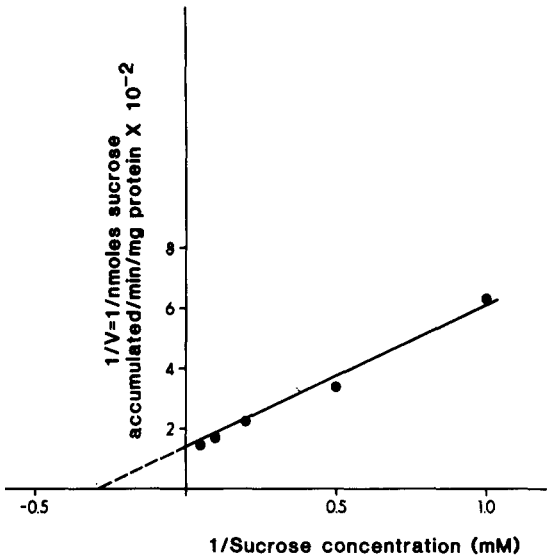

Fig. 6. Lineweaver-Burk plot of the uptake of labelled sucrose by log-phase glucose-adapted *S. mutans* NCTC 10449 monitored with sucrose concentrations ranging from 0.1 to 20 mM.

glucose-adapted cells as for sucrose-adapted ones (compare Fig. 6 and Fig. 2b). Similar data (not shown) were gathered for strain 6715-13 mutant 33.

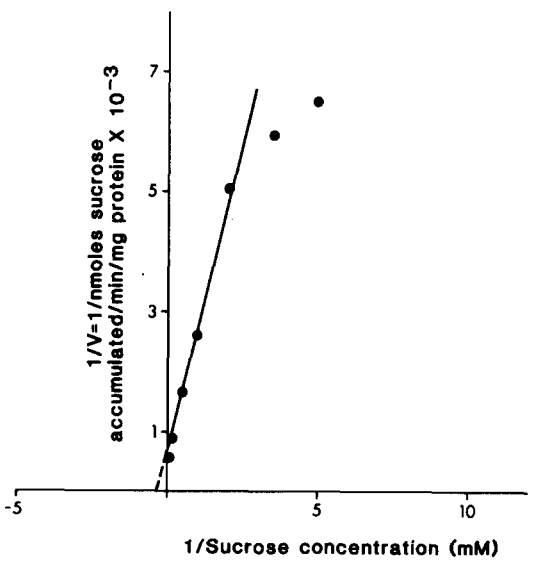

Fig. 7. Lineweaver-Burk plot of the uptake of labelled sucrose by late log-phase sucrose-adapted *S. mutans* NCTC 10449 PTS defective mutant 417-8:2, monitored with sucrose concentrations ranging from 0.1 to 20 mM.

Uptake kintetics for sucrose-specific phosphotransferase-deficient mutant

From the foregoing studies, the highest affinity sucrose transport system with apparent K_m of $7.1 \cdot 10^{-5}$ M was probably the previously described sucrose-specific phosphotransferase [6]. It, therefore, was expected that *S. mutans* NCTC 10449, mutant 417-8:2, with a known defect of its sucrose-specific phosphotransferase (although possessing normal glucose-specific phosphotransferase activity), was essentially unable to transport radiolabelled sucrose when presented at low concentrations, a condition previously shown to promote sucrose-specific phosphotransferase activity [9]. It could grow on sucrose if this carbohydrate was supplied at high concentrations. When washed and buffer resuspended cells of this mutant, grown to late logarithmic phase in 5 mM sucrose-supplemented FMC, were challenged with sucrose, the two highest affinity transport systems were no longer detectable. The lowest affinity system, with apparent K_m of $3.3 \cdot 10^{-3}$ M, however, persisted (Fig. 7).

Discussion

Relatively little is known of sucrose transport by *S. mutans* [6–9]. Yet sucrose is widely distributed in nature and is pivotal to dental caries, perhaps the most common chronic infection of man for which *S. mutans* is strongly implicated as the prime etiologic agent [32]. Because of the complexity of sucrose metabolism by this species, the present transport studies were performed using both wild type cells, a cell surface-associated glucan synthesis-defective mutant derived from a strain which fails to make significant intracellular polysaccharide and fructans, and a mutant lacking sucrose-specific phosphotransferase. All were adapted to a chemically-defined, carbohydrate-supplemented growth medium and, after harvest at the appropriate phase of growth, were challenged with substrate following their resuspension in phosphate buffer containing the glycolysis and intracellular polysaccharide synthesis inhibitor sodium fluoride [13]. Rapid uptake measurements permitted determination of the initial velocity of sucrose permeation, apparently prior to the depletion of energy pools consequent to the action of

sodium fluoride [27]. Appropriately labelled substrates and direct measurement of extracellular hexose formation allowed further discrimination between sucrose and hexose transport.

Of the three modes of sucrose transport made apparent by these studies, all are energy-dependent and two appear to be inducible phospho*enol*pyruvate-dependent sucrose group translocation processes. This conclusion is supported by a variety of evidence: (i) The initial velocity of sucrose uptake at 4°C is extremely slow and is a linear function of the sucrose concentration; however, at 37°C, the initial velocity of sucrose uptake is rapid and saturates with increasing sucrose concentrations. (ii) One of the transport components is comparable in apparent K_m to that determined previously for phospho*enol*pyruvate-dependent inducible sucrose-specific phosphotransferases for the same cells using an enzyme-couple assay [6,7]. Two transport components are absent from a mutant (417-8:2) defective in sucrose-specific phosphotransferase activity but having normal glucose-specific phosphotransferase activity, and they are also absent from its wild-type progenitor (10449) when adapted to defined medium supplemented with glucose, or when the sucrose-adapted wild type is studied at mid-logarithmic phase, conditions previously demonstrated to repress sucrose-specific phosphotransferase activity [9]. Thus, two phosphotransferase systems that can transport sucrose are apparent in *S. mutans*. The occurrence of two phosphotransferases for the same substrate, namely, a high and a low affinity, yet carbohydrate-specific phosphotransferase, is not unique to *S. mutans* or to sucrose, having been previously demonstrated in other microorganisms for the monosaccharides glucose and fructose [33,34]. Recent studies have also suggested that *S. mutans* appears to possess two phosphotransferases for glucose (Williamson, Keevil, Marsh and Ellwood, J. Dent. Res. 60: Spec. Issue B, Divisional Abstracts No. 209, 1981). Currently, it is unclear whether these two sucrose-specific phosphotransferases phosphorylate the same hexosyl moiety of the disaccharide, but preliminary studies suggest that each phosphotransferase succeeds in producing a monophosphate ester in which a different hexosyl is phosphorylated (Slee and Tanzer, unpublished data). *Aerobacter aerogenes* possesses two phosphotransferases for fructose which phosphorylate different carbons [35,36]. (iii) A lower affinity sucrose permeation system (with an apparent K_m of $3.3 \cdot 10^{-3}$ M) persists in a sucrose-specific phosphotransferase-defective mutant and in glucose-adapted and in sucrose-adapted cocci harvested from logarithmically growing cultures, conditions repressing sucrose-specific phosphotransferase activity [9]. It is, thus, a third transport system (TTS) for sucrose. The apparent K_m of this lowest affinity system is the same for glucose- and sucrose-adapted cocci. It, nonetheless, may be under different regulatory control, as its velocity differs with the sugar present during adaptation. The rates of sucrose uptake by glucose- versus sucrose-adapted cocci indicate that the third transport system is not classically inducible, although sucrose-adapted cells more rapidly take up the disaccharide compared to glucose-adapted cells. This situation is reminiscent of the specific activity of the intracellular sucrose catabolic enzyme invertase of *S. mutans* [16]. The precise mechanism, product(s) and energy donors for sucrose uptake by this low affinity system are currently unknown.

The apparent presence of the third transport system cannot be explained by hypothesizing that it merely reflects the uptake of free hexoses generated in the extracellular milieu by the activities of glucosyltransferase, fructosyltransferase or extracellular invertase. Several lines of evidence support this contention: (i) Serotype *d/g* cocci are devoid of fructosyltransferase activity [11]. (ii) Strain 6715-13 mutant 33 fails to make cell surface-associated glucans [4,11,25]. (iii) Washed cocci of strains 6715-13 mutant 33 and 10449, incubated with sucrose, fail to form detectable extracellular glucose and reducing sugar unless sonically disrupted, thereby freeing intracellular invertase. Nonetheless, the washed cocci accumulate sucrose. (iv) The velocity of the uptake of sucrose, as [*fructosyl*-U-^{14}C]sucrose, far exceeds the velocity of uptake of labelled fructose by the log-phase sucrose-adapted cocci; similarly, the velocity of sucrose uptake, measured using [*glucosyl*-U-^{14}C]sucrose, far exceeds that for labelled glucose by sucrose-adapted cocci harvested in log-phase, and the sum of the uptake velocities of both hexoses is lower than the uptake velocity of sucrose.

(v) There was no radiolabelled glucose or fructose contamination of the labelled sucrose used in these studies. Thus, the third transport system appears neither to be an artifact nor a reflection of one or both of glucose or fructose transport.

The apparent uptake of sucrose by the third transport system also cannot be attributable to intracellular polysaccharide synthesis: (i) The low level of intracellular polysaccharide found in *d/g* cocci does not increase on incubation with carbohydrate [20]. (ii) Fluoride is a powerful inhibitor of intracellular polysaccharide synthesis at very low concentrations [21]. (iii) The rate of intracellular polysaccharide synthesis [21] by *S. mutans* is far slower than the rate of the apparent sucrose uptake by the third transport system.

The current demonstration of the third transport system has not, of course, addressed the nature, energetic support, initial intracellular product, or route of the product's subsequent catabolism. It is tempting to speculate, however, that the product of the phospho*enol*pyruvate-dependent sucrose-specific phosphotransferase is catabolized via a phosphate hydrolase for which some preliminary evidence has already been given in *S. mutans* [37]. Similarly, one can further speculate that the product of the third transport system is metabolized via the intracellular invertase. Irrespective of the route of catabolism, the present data indicate that there are two phospho*enol*-pyruvate-dependent phosphotransferases which function to transport sucrose primarily under conditions of substrate paucity and slow growth; the third transport system functions under conditions supportive of more rapid growth and substrate abundance, consistent with previous indirect evidence [9].

The homofermentative microorganism *S. mutans* has as its primary ecological niche the tooth surface. This environment, under most normal conditions, receives only transient carbohydrate supply. One may postulate that if an organism possesses a high affinity transport system, it may have an ecological advantage during times of carbohydrate paucity. Roseman [38] has suggested that phospho*enol*pyruvate-dependent sugar-specific phosphotransferase can offer several physiological advantages to microorganisms. It would, naively perhaps, also appear energy efficient for sugar fermenting microorganisms if carbohydrates were transported and metabolized by interdependent processes which permit fine regulation and rapid adaptation to changing environmental conditions. Similarly, under conditions of limited energy supply, it would undoubtedly be advantageous if a transport mechanism(s) accomplished substrate permeation while concomitantly permitting the conservation of ATP. The transport of sucrose via two inducible phospho*enol*pyruvate-dependent sucrose-specific phosphotransferases of different K_m values is certainly consistent with such a physiological and ecological advantage. However, under ecological conditions in which there is an abundance of carbohydrate, as occurs intermittently in the oral cavity, such conservation of energy may no longer be a physiological necessity and the non-phosphotransferase transport system, with high apparent K_m, may be utilized to advantage to support rapid cell growth and metabolism. We envision such a role for the third transport system.

Acknowledgements

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