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Initial Characterization of Hexose and Hexitol Phosphoenolpyruvate-Dependent Phosphotransferases of *Staphylococcus aureus*

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The phosphoenolpyruvate sugar phosphotransferases of *Staphylococcus aureus* were surveyed biochemically to determine substrate range, inducibility and constitutivity, and requirements for soluble sugar-specific proteins. The substrate range is similar to that of the phosphotransferases of enteric bacteria, but the staphylococcal mannose and sorbitol systems are very inefficient. In addition, *S. aureus* has phosphotransferase activities for lactose and sucrose. The systems tested fell into two broad classes. Sugars for which there was substantial constitutive activity (fructose, mannose, sucrose, and glucose and its nonmetabolized analogues) did not require sugar-specific soluble factors for phosphorylation. Only in the case of fructose did growth in the presence of these constitutive sugars induce the corresponding phosphotransferase activity to higher levels. Kinetic experiments with each of these constitutive sugars yielded biphasic Hofstee plots; i.e., the kinetics were not characteristic of single enzymes. Preliminary experiments suggest that sucrose phosphorylation may involve the glucose and/or fructose systems. Truly inducible sugar phosphotransferase systems represent a second class; those for lactose and mannitol are the only members thus far identified. These systems are absent from uninduced cells, require soluble sugar-specific factors, and exhibit linear Hofstee plots. Sorbitol is apparently transported very poorly by intact cells but is an inducer of the mannitol system; it is phosphorylated efficiently in vitro by extracts of cells grown on either hexitol, but is taken up by intact cells at 0.1% of the mannitol rate.

In many bacterial genera, the uptake and phosphorylation of certain sugars is catalyzed by phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTS) (21). The reaction sequences depend on the bacterial species and sugar substrate involved but can be generalized as indicated below. (The nomenclature of the PTS has been described previously [13, 25].)

$$\text{PEP} + \text{HPr} \xrightleftharpoons[\text{Mg}^{2+}]{\text{enzyme I}} \text{pyruvate} + \text{P-HPr} \quad (1)$$

$$\text{P-HPr} + \text{sugar}_X \xrightarrow[\text{or enzyme II}^X \text{ complex}]{\text{factor III}^X + \text{enzyme II}^X} \text{HPr} + \text{sugar}_X\text{-P} \quad (2)$$

Reaction 1 involves only the nonspecific PTS proteins enzyme I and HPr and proceeds via a phospho-enzyme I intermediate (21, 29). Reaction 2 involves the sugar-specific PTS protein(s). In some cases these is a sugar-specific soluble protein (factor III) plus a corresponding membrane-bound component (enzyme II) (11, 27). In other cases there is a membrane-bound subsystem ("enzyme II complex"), which sometimes can itself be subdivided into two or more protein components (14). Reactions involving a soluble factor III proceed via a phospho-factor III intermediate (6, 20); in some cases phosphorylation of a component of an enzyme II complex has been observed (11).

The first biochemical identification of a PTS, by Kundig et al. (12), was in extracts of *Escherichia coli*. Distinct systems for glucose, fructose, mannose, mannitol, sorbitol (glucitol), galactitol, and β-methyl glucoside have subsequently been described by various groups (10, 13, 14, 16, 22). In general, the PTS of *E. coli* are marked by an absence of totally inducible sugar-specific soluble proteins (factors III), and, in several cases, the presence of both constitutive and inducible activities for the same sugar. Indirect evidence for a PTS in *Staphylococcus*

aureus was first reported by Egan and Morse (3, 4), who isolated a pleiotropic mutant that had lost the ability to utilize lactose, maltose, sucrose, galactose, fructose, mannitol, ribose, and trehalose; the mutant subsequently proved to lack the staphylococcal enzyme I, but only lactose, galactose, glucose, and mannitol have been reported as PTS sugars. Maltose apparently enters by diffusion and is hydrolyzed internally; the PTS acts only on the glucose liberated (1). The lactose PTS in *S. aureus* has been extensively studied (6, 8, 9, 24–26), but aside from preliminary characterizations of the glucose and mannitol systems (20, 27), none of the other systems has been investigated further (galactose is a substrate of the lactose PTS). There are factors III for both lactose and mannitol.

We report here an initial characterization of *S. aureus* PTS activities for sugars known to be PTS sugars in *E. coli* with respect to the following: (i) the substrate range of the PTS in *S. aureus*; (ii) the extent to which the systems are constitutive or inducible (or both); (iii) requirements for factors III; (iv) apparent Michaelis constants (K_m values). The results of preliminary study of the apparent sucrose PTS activity (which may involve the glucose or fructose systems, or both) are presented as well.

MATERIALS AND METHODS

Bacteria. The wild-type strain used in this study was *S. aureus* 5601. Although cells of strain 5601 remain completely suspended when grown in very rich media, e.g., 2% polypeptone plus 0.5% yeast extract, they tend to clump together and settle out when grown in less rich media, e.g., 2% Trypticase (without added carbohydrate) plus a vitamin mixture. Since it seemed desirable to test for induction of sugar PTS using media as free as possible from contaminating carbohydrates, we attempted to isolate a nonclumping derivative of 5601 by a multicycle enrichment procedure. We did not obtain a strain devoid of all clumping tendencies, but cells of the variant obtained (5601SF) do remain fairly well dispersed during exponential growth in liquid culture (with shaking). In contrast, 5601 cells settled to the bottom of the culture flask during growth on poor media. Strain C22 (26) is constitutive for the lactose PTS.

Growth of cells and preparation of membranes. For most experiments, the following "Trypticase-vitamins" medium was used. A solution containing 2% Trypticase (Baltimore Biological Laboratory) and 66 mM potassium phosphate buffer, pH 7.4, was autoclaved separately and then supplemented with a filtered solution of vitamins (0.01 volume) to the following final amounts (weight per liter of medium): biotin, 0.11 mg; thiamine, 2.2 mg; nicotinic acid, 2.2 mg; calcium pantothenate, 2.2 mg. Where indicated, carbon sources were sterilized by filtration and added to the medium; final concentrations were 0.5% unless otherwise indicated.

To obtain high densities of cells, a richer medium, containing 2% polypeptone, 0.5% yeast extract, and 50 mM potassium phosphate buffer, pH 7.4, was sometimes used. In these cases the pH was maintained near neutrality during growth, using a concentrated NH_3 solution (7).

Routinely, cells were harvested at the end of exponential phase by centrifugation, washed twice in buffer A [50 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, containing 0.15 M NaCl], and stored at −20°C.

Preparation and fractionation of extracts. Cell-free crude extracts were prepared (from 0.5 to 5 g of cells) by treatment with lysostaphin (Schwarz/Mann) and ultrasonic irradiation as described previously (7). The membrane fraction was obtained from the extracts as the pellet from ultracentrifugation (90 min at 270,000 × *g*) and was washed once in buffer C (1 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 8.3, containing 1 mM dithiothreitol and 1 mM ethylenediaminetetraacetate, pH 7.4). The washed pellet was resuspended in about 0.2 volume of buffer C. The final volume usually corresponded to 0.5 ml/original g of cells (wet weight). These membranes contain very little soluble PTS protein activity. Some cell wall material remains (25), and the lipid content is 370 nmol of lipid phosphorus per mg of protein. When viewed in the electron microscope (7) the preparation is seen to contain apparent cell wall fragments, membranous vesicles of various sizes, and some uncharacterized debris.

The high-speed supernatant fraction from the ultracentrifugation step was subjected to a second sedimentation to remove residual membrane fragments. To prepare the "acid pellet" fraction, this supernatant was titrated to pH 4.0 with 1 M acetic acid, stirred for 15 min at 0°C, centrifuged for 10 min at 12,000 × *g*, and resuspended in 0.2 volume of 50 mM potassium phosphate buffer, pH 7.4, containing 1 mM dithiothreitol and 0.1 mM ethylenediaminetetraacetate. The pH was readjusted to 7.4 with 1 M KOH.

Partial purification of enzyme I and HPr. Enzyme I and HPr were purified from extracts of cells of strain C22 grown in the presence of lactose, as described previously (7).

Standard in vitro phosphorylation assay. The assay procedure has been described (7). Briefly, ice-cold reaction mixtures were prepared by mixing stock solutions so that the final volumes (usually about 300 μl) contained the following components: PEP (3 mM); potassium phosphate buffer, pH 7.5 (10 mM); KF (3 mM); dithiothreitol (2 mM); $MgCl_2$ (4 mM); appropriate concentrations of radioactive sugar (3.3 mM, unless otherwise indicated), membrane fraction (enzyme II), enzyme I, and HPr. High-speed supernatant or acid pellet fraction, excess enzyme I, and amounts of HPr corresponding to partial (10 to 50%) saturation were used. Unless otherwise indicated, all protein fractions (except enzyme I and HPr) were obtained from cells grown on Trypticase-vitamins medium supplemented with

the sugars indicated. All incubations were for 30 min at 37°C. After the reaction was stopped by chilling on ice, the mixture was assayed for radioactive sugar phosphate by ion-exchange chromatography as previously described. The rate of sugar phosphate formation was in all cases directly proportional to membrane fraction (enzyme II) concentration (over the two- to fourfold range tested) and constant for at least 30 min. All values reported are the average of duplicate determinations; variations were usually less than 10%. Protein concentrations were determined by the method of Lowry et al. (18).

Cell transport experiments. Initial rates of uptake of sugars by whole cells were measured essentially as described previously (7). Equal portions of a mixture of radioactive sugar and cells (previously grown on Trypticase-vitamins medium in the presence of the indicated sugars and resuspended in 100 mM potassium phosphate buffer, pH 7.2, containing 0.4% sodium succinate) were quickly filtered and washed at approximately 10-s intervals during the first 1 to 2 min after mixing. For experiments with sorbitol, samples were removed at 1-min intervals during the first 5 min. Cell densities (usually about 2 mg/ml, dry weight) were estimated turbidimetrically using a conversion factor (1 Klett unit corresponds to 3 μg/ml) determined previously (7).

Determination of growth rates. Growth rates (generation times) were estimated from plots of turbidity increase versus time in the range 30 to 60 Klett units (red filter).

Chemicals. The following radioactive sugars were obtained from the indicated sources: [G-^{3}H]2-deoxyglucose, [1-^{14}C]mannitol, [1-^{14}C] mannose, [U-^{14}C]sorbitol, [1-^{14}C]ribose, [U-^{14}C]fructose, [^{14}C]-methylthio-β-galactoside, and [U-^{14}C]sucrose from New England Nuclear Corp.; [U-^{14}C]glucose and highly purified methyl α-[^{14}C]glucoside, gifts of Saul Roseman. Other materials used were unlabeled mannitol and sorbitol from Pfanstiehl, unlabeled mannose, arabinose, melibiose, ribose, and galactose from Sigma, unlabeled glucose and fructose from Baker, unlabeled sucrose from Schwarz/Mann, D-lactic acid from Fisher, and thiamine·HCl, biotin, calcium pantothenate, and nicotinic acid from Sigma. All hexoses are of the D-conformation, and all glycosides are pyranosides unless otherwise indicated.

RESULTS

Sugar substrates of the *S. aureus* PTS. Sugars were first tested for their effects on cell growth, when added to a Trypticase-vitamins medium. The generation time was 100 min in the unsupplemented medium. Substantial stimulations of growth (generation times, 40 to 50 min) were observed for glucose, lactose, fructose, mannitol, methyl β-glucoside, and sodium lactate. Arabinose and melibiose did not stimulate. Ribose, sorbitol, and mannose were slightly stimulatory (generation times, 85 to 90 min).

The results of in vitro sugar phosphorylation experiments are summarized in Table 1. The presence of a membrane-bound phosphorylation activity, dependent on added PEP, enzyme I, and HPr, is taken as evidence for a PTS. Since phosphorylation of mannitol and sorbitol requires an additional soluble component (see Table 3), specific activities for the two hexitols cannot be directly compared to those for other sugars. We were unable to study methyl β-glucoside phosphorylation, because putative methyl β-[^{14}C]glucoside from the only commercial source (Calatomic) proved to be more than 99% in an anionic form, as judged by its adherence to Dowex AG1-X2 (Cl^-form). Extracts of cells grown on ribose phosphorylated that sugar at a low rate, but the activity was stimulated by adenosine 5'-triphosphate rather than PEP and did not appear to be membrane bound.

Inducibility of PTS activities. The levels of

TABLE 1. *Dependence of sugar phosphorylation catalyzed by membrane fragments on the presence of enzyme I, HPr, and PEP*

Incubation mixture	Rate of phosphorylation of indicated sugars (nmol/min per mg of membrane protein)[a]				
	Fructose	Mannose	Mannitol	Sorbitol	Sucrose
Complete	33	18	7.9[b]	2.7[b]	27
Minus PEP	0.13	0.1	0.1	0.12	0.1
Minus PEP plus ATP[c]	0.12	0.1	0.1	0.13	0.46
Minus HPr	0.18	0.11	0.1	0.17	0.13
Minus enzyme I	0.23	0.42	—[d]	—[d]	0.17

[a] Standard *in vitro* sugar phosphorylation assays were performed using the indicated ^{14}C-labeled sugars and membrane fractions (40 to 600 μg of membrane protein) prepared from cells grown in the presence of the corresponding sugars, with omissions as indicated. The ^{14}C-labeled sugar concentrations were 3.3 mM in all cases, and specific activities were 0.1 μCi/μmol. Values correspond to the rate of sugar phosphate formation.

[b] Mannitol and sorbitol phosphorylation requires a soluble component in addition to enzyme I and HPr, so incubation mixtures were supplemented with acid pellet fractions (about 1 mg per assay) prepared from cells grown in the presence of mannitol and sorbitol, respectively (see text).

[c] ATP, Adenosine 5'-triphosphate.

[d] The acid pellet fraction added to the complete incubation mixture contained enzyme I.

activity in membranes prepared from cells grown on the Trypticase-vitamins medium (very low in carbohydrates) are compared to those from cells grown in the same medium supplemented with sodium lactate or the respective PTS sugars in Table 2. Activity for mannitol and sorbitol is undetectable unless hexitols are present in the growth medium; significant constitutive activity for lactose is similarly absent (9). There is, however, substantial constitutive fructose, mannose, sucrose, and glucoside activity. The fructose activity can be further induced sevenfold. The constitutive levels for fructose, mannose, and sucrose are depressed by growth in the presence of sodium lactate. We have observed a similar depression of the constitutive lactose PTS of strain C22 (26) in vigorously aerated cells. We cannot explain the apparent threefold depression of activity for methyl α-glucoside (and glucose) in cells of strain 5601SF grown on glucose, an effect that was not observed in strain C22. Methyl α-glucoside activity in the latter was the same in cells grown on lactate, glucose, or galactose. As Fig. 1 indicates, mannitol transport activity is present in mannitol (and sorbitol)-grown cells but absent in lactate-grown cells. There is a constitutive fructose uptake activity (Fig. 2) which can be induced to higher levels; thus, these results are consistent with the in vitro phosphorylation experiments. (Sorbitol uptake is not detectable under these conditions; see Table 4.)

Only mannitol and sorbitol require a fourth (soluble) component for phosphorylation. The lactose PTS of *S. aureus* consists of enzyme I, HPr, the membrane-bound enzyme II^{lac}, and a sugar-specific soluble protein, factor III^{lac} (6, 27); similar requirements for mannitol phosphorylation have previously been reported (27). Analogous soluble factors have been identified in other bacterial genera in only a few instances (11, 30). Since the staphylococcal lactose and mannitol factors are obligatory, addition of the appropriate high-speed supernatant fraction to a mixture of excess enzyme I, HPr, and the corresponding washed membranes results in a dramatic stimulation of sugar phosphorylation. An acid pellet fraction precipitated at about pH 4.0 can replace the high-speed supernatant fraction. As Table 3 indicates, only mannitol and sorbitol, of the sugars tested, require an additional soluble component for their phosphorylation. In the case of fructose, one experiment was performed at a very low (10 μM) sugar concentration, in order to test for the presence of a soluble fractor stimulatory only at subsaturating substrate concen-

TABLE 2. *Inducibility of phosphotransferase systems*

Supplemental carbon source in growth medium of cells used for membranes[a]	Sugar used for in vitro phosphorylation assay	Relative rate of sugar phosphorylation[b]
(Blank)[c]	Fructose	0.01
None		0.14
Sodium lactate		0.08
Fructose		(1.00)
(Blank)	Mannose	0.02
None		0.83
Sodium lactate		0.33
Mannose		(1.00)
(Blank)	Mannitol	0.01
None		0.01
Sodium lactate		0.01
Mannitol		(1.00)
(Blank)	Sorbitol	0.02
None		0.03
Sodium lactate		0.04
Sorbitol		(1.00)
(Blank)	Sucrose	0.09
None		0.91
Sodium lactate		0.64
Sucrose		(1.00)
(Blank)	Methyl α-glucoside[d]	0.03
None		0.83
Sodium lactate		0.83
Glucose		0.25
Galactose		(1.00)

[a] Membrane fractions prepared from cells grown on Trypticase plus the carbon sources indicated (concentration in medium, 0.5%) were used (40 to 600 μg of membrane protein per assay) for the in vitro phosphorylation assays with the corresponding sugar. For the mannitol and sorbitol experiments, 0.5 mg of the acid pellet fraction corresponding to the membrane fraction was added to the assay.

[b] Standard phosphorylation assays were performed using the indicated ^{14}C-labeled sugars (specific activities, 0.1 μCi/μmol); concentrations were 3.3 mM, except for sucrose (66 μM, 2.5 μCi/μmol). Relative rates of 1.00 correspond to the following rates of ^{14}C-labeled sugar phosphate formation, in nanomoles per minute per milligram of membrane protein: fructose, 54; mannose, 43; mannitol, 6.7; sorbitol, 3.6; sucrose, 56; methyl α-glucoside, 1.2; glucose, 140.

[c] Blank values correspond to the apparent amount of ^{14}C-labeled sugar phosphate (or other anionic derivative) present in assay mixtures incubated without proteins; the values were arbitrarily normalized using the membrane protein concentration of the lactate-grown cells.

[d] Similar results were obtained using glucose.

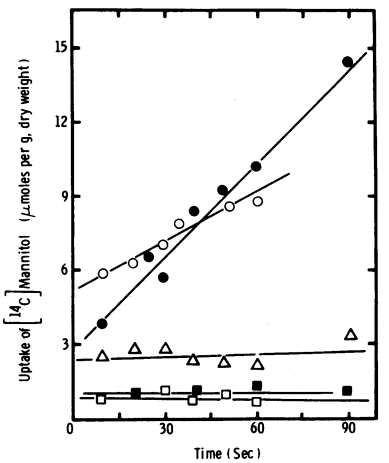

FIG. 1. *Uptake of mannitol by mannitol- and sorbitol-grown cells. Cell transport experiments were performed as described in the text using [14C]mannitol (specific activity, 0.2 μCi/μmol at the indicated concentrations and cells (1.4 to 3.0 mg/ml, dry weight) previously grown in the presence of the indicated sugars: (●) mannitol cells (0.76 mM [14C]mannitol); (○) sorbitol cells (2 mM [14C]mannitol); (△) sodium lactate cells (2 mM [14C]mannitol). (■, □) Corresponding blank determinations for mannitol and sorbitol cells in the presence of excess unlabeled mannitol.*

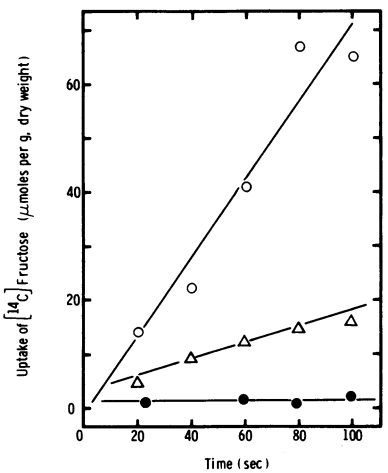

FIG. 2. *Uptake of fructose by fructose- and lactate-grown cells. Cell transport experiments were performed as described in the text using 320 μM [14C]fructose (specific activity, 0.2 μCi/μmol) and cells (2.2 to 2.4 mg/ml, dry weight) previously grown in the presence of the indicated sugar: (○) Fructose-grown cells; (△) sodium lactate-grown cells; (●) fructose-grown cells plus excess unlabeled fructose.*

trations; no significant stimulation was observed. Membranes prepared from fructose-grown cells were subjected to an additional intensive mechanical disruption-washing procedure; no soluble stimulatory factor was released. Further fractionation experiments were not attempted. The small stimulations observed for fructose, mannose, and sucrose could result from small membrane fragments remaining in the soluble fraction or, in the case of the sucrose PTS, the effects of sucrase (5).

Properties of the mannitol (sorbitol) PTS. In vitro complementation experiments (data not shown) indicate that growth of cells in the presence of either mannitol or sorbitol induces

TABLE 3. *Requirement of phosphorylation of various sugars for sugar-specific soluble components (factor III)*

Sugar	Supplement[a]	Relative rate of sugar phosphorylation[b]
Fructose	None	(1.00)
	Acid pellet, 0.5 mg	1.0
	High-speed supernatant, 0.5 mg	1.2
Mannose	None	(1.00)
	Acid pellet, 0.3 mg	1.1
	High-speed supernatant, 0.5 mg	1.0
Mannitol	None	(1.00)
	Acid pellet, 0.5 mg	25
	Acid pellet, 1.0 mg	50
Sorbitol	None	(1.00)
	Acid pellet, 1.0 mg	4.0
	Acid pellet, 2.0 mg	7.5
Sucrose	None	(1.00)
	Acid pellet, 0.5 mg	1.3
	Acid pellet, 1.0 mg	1.3
	High-speed supernatant, 0.5 mg	1.6
	Acid pellet, 0.5 mg[c]	1.3
	Acid pellet, 1.0 mg[c]	1.6
	High-speed supernatant, 0.5 mg[c]	1.4

[a] Crude high-speed supernatant and acid pellet fractions were prepared as described in the text, using cells grown in the presence of an 0.5% concentration of the sugar indicated; amount of protein added is indicated.

[b] Standard in vitro phosphorylation assays were performed using 3.3 mM ^{14}C-labeled sugars (specific activity, 0.1 μCi/μmol); the membrane fractions (40 to 600 μg per assay) were supplemented with high-speed supernatant and acid pellet fractions as indicated, enzyme I, and HPr. Relative rates of 1.00 correspond to the following rates of sugar phosphate formation, in nanomoles per minute per milligram of membrane protein: fructose, 37; mannose, 23; mannitol, 0.2; sorbitol, 2.0; sucrose, 69.

[c] Protein fractions from cells grown in the absence of sucrose.

phosphorylation activity for both sugars, and that the membrane fraction induced by either hexitol is active with either induced soluble factor. The sorbitol phosphorylation activity is always less than that for mannitol, regardless of the source of the components; the ratio is about 0.3, as judged by these and other experiments (Table 4). The apparent K_m values estimated for mannitol and sorbitol phosphorylation from several (initial velocity) – (substrate concentration) experiments are summarized in Table 5. Data from a typical experiment for mannitol are depicted in Fig. 3 using a Hofstee plot ($v/[S]$ versus v). An apparent K_m for mannitol uptake by intact cells was estimated from the data of Fig. 3 and similar experiments. Surprisingly, sorbitol uptake is virtually undetectable, despite the substantial phosphorylation activity observed in vitro. As Table 4 indicates, a very low rate of uptake can be demonstrated under extreme conditions (very high cell concentrations, very high sorbitol levels), but the uptake rate is only slightly (about 20%) above that observed using lactate-grown cells and only partially (20%) inhibited by the presence of excess unlabeled sorbitol. Thus, even the very inefficient sorbitol uptake observed may be mainly due to diffusion: it is directly proportional to sugar concentration over the

TABLE 4. *Michaelis constants and relative rates for in vitro phosphorylation and in vivo transport of mannitol and sorbitol*

Sugar	Phosphorylation in vitro		Uptake by intact cells	
	Apparent K_m[a] (μM)	Relative rate[b]	Apparent K_m[c] (μM)	Relative rate[d]
Mannitol	6 ± 3	(1.0)	20	(1.0)
Sorbitol	29 ± 16	0.28 ± 0.15		0.0004

[a] Apparent K_m values were determined from Lineweaver-Burk plots of initial velocity data obtained using the standard phosphorylation assays. Both the amounts of membrane fraction and acid pellet fraction and the cells used to prepare them were the same for any pair of determinations (mannitol phosphorylation versus sorbitol phosphorylation), but different preparations and concentrations were used for different experiments. There was no systematic variation in the results. Generally, 20 μg of membranes from mannitol-grown cells or 15 μg of membrane from sorbitol-grown cells, with 0.5 to 2.5 mg of acid pellet fraction from cells grown on mannitol or sorbitol, respectively, were used. Specific activities of ^{14}C-labeled sugars used were mannitol, 1 μCi/μmol, and sorbitol, 4 μCi/μmol. Both enzyme I and HPr were present in excess. The mannitol K_m value is the average of four experiments, and the sorbitol value is the average of two.

[b] The rate of phosphorylation catalyzed by a given membrane fraction (0.5 mg) and acid pellet fraction (1.0 mg) was compared to the rate of mannitol phosphorylation catalyzed by the same protein fractions. The indicated value is the average of results obtained using protein fractions prepared from mannitol-induced cells (three experiments) and sorbitol-induced cells (two experiments); there was no systematic variation in the results. Saturating concentrations (3.3 mM) of [^{14}C]mannitol and [^{14}C]sorbitol (specific activities, 0.1 μCi/μmol) were used in all cases.

[c] The apparent K_m for mannitol uptake was estimated from a plot of (initial uptake rate)$^{-1}$ versus (concentration)$^{-1}$, using the results of a series of whole-cell transport experiments similar to that of Fig. 1, performed using mannitol concentrations from 0.08 to 2.0 mM.

[d] Since sorbitol uptake was detected only at very high cell densities (8 mg/ml, dry weight) and sugar concentrations (0.5 to 3.6 mM) and was approximately first-order in sorbitol, whereas mannitol uptake was rapid and saturable, no rigorous comparison of the rates can be made. The ratio given corresponds to the ratio between the two limiting apparent first-order rate constants at low concentration, that is, between [(maximum velocity)/(K_m)] for mannitol uptake and the observed rate constant for sorbitol uptake. The sorbitol uptake rate was corrected using a control value corresponding to the rate of uptake of [^{14}C]sorbitol in the presence of a 100-fold excess of unlabeled sorbitol. This control value was about 80% of the rate actually observed.

TABLE 5. *"Limiting" Michaelis constants for PTS with complex initial velocity-substrate concentration curves*

Sugar phosphorylated[a]	Apparent K_m values (mM)[b]	
	$K_m(1)$	$K_m(2)$
2-Deoxyglucose	<0.06[c]	5
Glucose	<0.02[c]	>2[c]
Methyl α-glucoside	0.3	10
Mannose	1.6	30
Sucrose	0.015	>40[c]
Fructose	0.02	0.3

[a] Standard in vitro sugar phosphorylation assays were performed using the amounts of membrane fraction indicated below; the carbon source present in the growth medium used to prepare the membranes is indicated below as "glucose membranes," etc: 2-deoxyglucose assays, 10 μg of glucose membranes; glucose assays, 2.2 μg of glucose membranes; methyl α-glucoside assays, 200 μg of lactate membranes (glucose membranes gave similar results); mannose assays, 300 μg of mannose membranes; sucrose assays, 2.2 μg of sucrose membranes; fructose assays, 2.2 μg of fructose membranes (lactate membranes gave similar results). Specific activities of radioactive sugars were [^{3}H]deoxyglucose, 0.3 μCi/μmol; [^{14}C]glucose, 5 μCi/μmol; methyl α-[^{14}C]glucoside, 0.3 μCi/μmol; [^{14}C]-mannose, 0.05 μCi/μmol; [U-^{14}C]sucrose, 2.5 or 0.1 μCi/μmol; [^{14}C]fructose, 1 μCi/μmol. The K_m estimates were not substantially affected by variation of HPr concentrations.

[b] Apparent K_m values were estimated from Hofstee plots, i.e., from the limiting slopes of plots of (initial velocity)/(sugar concentration) versus (sugar concentration) (2). $K_m(1)$ corresponds in each case to the slope of the linear portion of the plot at low sugar concentrations, and $K_m(2)$ to the slope of the linear portion at high concentrations.

[c] Hofstee plot was nonlinear at lowest or highest concentrations. Upper or lower limits to $K_m(1)$ or $K_m(2)$ correspond to slope through the two lowest or highest data points.

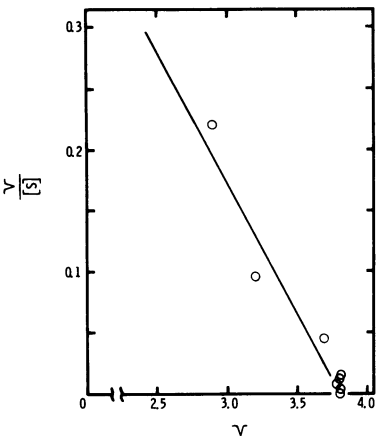

FIG. 3. *Kinetic data for phosphorylation of mannitol in vitro. The rate of phosphorylation of [^{14}C]mannitol (specific activity, 1 μCi/μmol) was determined by the standard assay, using 20 μg of membrane fraction (from cells grown in the presence of mannitol) and 0.5 mg of acid pellet fraction from the same cells. In these experiments HPr was in excess. The symbol v corresponds to the initial rate of mannitol phosphate formation in nanomoles per milligram of membrane protein, and the symbol [S] corresponds to the concentration of [^{14}C]mannitol (micromolar). The line is the best least-squares fit.*

range tested (0.5 to 3.6 mM). In a preliminary competition experiment (data not shown), sorbitol was found to be a competitive inhibitor of mannitol uptake in vivo (K_i approximately 0.2 mM).

To determine the relative efficiencies of mannitol and sorbitol as inducers of PTS activity, cells were grown (almost to stationary phase) at a series of hexitol concentrations. Membranes prepared from these cells were assayed for mannitol phosphorylation activity. The results indicate that at a low concentration (0.026 mM) sorbitol is about 15% as effective an inducer as mannitol, but that at a high concentration (2.6 mM), the same level of activity is induced.

Biphasic kinetic plots for "constitutive" PTS sugars. When (initial velocity) – (substrate concentration) data for mannitol are analyzed by either Lineweaver-Burk or Hofstee plots (Fig. 3), the points fit a single straight line (within experimental error). Experiments with the lactose PTS gave similar results (20, 24). A different pattern is obtained for all of the sugars listed in Table 5. The plots are biphasic; the deviation from linearity is more apparent in the Hofstee plots. Typical results are shown for methyl α-glucoside in Fig. 4. We have estimated the apparent K_m values from the limiting slopes of the Hofstee plots (as in Fig. 4), assuming one high-affinity system [K_m (1)] and one low-affinity system [K_m (2)] for each sugar. In several instances, the plots did not become linear even at the highest and lowest substrate concentrations, so that the estimates for K_m (1) and K_m (2) represent only upper and lower limits, respectively.

Apparent PTS activity for sucrose. Sucrose substantially stimulates the growth of these cells. There is an apparent constitutive PTS activity for this sugar; no soluble factor in addition to enzyme I and HPr is required. Plots of the kinetic data are markedly biphasic (Table 5). Our results are consistent with indirect evidence for utilization of sucrose via the PTS reported by Egan and Morse (5), but in neither that study nor the present report has the actual substrate or product of the phosphorylation reaction been identified. In preliminary competition experiments (data not shown) we found that both glucose and fructose inhibited the phosphorylation of sucrose in vitro (even when the data were extrapolated to infinite HPr concentration), and that the effects for the two inhibitors were roughly additive. These results cannot be taken as direct evidence for involvement of the glucose or fructose PTS in sucrose phosphorylation, however. Identification of the actual sugar phosphate formed should be the first step in further characterization of this potentially interesting system.

DISCUSSION

General characteristics of the staphylococcal PTS. All of the substrates of the *E. coli*

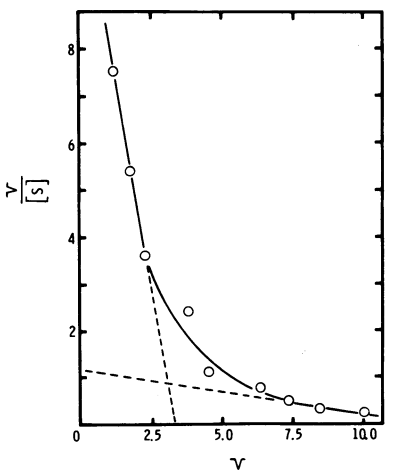

FIG. 4. *Kinetic data for phosphorylation of methyl α-glucoside in vitro. The rate of phosphorylation of methyl α-glucoside (specific activity, 0.3 μCi cpm/μmol) was determined by the standard assay, using 200 μg of membrane fraction (from cells previously grown in the presence of sodium lactate). The symbol v is defined in the legend to Fig. 3. The symbol [S] corresponds to the concentration of methyl α-glucoside (millimolar).*

phosphotransferases tested here were substrates for *S. aureus* systems as well. However, the mannose PTS activity is very inefficient, both in vitro and in vivo, and the sorbitol system is essentially inoperative in vivo. Additional PTS activities in *S. aureus*, for which there are no *E. coli* equivalents, include the lactose (galactose) system (9, 25) and a PTS-like activity for sucrose. Further studies, including product analyses, are necessary to determine the relationship of the sucrose activity to the glucose and fructose systems. The only truly inducible systems thus far identified are those for mannitol (sorbitol) and for lactose (galactose). Rate-concentration experiments with the inducible systems yield the kinetic plots expected for single saturable enzymes (Fig. 3). The apparent K_m observed here for mannitol (6 μM) is comparable to those for substrates of the lactose system (26). Both of the inducible systems require (distinct) sugar-specific soluble factors (27).

In contrast to the above, the PTS activities for fructose, mannose, sucrose, and glucose and its nonmetabolized analogues are present in lactate-grown cells and (except for glucose) are induced to higher levels by growth on the corresponding substrate. Rate-(substrate concentration) experiments with all of these constitutive systems yield biphasic kinetic plots. These experiments were performed using partially purified protein preparations; the values obtained for the kinetic parameters correspond to complex systems and do not represent absolute determinations of K_m values. Further experiments, including the effects of HPr concentration and of inhibitors, are necessary to determine whether the biphasic plots are the result of two distinct enzymes for each sugar, a smaller number of enzymes with overlapping specificities, or single enzymes exhibiting negative cooperativity. The *E. coli* systems for these constitutive sugars are similarly complex (21). The apparent high K_m and slow growth rate for mannose suggest that there is no mannose PTS per se, but that inefficient utilization of another system is involved.

Mannitol (sorbitol) PTS. The results suggested that only a single (mannitol) PTS catalyzes hexitol phosphorylation but that it is induced by sorbitol. Apparently the in vivo uptake process is much more selective than the in vitro reaction; the sorbitol-mannitol activity ratio is 10^{-3} for the former and 0.3 for the latter. The level of mannitol PTS activity induced by sorbitol is well above that which could be accounted for by contaminating mannitol. It seems paradoxical that sorbitol, which is a poor substrate for growth and whose uptake is barely detectable, is a fairly good inducer. A somewhat similar phenomenon was observed by Solomon and Lin (28) with the mannitol PTS in *E. coli*. In this organism the enzyme II^{mtl} and a mannitol phosphate dehydrogenase are coordinately induced. A mutant strain, unable to ferment mannitol and lacking enzyme II^{mtl} activity (as measured by an in vitro phosphorylation assay), could nevertheless induce the dehydrogenase to about one-third of the normal level. Our results with sorbitol and the *E. coli* results thus suggest that induction may result from binding of the substrate to a membrane receptor, whether or not it is actually transported into the cell. We do not know if the slight stimulation of growth at high (2.6 mM) sorbitol concentration is due to the slow diffusive entry of sorbitol or utilization of trace amounts of mannitol, or both.

The substrate range of the *E. coli* hexitol PTSs is different from that for *S. aureus* (15). In the former organism there are genetically and biochemically distinct systems for mannitol, sorbitol, and galactitol, although the substrate specificities of these three systems are not absolute (15–17). Similarly, the mannitol PTS in *Streptococcus mutans* is biochemically distinct from that for sorbitol (19). Our results are quite similar to those obtained by Saier and Newman using *Spirochaeta aurantia* (23). Cells of this organism, grown in the presence of mannitol, induce a PTS that catalyzes the PEP-dependent in vitro phosphorylation of mannitol and sorbitol with equal efficiencies. However, the rate of sorbitol uptake by mannitol-grown intact cells is less than 2% of the rate for mannitol. A low level of fructose phosphorylation activity (but not transport) is also induced by mannitol in this organism. Curiously, we find that fructose PTS activity (both in vitro phosphorylation and in vivo transport) is induced in *S. aureus* when autoclaved sorbitol is added to the growth medium, but no such induction is observed when the sugar is sterilized by filtration instead.

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