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Fractionation and Characterization of the Phosphoenolpyruvate:Fructose 1-Phosphotransferase System from *Pseudomonas aeruginosa*

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The initial reactions involved in the catabolism of fructose in *Pseudomonas aeruginosa* include the participation of a phosphoenolpyruvate:fructose 1-phosphotransferase system (F-PTS). Fractionation of crude extracts of fructose-grown cells revealed that both membrane-associated and soluble components were essential for F-PTS activity. Further resolution of the soluble fraction by both size exclusion and ion-exchange chromatography revealed the presence of only one component, functionally analogous to enzyme I. Enzyme I exhibited a relative molecular weight of 72,000, catalyzed the pyruvate-stimulated hydrolysis of phosphoenolpyruvate to pyruvate, and mediated the phosphorylation of fructose when combined with a source of enzyme II (washed membranes). No evidence for the requirement of a phosphate carrier protein, such as HPr, could be demonstrated. Thus, the F-PTS requires a minimum of two components, a soluble enzyme I and a membrane-associated enzyme II complex, and both were shown to be inducible. Reconstituted F-PTS activity was specific for phosphoenolpyruvate as a phosphate donor (K_m, ~0.6 mM) and fructose as the sugar substrate (K_m, ~18 μM). Components of the *Pseudomonas* F-PTS did not restore activity to extracts of deletion mutants of *Salmonella typhimurium* deficient in individual proteins of the PTS or to fractionated membrane and soluble components of the F-PTS of *Escherichia coli*. Similarly, membrane and soluble components of *E. coli* and *S. typhimurium* would not cross-complement the F-PTS components from *P. aeruginosa*.

The intracellular accumulation of sugars as sugar-phosphate derivatives is mediated by the phosphoenolpyruvate (PEP):phosphotransferase system (PTS) in numerous species of bacteria (for reviews, see 2, 3, 10, 11). Extensive biochemical, physiological, and genetic studies have revealed that the PTS is a multicomponent system composed of both soluble and membrane-associated enzymes and phosphocarrier proteins. These components catalyze the hydrolysis of PEP to pyruvate concomitant with the sequential transfer of the phosphoryl moiety of PEP to sugar substrate, resulting in the transmembrane accumulation of phosphorylated sugar. The general reaction sequence is as follows:

$$\text{PEP} + \text{HPr} \xrightarrow[\text{MgCl}_2]{\text{enzyme I}} \text{phospho-HPr} + \text{pyruvate}$$

$$\text{Phospho-HPr} + \text{sugar} \xrightarrow[\text{complex}]{\text{enzyme II}} \text{sugar-P} + \text{HPr}$$

Initial phosphoryl transfer is catalyzed by enzyme I and the heat-stable, low-molecular-weight phosphocarrier protein HPr. These are designated "general" proteins of the PTS and are synthesized constitutively or at only slightly elevated levels during growth on PTS-sugars (2). Recently, partial reactions mediated by enzyme I have been demonstrated (15); enzyme I catalyzed an exchange phosphoryl transfer reaction between PEP and pyruvate as well as the hydrolysis of PEP to pyruvate in the presence of HPr. Membrane-associated enzyme II complexes are sugar-specific recognition proteins which catalyze the phosphorylation and membrane translocation of the sugar substrate (2, 10, 12). Enzyme II also mediates the vectorial uptake of sugars via an exchange group translocation (sugar phosphate:sugar transphosphorylation) that is independent of the soluble components of the PTS (2, 4, 8, 12, 14). Uptake of certain sugars by some bacterial species requires an additional phosphocarrier protein, enzyme III, which may be either membrane bound or soluble (2, 3, 10, 17).

Recent studies (1, 16, 18) have shown that crude extracts of certain species of the genus

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Pseudomonas contain a fructose-specific PTS (F-PTS). In *Pseudomonas aeruginosa* the F-PTS catalyzed PEP-dependent phosphorylation of fructose to fructose 1-phosphate, which is further metabolized primarily through the Entner-Doudoroff pathway and to a lesser extent through the Embden-Meyerhof pathway (9). In this paper, we describe the general characteristics of the F-PTS from *P. aeruginosa* and present evidence that it apparently is composed only of enzyme I and enzyme II complex. Furthermore, data are presented which demonstrate that components of the F-PTS from *Pseudomonas* do not cross-complement the PTS components of enteric bacteria.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* PAO was obtained from B. W. Holloway. *Salmonella typhimurium* SB3507 (*trpB223*), *S. typhimurium* SB3750 (*trpB223 ptsI181*), and *S. typhimurium* NCR 127 (*trpB223 ptsH196 crp*) were kindly provided by Thoyd Melton (North Carolina State University). *Escherichia coli* K-12 was obtained from Martha Zuniga (Yale University). Stock and working cultures were maintained as described elsewhere (9).

Medium and growth conditions. Bacterial strains were cultured on basal salts medium (pH 7.0) with the following composition, per liter: Na_2HPO_4, 1.77 g; KH_2PO_4, 1.7 g; $(NH_4)_2SO_4$, 1 g; $MgCl_2 \cdot 6H_2O$, 0.16 g; and $FeSO_4 \cdot 7H_2O$, 5 mg. Carbon sources were prepared separately as 1 or 2 M stock solutions, filter sterilized, and added to the medium to a final concentration of 20 mM for fructose and 40 mM for lactate.

A 1% inoculum of cells preadapted to growth on a particular carbon source was used to initiate growth of microorganisms in 1-liter Erlenmeyer flasks containing 250 ml of basal salts medium plus the appropriate substrate. Cells were grown at 37°C in a New Brunswick environmental shaker with aeration (250 rpm) and were harvested at late exponential growth phase by centrifugation at 10,000 × *g* for 5 min at 4°C. Cultures were washed with 50 mM Na_2HPO_4-KH_2PO_4 buffer (pH 7.0) containing 1 mM dithiothreitol and were stored at −40°C until use.

Preparation and fractionation of crude extracts. Cell pastes were suspended in approximately 2 volumes of 50 mM Na_2HPO_4-KH_2PO_4 buffer (pH 7.0) containing 1 mM dithiothreitol and disrupted by passage through a French pressure cell at 18,000 lb/in^2 at 4°C. Cell debris and unbroken cells were removed by centrifugation at 10,000 × *g* for 20 min. The supernatant fraction thus obtained was used as a source of crude extract. Further fractionation of crude extract was accomplished after ultracentrifugation at 188,000 × *g* for 2 h at 4°C. The supernatant fraction is designated soluble fraction throughout the text. The pellet (membrane-enriched fraction) was suspended in phosphate buffer and homogenized with a Teflon tissue homogenizer. For most experiments, the homogenized membrane fractions were passed over DEAE-cellulose columns (1.5 by 4 cm) equilibrated in phosphate buffer to remove the majority of contaminating soluble PTS components. Both soluble and membrane fractions were used immediately or stored at −80°C until use.

Enzyme assays. Phosphotransferase assays were done as described by Kundig and Roseman (6). Reaction mixtures (200 μl) were incubated at 32°C for 30 min and stopped by the addition of 0.5 ml of ice-cold distilled water. Sugars and sugar-phosphates were separated by Bio-Rad AG 1-X2 column radiochromatography as previously described (6).

Enzyme I-mediated hydrolysis of PEP to pyruvate was performed as described elsewhere (15). Reaction mixtures were a modification of that used for the PEP-pyruvate exchange reaction described by Saier et al. (15). Addition of low concentrations of pyruvate (2 mM and less) stimulated the membrane-independent hydrolysis of PEP to pyruvate catalyzed by the soluble fraction. Thus, 2 mM pyruvate was routinely added to the reaction mixtures.

Protein was determined by the method of Lowry et al. (7), using bovine serum albumin as a standard, or by the spectrophotometric method of Kalb and Bernlohr (5).

Materials: [*U*-^{14}C]fructose and phosphoenol[1-^{14}C]pyruvate (cyclohexylammonium salt) were purchased from Amersham Corp. All other chemicals were of the highest purity commercially available.

RESULTS

Fractionation of the F-PTS. Previous reports (1, 16, 18) have demonstrated that crude extracts obtained from numerous species of *Pseudomonas* contain an inducible fructose-specific PTS when grown on fructose. Crude extracts of *P. aeruginosa* convert fructose to fructose 1-phosphate (9) only with the addition of PEP to reaction mixtures (Table 1). Further resolution of crude extracts into soluble and membrane fractions via ultracentrifugation revealed that these fractions alone exhibited negligible phosphotransferase activity. However, reconstitution of the complete system (membrane plus soluble fractions) resulted in total recovery of phosphotransferase activity, which was apparent in crude extracts. Thus, the results indicate

TABLE 1. Fractionation of the PEP:F-PTS from *P. aeruginosa*[a]

Fraction	Addition	Total nmol of fructose 1-phosphate formed/min per ml of extract
Crude extract	+PEP	13.8
Crude extract	−PEP	0.17
Soluble fraction	+PEP	0.39
Membrane fraction	+PEP	1.60
Soluble + membrane fraction	+PEP	14.77

[a] Cells were grown on 20 mM fructose, harvested, and disrupted by passage through a French pressure cell at 18,000 lb/in^2. Crude extract was the supernatant fraction after centrifugation at 10,000 × *g* for 20 min. Membrane and soluble fractions were obtained after ultracentrifugation at 188,000 × *g* for 2 h. PTS activity was assayed by Bio-Rad AG 1-X2 column radiochromatography.

TABLE 2. Induction of soluble and membrane fraction components of the PEP:F-PTS[a]

Cell extract fractions from cells cultured on:[b]	pmol of fructose 1-phosphate formed/min per reaction mix
Soluble (Fru)	4
Membranes (Fru)	36
Soluble (Fru) + membranes (Fru)	116
Soluble (Lact)	4
Membranes (Lact)	8
Soluble (Lact) + membranes (Lact)	7
Soluble (Lact) + membranes (Fru)	8
Soluble (Fru) + membranes (Lact)	14

[a] Preparation and fractionation of crude extracts and PTS assays were performed as described in Table 1.

[b] Cells were cultured on either 20 mM fructose (Fru) or 40 mM lactate (Lact).

that the F-PTS is multicomponent and that both membrane-bound and soluble components are needed for the phosphorylation of fructose.

Induction of soluble and membrane components of the F-PTS. In *P. aeruginosa* F-PTS activity is detected only in crude extracts of cells cultured on fructose and to a lesser extent on mannitol (9). To determine the inducibility of the soluble and membrane-associated components of the F-PTS from *P. aeruginosa*, membrane and soluble fractions were obtained from lactate- and fructose-grown cells. Membrane and soluble fractions prepared from cells grown on either substrate did not contain PTS activity alone (Table 2); as expected, recombination of the fractions prepared from fructose-grown cells resulted in activity, whereas recombination of those fractions prepared from lactate-grown cells did not. Addition of membranes obtained from fructose-grown cells (source of enzyme II) to the soluble fraction from lactate-grown cells did not restore F-PTS activity (Table 2), suggesting that the soluble component(s) of the F-PTS is inducible. Similar results were observed when the soluble fraction prepared from cells grown on fructose was added to membranes obtained from lactate-grown cells. Nearly identical results were obtained when cells were grown on Casamino Acids rather than lactate (data not shown). Therefore, the F-PTS from *P. aeruginosa* differs from most bacterial PTS in that both soluble and membrane components are inducible.

Properties of the F-PTS. Hyperbolic kinetics were observed when the PEP-dependent phosphorylation of fructose was examined as a function of either membrane or soluble protein with one component limiting (Fig. 1). Phosphotransferase activity required Mg^{2+}, and activity was maximal at pH 7.4 (data not shown). At pH 7.4, the apparent K_m values for PEP and fructose were 0.6 mM and 18 μM, respectively.

The ability of various phosphate esters to serve as phosphoryl donors for the phosphorylation of fructose is shown in Table 3. When tested at concentrations of 5 mM, ATP, ADP, GTP, 2-phosphoglycerate, and UTP exhibited low activity (≤7% that of PEP) when substituted as a phosphoryl donor. These compounds conceivably may be slowly converted to or cause the generation of PEP in crude extracts. AMP and other sugar phosphates were inactive (Table 3).

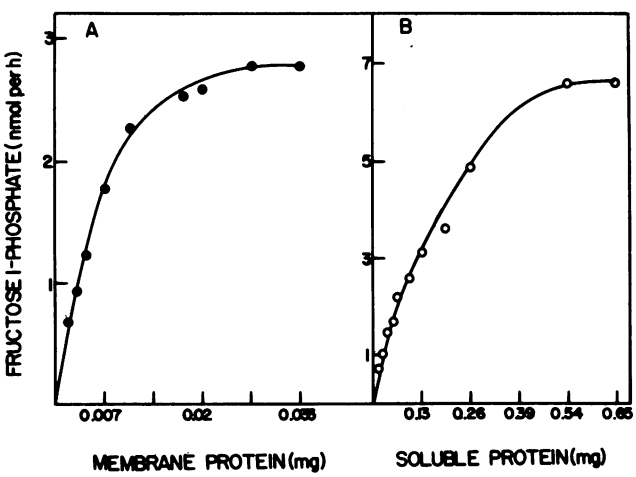

FIG. 1. PEP-dependent phosphorylation of fructose as a function of either membrane or soluble protein. (A) Various concentrations of membrane protein were added to a constant amount of soluble protein (0.25 mg). (B) Various concentrations of soluble protein were added to 0.026 mg of membrane protein. [^{14}C]fructose 1-phosphate formation was determined as described in Table 1.

TABLE 3. Phosphate donor specificity of PEP:F-PTS of *P. aeruginosa*[a]

Phosphate donor	Activity relative to PEP (%)
PEP	100
ATP	7
ADP	5
GTP	4
2-Phosphoglycerate, UTP	1
AMP, fructose 1-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, glucose 1-phosphate, glucose 6-phosphate, mannose 1-phosphate, mannose 6-phosphate, α-glycerol phosphate, 3-phospho-glycerate, ribose 5-phosphate	<1

[a] Phosphate donors were added individually to a final concentration of 5 mM. Reactions were started by addition of dialyzed crude extracts (0.98 mg of protein per ml) from fructose-grown cells. PTS assays were done as described in Table 1.

Resolution of the soluble fraction. To ascertain whether the soluble fraction from *P. aeruginosa* possessed typical soluble PTS proteins such as enzyme I and HPr, the soluble protein fraction from fructose-grown cells was concentrated by precipitation with ammonium sulfate (30 to 75%) and applied to a Sephadex G-100 column (2.5 by 80 cm) previously calibrated with standard molecular weight proteins. Recombination of washed membranes with those fractions eluting directly after the void volume (enzyme I) together with those fractions with an elution volume corresponding to a molecular size similar to HPr (~10,000) should result in recovery of F-PTS activity. Surprisingly, only the recombination of those fractions with an elution volume corresponding to a relative molecular weight of 72,000 was required to restore phosphotransferase activity when added to membrane suspensions (Fig. 2). Protein eluting in these fractions also catalyzed the pyruvate-stimulated hydrolysis of PEP to pyruvate. Those fractions that eluted where one might expect HPr to elute did not restore F-PTS activity when combined with membranes, nor did they enhance phosphotransferase activity when added to membranes plus enzyme I (protein eluting at 140 ml; Fig. 2).

Similar results were obtained after DEAE-cellulose column chromatography of the soluble fraction. Phosphotransferase activity (fractions plus membranes) and enzyme I-mediated PEP hydrolysis activity were observed to coelute from a DEAE-cellulose column with a linear gradient of KCl (Fig. 3). The most likely interpretation of the results presented in Fig. 2 and 3 is that the soluble fraction contains a single PTS component, functionally analogous to enzyme I,

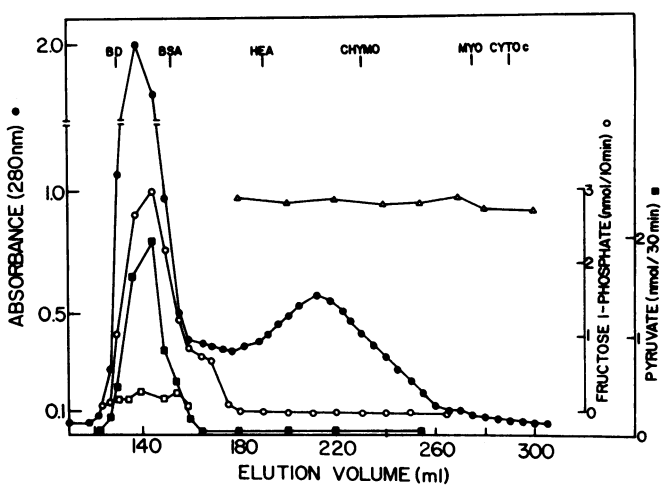

FIG. 2. Fractionation of the soluble proteins by Sephadex G-100 column chromatography. Concentrated soluble fraction (30 to 75% ammonium sulfate precipitation) was applied to a Sephadex G-100 column (2.5 by 80 cm), and protein was monitored at 280 nm (●). Samples of various fractions or combinations of fractions were added to membrane preparations and assayed for phosphotransferase activity. Fractions eluting between 120 and 175 ml exhibited phosphotransferase activity when combined with membranes (○), but possessed negligible activity alone (□). Proteins eluting between 180 and 300 ml were combined with membranes and a sample of the fraction eluting at 140 ml; no stimulation of phosphotransferase activity was observed (△). The fractions which restored phosphotransferase activity when combined with membranes were also observed to catalyze the hydrolysis of PEP to pyruvate in the absence of membrane protein (■). Abbreviations: BD, blue dextran; BSA, bovine serum albumin: HEA, hen egg albumin; CHYMO, chymotrypsinogen A: MYO, myoglobin; CYTOc. cytochrome *c*.

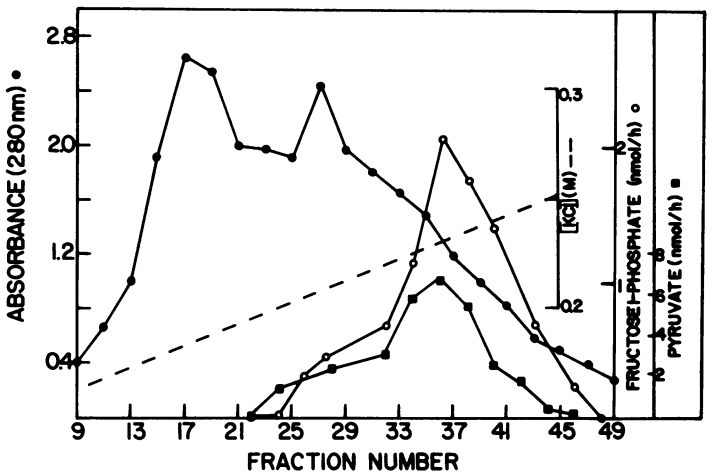

FIG. 3. DEAE-cellulose column chromatography of soluble protein. Soluble protein (176 mg) was applied to a DEAE-cellulose column (1.5 by 10 cm) equilibrated with 10 mM Tris-hydrochloride (pH 7.8) containing 0.1 M KCl and 1 mM dithiothreitol. Enzyme I was eluted with a linear gradient of 0.1 to 0.4 M KCl in 10 mM Tris-hydrochloride (pH 7.8). Both enzyme I-mediated (membrane-dependent) phosphotransferase activity (○) and PEP hydrolysis activity (■) were eluted by 0.22 to 0.3 M KCl.

and that addition of this component to membrane preparations results in the PEP-dependent phosphorylation of fructose in the absence of any smaller-molecular-weight protein (i.e., phosphocarrier protein such as HPr). If an intermediary phosphocarrier protein(s) is present in the soluble fraction, it is highly unlikely that it would coelute with enzyme I in both size exclusion and DEAE-cellulose chromatography. However, HPr or some similar phosphocarrier protein may be an integral component of the membrane. To test this, soluble and membrane fractions were heat treated to denature enzyme I and enzyme II, respectively. Addition of these heat-treated extracts to reconstituted F-PTS (membrane plus soluble proteins) failed to significantly enhance phosphotransferase activity (Table 4), providing further evidence that the F-PTS of *P. aeruginosa* does not require a heat-stable HPr-like phosphocarrier protein.

Complementation experiments. To determine whether soluble component fractions of the F-PTS from *P. aeruginosa* would cross-complement the PTS from enteric bacteria, crude extracts of deletion mutants of *S. typhimurium* deficient for enzyme I (*ptsI*) and HPr (*ptsH*) were utilized. Addition of soluble fraction from wild-type *S. typhimurium* or *E. coli* to both *ptsI* and *ptsH* mutants resulted in restoration of phosphotransferase activity (Table 5). In contrast, supplementation of the extracts from *Salmonella* deletion mutants with the soluble fraction from *P. aeruginosa* did not restore phosphotransferase activity. Identical results were obtained when the experiment was slightly modified. Membranes prepared from wild-type *S. typhimurium* and *E. coli* were complemented with the addition of soluble fractions of either *E. coli* or *S. typhimurium*, and cross-complementation was observed (Table 6). However, addition of soluble protein from *P. aeruginosa* was ineffective in restoring PTS activity to either *S. typhimurium* or *E. coli* membranes. Similarly, mixtures of soluble fractions obtained from *E. coli* and *S. typhimurium* plus membranes prepared from *P. aeruginosa* did not contain phosphotransferase activity (Table 6). Thus, both soluble (enzyme I) and membrane (enzyme II) components of the F-PTS from *P. aeruginosa* failed to complement components of the F-PTS from *E. coli* or *S. typhimurium*, although components from the latter two organisms readily cross-complemented each other.

DISCUSSION

Several investigators (1, 9, 16, 18) have demonstrated recently that the initial reactions involved in fructose catabolism in most but not all *Pseudomonas* spp. involve the PEP-dependent phosphorylation of fructose to fructose 1-phosphate with subsequent ATP-dependent phosphorylation to yield fructose 1,6-bisphosphate. The latter compound is further metabolized primarily through the Entner-Doudoroff pathway (9, 16). Thus far, F-PTS activity in *Pseudomo-*

TABLE 4. Effect of heat-treated membrane and soluble fractions on phosphotransferase activity[a]

Fraction	Addition (μg of protein)	pmol of fructose 1-phosphate formed per min
Soluble + membrane	None	82 (100)[b]
Soluble + membrane	Heat-treated soluble (52)	72 (88)
Soluble + membrane	Heat-treated membrane (40)	78 (95)
Soluble + membrane	Heat-treated membrane (80)	90 (110)
Soluble	None	<0.01
Membrane	None	2
Heat-treated soluble	None	<0.01
Heat-treated membrane	None	<0.01

[a] Soluble and membrane protein of fractionated crude extracts prepared from fructose-grown cells of *P. aeruginosa* were heat treated to 100°C for 1 min, rapidly cooled, and clarified by centrifugation at 35,000 × *g* for 45 min. The supernatant fluids were added to assay mixtures, and phosphotransferase activities were measured. Assay mixtures contained unheated soluble (30 μg) and membrane (50 μg) protein as indicated.

[b] Number in parentheses is percent activity relative to the complete system (soluble plus membrane) with no additions.

nas spp., including *P. aeruginosa*, has been demonstrated only in membrane-containing crude extracts. In this study, we have fractionated extracts of *P. aeruginosa* and have demonstrated that the F-PTS is composed of both soluble and membrane-bound components (Table 1). It should be noted, however, that membrane preparations alone generally possess some detectable phosphotransferase activity, indicating that the soluble component may be a peripheral membrane protein. This component may be released during cell disruption and thus appear as a soluble constituent of the cell. Further fractionation of the soluble protein by size-exclusion chromatography revealed a single peak of fractions which, when combined with washed membranes, exhibited phosphotransferase activity (Fig. 2). The peak activity of these fractions eluted at an elution volume equivalent to 72,000, a molecular weight similar to that of enzyme I of the PTS from eubacterial species (2, 10). In addition to restoring phosphotransferase activity to washed membranes, these fractions also catalyzed the pyruvate-stimulated hydrolysis of PEP to pyruvate. Thus, it appears that the soluble component is structurally and functionally analogous to enzyme I, and we have adopted this terminology.

The coelution of both enzyme I functions during both size-exclusion (Fig. 2) and ion-exchange (Fig. 3) chromatography indicates that the F-PTS from *P. aeruginosa* contains a minimum of two components, one soluble and at least one membrane associated. Efforts to demonstrate a separate intermediary phosphocarrier protein such as HPr have proven unsuccessful

TABLE 5. Complementation of *ptsH* (HPr) and *ptsI* (enzyme I) deletion mutants of *S. typhimurium*[a]

Crude extract of:	Soluble fraction supplement	mg of protein	pmol of fructose 1-phosphate formed per min
S. typhimurium (*ptsI*)	No supplement		4
	S. typhimurium (W^+)	0.12	79
	E. coli	0.24	104
	P. aeruginosa	0.09	<1
		0.23	<1
		0.45	<1
S. typhimurium (*ptsH*)	No supplement		2
	S. typhimurium (W^+)	0.12	60
	E. coli	0.24	94
	P. aeruginosa	0.09	<1
		0.23	<1
		0.45	<1

[a] Soluble protein from *S. typhimurium* SB3507 (*trpB223*), *E. coli* K-12, and *P. aeruginosa* was added to crude extract (0.15 mg of protein) of *S. typhimurium* SB3750 (*trpB223 ptsI181*) and to crude extract (0.20 mg of protein) of *S. typhimurium* NCR 127 (*trpB223 ptsH196 crp*). Cells were cultured in basal salts medium containing 20 mM lactate and 20 mM fructose. Phosphotransferase activity was determined as described in Table 1.

TABLE 6. Cross-complementation of membrane and soluble components of *P. aeruginosa*, *S. typhimurium*, and *E. coli*[a]

Source of membrane fraction	Soluble fraction supplement	mg of protein	pmol of fructose 1-phosphate formed per min
P. aeruginosa	No supplement		23
	P. aeruginosa	0.10	114
	S. typhimurium	0.21	23
		0.42	21
	E. coli	0.30	25
		0.60	34
S. typhimurium	No supplement		52
	S. typhimurium	0.14	123
	E. coli	0.12	149
		0.24	325
	P. aeruginosa	0.25	35
		0.50	30
E. coli	No supplement		58
	E. coli	0.12	268
	S. typhimurium	0.11	121
		0.22	314
	P. aeruginosa	0.25	46
		0.50	31

[a] Soluble protein from *P. aeruginosa*, *S. typhimurium* SB3507, and *E. coli* K-12 was added to membrane fractions of *P. aeruginosa* (0.08 mg of protein), *S. typhimurium* SB3507 (0.09 mg of protein), and *E. coli* K-12 (0.09 mg of protein). Cells were cultured on basal salts medium containing 20 mM lactate and 20 mM fructose.

(Fig. 2; Table 4), but these results do not exclude the possibility that an HPr-like component may be linked covalently to enzyme I. Thus, the F-PTS from *P. aeruginosa* resembles those found in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* (13), which also have been shown to be devoid of a separate phosphocarrier protein and apparently are comprised of two components. In *R. rubrum*, fructose-phosphotransferase activity appears to be particulate. However, one component with a relative molecular weight of 200,000 can be readily extracted from the membrane after two water washes. Similarly, *R. sphaeroides* requires two protein fractions for fructose-phosphotransferase activity, one being particulate and the other a soluble protein with a molecular weight close to that observed for *R. rubrum*. Whether the extractable components from these photosynthetic bacteria catalyze enzyme I partial reactions (15) is unknown.

The evidence presented herein (Table 2) indicates that both enzyme I and enzyme II complex are inducible in *P. aeruginosa*. This is in contrast to the PTS observed in most eubacterial genera (10), but is similar to the mannitol-PTS of *Spirochaeta aurantia* (14). The physiological significance of the F-PTS in *P. aeruginosa* has recently been established. Mutants deficient in F-PTS activity are unable to take up [^{14}C]fructose or to utilize exogenously provided fructose as a carbon source. The mutations in these fructose-negative strains, designated *pts*, have been mapped at 55 min by plasmid R68.45-mediated conjugational analysis and transductional analysis with phage F116L (R. A. Roehl, P. V. Phibbs, Jr., and T. W. Feary, Abstr. Am. Soc. Microbiol. 1980, K107, p. 144).

The PTS is widely distributed in bacteria, and detailed enzymological analysis of soluble proteins of the PTS demonstrate a considerable amount of evolutionary conservation. Enzyme I and HPr from diverse sources possess similar oligomeric structures and cross-react serologically (2, 3, 10). Moreover, these enzymes may be interchanged among divergent systems such that heterologous phosphotransferase activities are observed (3, 10). The results presented in this communication indicate that both the soluble and membrane-associated constituents of the F-PTS from *P. aeruginosa* do not cross-complement the respective systems in *S. typhimurium* and *E. coli* and vice versa. Similar results were observed with complementation assays between *R. rubrum* and *S. typhimurium* PTS (13). These results suggest that the F-PTS of *P. aeruginosa* and photosynthetic bacteria are widely divergent from those of enteric microorganisms.

Fructose is the most universal PTS substrate, and many diverse bacteria possess only fructose-specific phosphotransferase activity. More-

over, that the PTS of *P. aeruginosa* and photosynthetic bacteria appear to be less complex than those observed for many eubacterial species suggests that the fructose-specific PTS may have an early evolutionary origin. The interesting possibility that the multiple, sugar-specific PTS in many eubacteria may have arisen via gene duplication of fructose-specific enzyme II complexes and related proteins merits further comparative biochemical studies.

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LITERATURE CITED

1. **Baumann, P., and L. Baumann.** 1975. Catabolism of D-fructose and D-ribose by *Pseudomonas doudoroffii*. I. Physiological and mutant analysis. Arch. Microbiol. **105**:225–240.
2. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **44**:385–418.
3. **Hays, J. B.** 1978. Group translocation transport systems, p. 43–102. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, New York.
4. **Hudig, H., and W. Hengstenberg.** 1980. The bacterial phosphoenolpyruvate-dependent phosphotransferase system (PTS): solubilization and kinetic parameters of the glucose-specific membrane bound Enzyme II component of *Streptococcus faecalis*. FEBS Lett. **114**:103–106.
5. **Kalb, V. F., Jr., and R. W. Bernlohr.** 1977. A new spectrophotometric assay for protein in cell extracts. Anal. Biochem. **82**:362–371.
6. **Kundig, W., and S. Roseman.** 1971. Sugar transport. II. Characterization of constitutive membrane-bound Enzyme II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **246**:1407–1418.
7. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
8. **Perret, J., and P. Gay.** 1979. Kinetic study of a phosphoryl exchange reaction between fructose and fructose 1-phosphate catalyzed by the membrane-bound Enzyme II of the phosphoenolpyruvate-fructose 1-phosphotransferase system of *Bacillus subtilis*. Eur. J. Biochem. **102**:236–246.
9. **Phibbs, P. V., Jr., S. M. McCowen, T. W. Feary, and W. T. Blevins.** 1978. Mannitol and fructose catabolic pathways of *Pseudomonas aeruginosa* carbohydrate-negative mutants and pleiotropic effects of certain enzyme deficiencies. J. Bacteriol. **133**:717–728.
10. **Postma, P., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate:sugar phosphotransferase system. Biochem. Biophys. Acta **457**:213–257.
11. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate: sugar phosphotransferase systems: structural, functional, and evolutionary interrelationships. Bacteriol. Rev. **41**:856–871.
12. **Saier, M. H., Jr.** 1979. Sugar transport mediated by the bacterial phosphoenolpyruvate-dependent phosphotransferase system, p. 72–75. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
13. **Saier, M. H., Jr., B. U. Feucht, and S. Roseman.** 1971. Phosphoenolpyruvate-dependent fructose phosphorylation in photosynthetic bacteria. J. Biol. Chem. **246**:7819–7821.
14. **Saier, M. H., Jr., M. J. Newman, and A. W. Rephaeli.** 1977. Properties of a phosphoenolpyruvate:mannitol phosphotransferase system in *Spirochaeta aurantia*. J. Biol. Chem. **252**:8890–8898.
15. **Saier, M. H., Jr., M. R. Schmidt, and P. Lin.** 1980. Phosphoryl exchange reaction catalyzed by Enzyme I of the bacterial phosphoenolpyruvate:sugar phosphotransferase system: kinetic characterization. J. Biol. Chem. **255**:8579–8584.
16. **Sawyer, M. H., P. Baumann, S. J. Berman, J. L. Canovas, and R. H. Berman.** 1977. Pathways of D-fructose catabolism in species of *Pseudomonas*. Arch. Microbiol. **112**:49–55.
17. **Simoni, D., M. F. Smith, and S. Roseman.** 1968. Resolution of a staphylococcal phosphotransferase system into four protein components and its relation to sugar transport. Biochem. Biophys. Res. Commun. **32**:804–811.
18. **Van Dijkin, J. P., and J. R. Quayle.** 1977. Fructose metabolism in four *Pseudomonas* species. Arch. Microbiol. **114**:281–286.