Characterization and Genetic Mapping of Fructose Phosphotransferase Mutations in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa transports and phosphorylates fructose via a phosphoenolpyruvate-dependent fructose phosphotransferase system (PTS). Mutant strains deficient in both PTS activity and glucose-6-phosphate dehydrogenase activity were isolated and were used to select mannitol-utilizing revertant strains singly deficient in PTS activity. These mutants were unable to utilize fructose as a carbon source and failed to accumulate exogenously provided [^{14}C]fructose, and crude cell extracts lacked phosphoenolpyruvate-dependent fructose PTS activity. Thus, the PTS was essential for the uptake and utilization of exogenously provided fructose by *P. aeruginosa*. Mutations at a locus designated *pts*, which resulted in a loss of PTS activity, exhibited 57% linkage to *argF* at 55 min on the chromosome in plasmid R68.45-mediated conjugational crosses. The *pts* mutations in four independently isolated mutant strains exhibited from 11 to 20% linkage to *argF*, and one of these mutations exhibited 3% linkage to *lys-9015* in phage F116L-mediated transductional crosses.

Wild-type strains of *Pseudomonas aeruginosa* form phosphoenolpyruvate:fructose-1-phosphotransferase activity during growth on fructose minimal medium (29). Metabolism of intracellular fructose 1-phosphate occurs via an ATP-dependent 1-phosphofructokinase (EC 2.7.1.56), forming fructose 1,6-bisphosphate, which is catabolized primarily through the Entner-Doudoroff pathway (Fig. 1) and to a lesser extent through the Embden-Meyerhof-Parnas pathway (21). The induction of fructose phosphotransferase system (PTS) and 1-phosphofructokinase activities during growth of various species of *Pseudomonas* on fructose has been established previously (1, 21, 29, 31). Soluble and membrane-associated components of the fructose PTS in *P. aeruginosa* have been identified and characterized recently (4), but the genetic organization of the structural and regulatory genes involved in the formation of the PTS components remains unknown. Furthermore, the essential physiological role of the PTS in the transport and phosphorylation of fructose has not been demonstrated by analysis of mutant strains defective in PTS activity. In this report we describe the isolation of PTS-negative mutant strains of *P. aeruginosa* and characterize the growth properties of these strains. In addition, we identify chromosomal loci for the mutations which impair formation of PTS activity in these mutants.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the bacterial strains used in this study were derived from *P. aeruginosa* PAO (10); they are listed in Table 1. Complex culture medium contained the following (per liter): 10 g of tryptone, 2.5 g of yeast extract, and 2.5 g of NaCl. Basal salts medium (BSM) had the following composition: 50 mM potassium phosphate (pH 7.0), 15 mM $(\text{NH}_4)_2\text{SO}_4$, 0.81 mM MgCl_2 , 2 μM FeSO_4 , 0.18 mM CaCl_2 , 8 μM NaMoO_4 , 5 μM MnCl_2 . Carbon sources were used at concentrations of 20 mM unless indicated otherwise, and L-amino acid supplements were used at concentrations of 25 $\mu\text{g}/\text{ml}$. Solidified medium contained 1.5% agar. Cells were cultured aerobically at 37°C in a New Brunswick environmental shaker (250 rpm), and growth was measured in cuvettes (1.4 by 9.3 cm) with a Klett-Summerson colorimeter equipped with a no. 66 filter.

Mutagenesis and isolation of mutant strains. Four independent *pts* mutant strains were isolated from separate exponential-phase cultures of *zwf-1* strain PFB98. Ethyl methane sulfonate was added (0.2 ml of ethyl methane sulfonate per 20 ml of culture) when the culture absorbance reached approximately 200 Klett units, and the cultures were incubated with gentle shaking for 60 min at 37°C. The cells were harvested by centrifugation at $4,300 \times g$, washed twice with BSM, and suspended in 20 ml of complex broth. Samples (0.5 ml) were diluted into 4.5-ml portions of complex broth and incubated at 37°C with aeration overnight. These cultures of mutagenized cells were enriched for fructose-negative mutants by counterselection in D-cycloserine and carbenicillin, essentially as described by Ornston et al. (19). Dilutions of the

enriched cultures were spread on minimal agar medium containing 1.0 mM lactate and 20 mM fructose and incubated for 96 h at 37°C. Small translucent colonies were picked and replica-plated on minimal agar media containing various carbon sources to identify fructose-negative *zwf-1 pts* double mutants. From these strains mutants deficient only in PTS activity were obtained by selecting *zwf-1*⁺ revertants of *zwf-1 pts* strains PRP900 and PRP901. Exponential-phase cultures (approximately 150 Klett units) of these strains were treated with ethyl methane sulfonate (50 µl/2.5 ml of culture) for 1 h at 37°C without agitation. The treated cells were harvested, suspended in 0.2 ml of BSM, and spread on mannitol minimal agar medium. The colonies which appeared were purified and tested for retention of the fructose-negative trait, and two *pts* strains (PRP809 and PRP810) were chosen for further study. Nalidixic acid-resistant strain PRP811 was selected by spreading approximately 10⁹ cells of strain PAO25 on succinate minimal agar medium containing nalidixic acid (400 µg/ml).

Conjugation procedures. The chromosome-mobilizing plasmids pRO271 and R68.45 were conjugally transferred to the *pts* strains. A plasmid-containing donor strain [PAO25(pRO271) or PAO8(R68.45)] and a *pts* recipient strain were grown separately in complex broth to densities of approximately 200 Klett units. Equal volumes of the two cultures were mixed, and a 0.1-sample of the mixture was spread on complex agar medium and incubated overnight at 37°C. A large loopful of cells from the resulting growth was suspended in 1.0 ml of BSM, and 0.1-ml samples of serial

dilutions in BSM were spread on plates of succinate minimal agar medium containing sodium carbenicillin (500 µg/ml). Carbenicillin-resistant *pts* exconjugants were purified and tested for retention of the fructose-negative trait.

Matings involving chromosome transfer were done in liquid medium when the donor strains carried pRO271 and on solid medium when the donor strains carried R68.45. In pRO271-mediated matings, 4.0 ml of an exponential-phase donor culture and 4.0 ml of a recipient cell culture in complex broth (approximately 10⁹ cells per ml) were combined in a 50-ml centrifuge tube, gently mixed, and incubated for 2 h at 37°C without agitation. The cells were then harvested and resuspended in BSM, and serial dilutions were spread on selective minimal medium containing nalidixic acid (400 µg/ml) to counterselect donor cells. In R68.45-mediated matings cells were grown overnight on complex agar medium, and a large loopful of growth was suspended in 2.0 ml of complex broth. Equal volumes of donor and recipient cell suspensions (approximately 10¹⁰ cells per ml) were mixed, and 0.1-ml samples of the mixed suspension were spread on complex agar medium and incubated overnight at 37°C to permit conjugation. A large loopful of cells from the resulting growth was suspended in 2.0 ml of BSM, and dilutions of the cell suspension were spread on selective medium. In all matings, identical portions of individual donor and recipient control cultures were spread on selective medium to confirm that selected colonies were recombinants.

Bacteriophage propagation and transduction proce-

TABLE 1. Description of wild-type and mutant strains of *P. aeruginosa*

Strain		Genotype ^a	Derivation or source ^b	Reference
PAO	FP ⁻	prototroph	Laboratory stock	10
PAO8	(R68.45)	<i>met-28 ilv-202 str-1/R68.45</i> (Cb ^r Tc ^r Km ^r)	Olsen ^c	7
PAO25	FP	<i>argF10 leu-10</i>	Olsen ^c	7
PAO25	(pR0271)	<i>argF10 leu-10/FP2::TnI</i> (Cb ^r Hg ^r)	Olsen ^c	18
PAO236	FP ⁻	<i>his-4 ilv-226 lys-12 met-28 nalA2 pro-82 trp-6</i>	Olsen ^c	7
PAO2196	FP ⁻	<i>catA1 chu-9002 lys-9015 met-9020 nar-9011 trp-9029</i>	Matsumoto ^d	
PFB98	FP ⁻	<i>zwf-1</i>	Laboratory stock	21
PRP809	FP	<i>pts-1</i>	PRP900, Mtl ⁺ , EMS	
PRP809	(pR0271)	<i>pts-1/FP2::TnI</i> (Cb ^r Hg ^r)	PRP809, Cb ^r , conj. PAO25(pR0271)	
PRP810	FP ⁻	<i>pts-2</i>	PRP901, Mtl ⁺ , EMS	
PRP810	(R68.45)	<i>pts-2/R68.45</i> (Cb ^r Tc ^r Km ^r)	PRP810, Cb ^r , conj. PAO8(R68.45)	
PRP811	FP ⁻	<i>argF10 leu-10 nal-54</i>	PAO25, Nal ^r , spon. mut.	
PRP818	FP ⁻	<i>argF10 leu-10 nal-54 rif-50</i>	PRP811, Rif ^r , spon. mut.	
PRP900	FP	<i>pts-1 zwf-1</i>	PFB98, Fru ⁻ , EMS	
PRP901	FP ⁻	<i>pts-2 zwf-1</i>	PFB98, Fru ⁻ , EMS	
PRP902	FP	<i>pts-3 zwf-1</i>	PFB98, Fru ⁻ , EMS	
PRP905	FP ⁻	<i>pts-6 zwf-1</i>	PFB98, Fru ⁻ , EMS	

^a All gene designations are according to Holloway et al. (10). *pts* is tentatively proposed as the described locus for PTS activity, following the nomenclature recommendations of Lin (16).

^b Abbreviations: EMS, ethyl methane sulfonate; conj., conjugation; spon. mut., spontaneous mutation; Mtl⁺, mannitol utilization; Fru⁻, lack of fructose utilization. Strain derivation key: parent strain, selected trait, method (i.e., mutation or conjugation); for example, PRP809 was derived from parent strain PRP900 by EMS mutagenesis and selection for mannitol utilization.

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dure. Phage F116L was used in transductions as a generalized transducing phage; this phage carries up to 1 to 2% of the bacterial chromosome (13). F116L was propagated on *pts* strains by adding approximately 10^7 PFU of phage to exponential-phase cells (approximately 10^8 cells per ml) grown in 10 ml of complex broth. When visible cell lysis occurred (within approximately 4 h), the cultures were treated with 0.2 ml of chloroform and centrifuged at $4,300 \times g$ for 20 min at 4°C , and the phage-containing supernatants were collected. Recipient cells for transductions were grown overnight in 10 ml of complex broth, harvested, suspended in 1.0 ml of BSM (approximately 2×10^{10} cells per ml), and incubated for approximately 15 min at 37°C . A phage suspension (0.1 ml, containing 10^9 PFU) was then added to each cell suspension, and the mixtures were incubated for 15 min at 37°C . The transduction mixtures were centrifuged, suspended in 1.0 ml of BSM, and spread on selective medium. Control samples of uninfected cells and of phage suspensions were spread on selective medium to confirm that selected colonies were recombinants.

Cell extracts and enzyme assays. Cells were cultured in BSM containing specified carbon sources and harvested in late exponential phase (175 Klett units) by centrifugation. Cells were washed twice with BSM and stored as cell sediments at -40°C . Cell extracts for assays of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) were prepared by suspending thawed cell sediments in 1.5 ml of 100 mM Tris-hydrochloride (pH 8.0) containing 10 mM 2-mercaptoethanol and disrupting the cells by ultrasonic oscillation, as previously described (11). Soluble extracts were obtained from the supernatant fraction after centrifugation at $105,000 \times g$ for 2 h at 4°C . These soluble fractions contained negligible NAD(P)H oxidase activity. A previously described spectrophotometric method was used to assay for NAD(P)-linked glucose-6-phosphate dehy-

drogenase activity (11). Cell extracts for assays of phosphoenolpyruvate:fructose-1-phosphotransferase activity were prepared from washed cell sediments as described previously (4). The fructose PTS assay mixtures (200 μl) contained 50 mM potassium phosphate buffer (pH 7.4), 0.05 mM [$U\text{-}^{14}\text{C}$]fructose (2.5 mCi/mmol), 50 mM phosphoenolpyruvate, 18 mM MgCl_2 , 36 mM KF, and 3.6 mM dithiothreitol. The reactions were started by adding crude extract, incubated for 30 min at 32°C , and terminated by adding 0.5 ml of ice-cold water. The phosphorylated sugar product was separated from excess free fructose by the ion-exchange chromatography procedure described by Kundig and Roseman (14). The protein concentrations in cell extracts were determined by the spectrophotometric assay of Kalb and Bernlohr (12) or by the method of Lowry et al. (17).

Uptake of [$U\text{-}^{14}\text{C}$]fructose. Cells were induced for fructose uptake activity by subculturing 20 ml of early stationary-phase, lactate-grown cells into 200 ml of BSM containing 10 mM fructose and 10 mM lactate. Cultures were incubated in a rotary shaker at 37°C for slightly more than one cell generation. The cells were harvested by centrifugation, washed once with cold BSM, and suspended in sufficient BSM (containing 50 μg of chloramphenicol per ml) to yield an absorbance of 175 Klett units. These cell suspensions were held in an ice bath and then incubated at 37°C for 15 min before use in assays. Uptake of [$U\text{-}^{14}\text{C}$]fructose was measured by using a standard membrane filter assay (21). Uptake was initiated by adding [$U\text{-}^{14}\text{C}$]fructose (2.9 mCi/mmol; final concentration, 0.1 mM). The protein concentrations in whole-cell suspensions were determined by the method of Lowry et al. (17), using crystalline bovine serum albumin as a standard.

Chemicals. The pyridine nucleotides and substrates used in the enzyme assays were of the highest chemical purity available from either P-L Biochemicals,

FIG. 1. Carbohydrate catabolic pathways in *P. aeruginosa*. Abbreviations: pgi, phosphoglucosomerase; zwf, glucose-6-phosphate dehydrogenase; edd, 6-phosphogluconate dehydratase; eda, 2-keto-3-deoxy-6-phosphogluconate aldolase; tpi, triosephosphate isomerase; fda, fructose diphosphate aldolase; fdp, fructose diphosphatase; fpk, fructose-1-phosphate kinase; pts, phosphotransferase system; PEP, phosphoenolpyruvate.

TABLE 2. Growth characteristics of *P. aeruginosa* strains and their mutant derivatives

Strain	Genotype	Growth on the following carbon sources: ^a				
		Mannitol	Fructose	Glycerol	Succinate	Gluconate
PAO	Wild type	++	++	++	++	++
PFB98	<i>zwf-1</i>	-	+	+	++	++
PRP900	<i>zwf-1 pts-1</i>	-	-	+	++	++
PRP901	<i>zwf-1 pts-2</i>	-	-	+	++	++
PRP902	<i>zwf-1 pts-3</i>	-	-	+	++	++
PRP905	<i>zwf-1 pts-6</i>	-	-	+	++	++
PRP809	<i>pts-1</i>	++	-	++	++	++
PRP810	<i>pts-2</i>	++	-	++	++	++

^a Fresh inocula were streaked on solid medium containing single carbon sources (20 mM) and then incubated at 37°C for 48 h. Growth was scored as follows: ++, wild-type growth (large colonies); +, weak growth (small colonies); -, no growth.

Inc., Milwaukee, Wis., or Sigma Chemical Co., St. Louis, Mo. [U - ^{14}C]fructose was purchased from Amersham Corp., Arlington Heights, Ill. Other chemicals were of reagent-grade purity and were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J., or Sigma Chemical Co. Tryptone, yeast extract, and nutrient agar were products of Difco Laboratories, Detroit, Mich.

RESULTS

Construction and growth characteristics of *pts* strains. Bacterial strains defective in PTS activity were selected as fructose-negative derivatives of *zwf-1* mutant strain PFB98. Mutant strain PFB98 lacks glucose-6-phosphate dehydrogenase activity, fails to utilize mannitol, and exhibits slow growth on fructose or glycerol minimal medium (21). The presumptive *zwf-1 pts* double mutant derivatives (PRP900 through PRP905) failed completely to grow on fructose minimal medium but retained the other growth characteristics of parental strain PFB98 (Table 2). Derivative mutant strains deficient only in PTS activity were then selected as mannitol-utilizing *zwf-1*⁺ revertants of PRP900 and PRP901. Two presumptive *zwf-1*⁺ revertant *pts* strains (PRP809 and PRP810) were isolated; both of these strains were unable to utilize fructose, but they did exhibit wild-type growth on mannitol and glycerol (Table 2).

The effect of *pts* mutations on the growth rates of mutant strains was tested in diauxic growth experiments (Fig. 2). Wild-type strain PAO grew preferentially on succinate and, after a diauxic lag period, resumed exponential growth when the medium contained fructose, mannitol, or glycerol as an additional carbon source (Fig. 2A). Mutant strain PFB98 (*zwf-1*) utilized succinate, but failed to resume growth on mannitol and grew at significantly reduced rates on fructose and glycerol (Fig. 2B), in accord with previous results (21). The *zwf-1 pts-1* mutant strain PRP900 exhibited diauxic growth curves similar to those of *zwf-1* strain PFB98, except for

the failure of PRP900 to resume growth on fructose (Fig. 2C). The recovery of glucose-6-phosphate dehydrogenase activity in *pts-1* strain PRP809 permitted this strain to utilize mannitol and glycerol at wild-type rates, but no growth occurred on fructose (Fig. 2D). Thus, the *pts-1* mutation was responsible for the lack of growth of strains PRP900 and PRP809 on fructose. Mutant strains PRP901 (*zwf-1 pts-2*) and PRP810 (*pts-2*) exhibited diauxic growth patterns that were essentially identical to those of strains PRP900 (*zwf-1 pts-1*) and PRP809 (*pts-1*), respectively (data not shown).

Enzyme activities and fructose uptake in *pts* mutants. Glucose-6-phosphate dehydrogenase and fructose PTS activities were determined in extracts of the wild-type and mutant strains. Soluble extracts prepared from gluconate-grown cells of the strains containing the *zwf-1* lesion lacked both NAD- and NADP-linked glucose-6-phosphate dehydrogenase activities, whereas extracts prepared from wild-type strain PAO and *zwf-1*⁺ revertant *pts* strains PRP809 and PRP810 contained induced levels of these activities (Table 3). When these strains were cultured in the presence of fructose, crude extracts of wild-type strain PAO and *zwf-1* strain PFB98 contained induced levels of fructose PTS activity, whereas crude extracts of the *zwf-1 pts* strains and the *pts* strains were markedly deficient in this activity (Table 3).

The fructose PTS of *P. aeruginosa* is a multi-component system that has been fractionated into a single soluble component (enzyme I) and a membrane-bound enzyme II component (4). The activities of the PTS components formed by the *pts* mutants were determined by measuring the extents of complementation of mutant crude extracts with either soluble or particulate fractions prepared from wild-type strain PAO (Table 4). Crude extracts of all four *pts* mutants exhibited marked deficiencies in both soluble and membrane-associated PTS component activities. However, mutant PRP902 expressed a low level

FIG. 2. Diauxic growth curves of *P. aeruginosa*. The conditions for growth are described in the text. (A) Strain PAO. (B) Strain PFB98. (C) Strain PRP900. (D) Strain PRP809. The curves correspond to growth in media containing the following carbon sources: 5 mM succinate alone (●); 5 mM succinate plus either 10 mM mannitol (▲), 10 mM fructose (■), or 20 mM glycerol (●).

of membrane-associated enzyme II activity, as indicated by specific complementation with the PAO soluble fraction, and mutant PRP905 crude extract contained low enzyme I activity that exhibited specific complementation with the PAO membrane fraction (Table 4).

The impaired growth of *pts* strains on fructose and the absence of PTS activity in cell extracts indicated that these mutant cells should be deficient in PTS-mediated fructose transport. Uptake of [14 C]fructose by washed cells of wild-type and *pts* mutant strains cultured in the presence of fructose was also tested (Fig. 3). Wild-type strain PAO and *zwf-1* mutant PFB98 exhibited fructose uptake activity, whereas *pts* mutant strains PRP900, PRP901, PRP809, and PRP810 failed to accumulate detectable amounts of intracellular 14 C-labeled substrate.

TABLE 3. Enzyme activities in extracts of *P. aeruginosa*

Strain	Genotype	Glucose-6-phosphate dehydrogenase sp act ^a		Fructose PTS sp act ^b
		NAD ⁺	NADP ⁺	
PAO	Wild type	112	151	805
PFB98	<i>zwf-1</i>	<1	1	771
PRP900	<i>zwf-1 pts-1</i>	<1	3	5
PRP901	<i>zwf-1 pts-2</i>	<1	1	4
PRP902	<i>zwf-1 pts-3</i>	ND ^c	ND	4
PRP905	<i>zwf-1 pts-6</i>	ND	ND	4
PRP809	<i>pts-1</i>	139	197	1
PRP810	<i>pts-2</i>	103	160	2

^a Specific activities (in nanomoles per minute per milligram of protein) of NAD⁺- and NADP⁺-linked glucose-6-phosphate dehydrogenase in soluble extract fractions. Cells were harvested from 50-ml exponential-phase cultures grown in BSM supplemented with 40 mM gluconate.

^b Specific activity (in picomoles per minute per milligram of protein) of the fructose PTS in crude cell extracts. Cells were harvested from 250-ml cultures which were grown in BSM supplemented with 20 mM lactate to a Klett reading of 80 and then induced by the addition of 10 mM lactate plus 20 mM fructose and further incubation for approximately 3 h.

^c ND, Not determined.

TABLE 4. In vitro complementation analysis of crude extracts of *pts* mutants with soluble and membrane components of wild-type *P. aeruginosa*^a

Mutant crude extract		Wild-type component addition ^c	Fructose PTS sp act ^d
Strain	Amt of protein (μg) ^b		
None		Soluble + membrane	647
PRP900	300	Soluble	32
		Membrane	13
PRP901	370	Soluble	20
		Membrane	8
PRP902	340	Soluble	71
		Membrane	23
PRP905	340	Soluble	5
		Membrane	72

^a Crude extracts of *pts* mutants and soluble and membrane components of wild-type strain PAO were prepared as described previously (4) from cells grown in BSM supplemented with 30 mM lactate and 20 mM fructose.

^b Amount of protein added to standard 200-μl reaction mixtures.

^c In all cases the amounts of wild-type protein added were as follows: soluble component, 40 μg; membrane component, 20 μg.

^d Specific activity expressed as picomoles of fructose 1-phosphate formed per minute per milligram of total protein in reaction mixtures.

FIG. 3. Uptake of [14 C]fructose by wild-type and *pts* mutant strains. The conditions for growth and fructose uptake determinations are described in the text. Symbols: ●, strain PAO; ■, strain PFB98; ○, strain PRP900; □, strain PRP901; △, strain PRP809; ◇, strain PRP810.

Conjugational mapping of *pts*. The genetic loci for the *pts* mutations in strains PRP809 and PRP810 were determined by identifying other previously characterized mutations on the *P. aeruginosa* chromosome with which *pts* showed linkage in conjugational crosses. Chromosome transfer was mediated by the chromosome-mobilizing plasmids pRO271 (18) and R68.45 (7-9). Plasmid pRO271 is a TnI-containing derivative of plasmid FP2, which confers carbenicillin resistance and mobilizes the transfer of large segments of chromosome originating at 0 min on the chromosome map (9, 18) (Fig. 4). A carbenicillin-resistant derivative of *pts-1* mutant PRP809 carrying plasmid pRO271 was constructed as described above, and this derivative was used as a donor in crosses with recipient strain PAO236. Exconjugants were selected for repair of each of the six auxotrophic lesions in PAO236 in the 0- to 40-min region of the chromosome and were scored for coinheritance of the fructose-negative phenotype. No linkage of *pts-1* with *ilv-226*, *his-4*, *lys-12*, or *met-28* was observed (data not shown), but a low frequency of linkage with both *trp-6* and *pro-82* was detected (Table 5 and Fig. 4). Three-point analyses of *pts-1*, *pro-82*, and *trp-6* markers in exconjugants indicated that *pts-1* was located in the region of the chromosome beyond 40 min (data not shown). Linkage of *pts* with genes in the late region of the chromosome was tested with plas-

mid R68.45, which typically mediates transfer of 5 to 10 min of chromosome from multiple points of origin (7-9). The *pts-2* strain PRP810 carrying chromosome-mobilizing plasmid R68.45 was crossed with strain PRP811, and *argF10*⁺ exconjugants were selected and scored for coinheritance of *pts-2* (Table 5). Our results showed that *pts-2* exhibited 57% linkage with *argF10*, near 55 min on the chromosome map (Fig. 4).

Transductional mapping of *pts*. The linkage of *pts-2* with *argF10* in R68.45-mediated crosses indicated that *pts-2* and the other *pts* mutations might be located sufficiently close to *argF10* to exhibit transductional linkage mediated by the generalized transducing phage F116L. Phage F116L was propagated on each of the four independently isolated *pts* mutant strains and was used to select *argF10*⁺ in strain PRP818. The *argF10*⁺ transductants were scored for *pts* by testing for growth on fructose minimal medium. We found that the *pts* mutations exhibited between 11 and 20% linkage to *argF10* in all four transductional crosses (Table 6). The marker *lys-9015* is also located close to *argF10* (25) and thus might be expected to exhibit linkage with *pts*. Strain PAO2196 was transduced to *lys-9015*⁺ by using F116L propagated on *pts-1* strain PRP809, and transductants were scored for growth on fructose minimal medium. Our results

FIG. 4. Chromosomal map of *P. aeruginosa* PAO according to Royle et al. (25). Linked genetic markers are indicated by brackets to indicate the approximate locus in minutes and in cases where the absolute orientation of markers relative to an outside marker is unknown.

TABLE 5. Conjugational mapping of *pts* mutations on the *P. aeruginosa* chromosome^a

Donor	Recipient	Selection	Coinheritance frequency of <i>pts</i>
PRP809(pR0271)	PAO236	<i>trp-6</i> ⁺	0.08 (7/93) ^b
		<i>pro-82</i> ⁺	0.07 (5/72)
PRP810(R68.45)	PRP811	<i>argF10</i> ⁺	0.57 (50/87)

^a Conjugations with chromosome-mobilizing plasmids pR0271 and R68.45 were performed as described in the text.

^b The numbers in parentheses are number unselected/number selected.

showed that the *pts-1* locus exhibited 3% co-transductional linkage to *lys-9015* (Table 6). Thus, the *pts-1* locus appears to be located closer to *argF10* than to *lys-9015*, although the relative gene order for these three markers is not yet clear.

DISCUSSION

Diauxic growth curves of mutant strains lacking glucose-6-phosphate dehydrogenase activity confirmed previous results which showed that *zwf-1* mutants are unable to grow on mannitol due to an inability to metabolize fructose and hexose monophosphates that are generated intracellularly from mannitol catabolism (21). However, *zwf-1* mutants retain the ability to metabolize exogenously provided fructose slowly through the lower portion of the Embden-Meyerhof-Parnas pathway (Fig. 1). Impairment of PTS function by mutation at *pts* entirely prevented fructose utilization in both *zwf-1* strains and *zwf-1*⁺ revertant strains that contained a functional Entner-Doudoroff pathway. Thus, the PTS is essential for initial metabolism of exogenously provided fructose to fructose 1-phosphate, which is phosphorylated to fructose 1,6-bisphosphate and then metabolized primarily through the Entner-Doudoroff pathway or very slowly through the Embden-Meyerhof-Parnas pathway (Fig. 1).

The inability of *pts* mutants to exhibit uptake of radiolabeled fructose demonstrates that *P. aeruginosa* does not contain an alternate, non-PTS route of entry for fructose during growth on this substrate. Metabolism of fructose transported via a non-PTS system would normally require phosphorylation by fructokinase. Although *P. aeruginosa* contains an inducible ATP-dependent fructokinase (20), this enzyme is specifically induced only during growth on mannitol and is required for the phosphorylation of intracellular fructose generated from mannitol oxidation (21). Thus, the specific induction of fructokinase by mannitol is consistent with the apparent lack of

an alternate route of entry for exogenously provided fructose.

All four *pts* mutants failed to grow on exogenously provided fructose and were deficient in fructose uptake activity, and crude extracts failed to catalyze phosphoenolpyruvate-dependent fructose phosphorylation. These results demonstrate the essential physiological role of the PTS in transport and phosphorylation of exogenously provided fructose in *P. aeruginosa*, and this role is shared by numerous other bacterial species (5, 6, 23, 26, 27). Our results do not support the suggestion that the PTS in *Pseudomonas* may participate in the catabolism of intracellular fructose generated from mannitol catabolism (32).

In other bacterial species, *ptsI* mutants lack a soluble enzyme I component of the PTS, are unable to transport and metabolize fructose and a wide range of PTS and non-PTS substrates, are highly sensitive to repression of cyclic adenosine 3',5'-monophosphate-dependent enzyme synthesis, and are deficient in other physiological functions (23, 26, 28). The complementation analysis of PTS activity in the *pts* mutants of *P. aeruginosa* described above indicated that these mutants are deficient in both the soluble (enzyme I) and membrane-associated (enzyme II) components of the PTS. However, these mutants do not exhibit the pleiotropic growth defects observed in *ptsI* mutants of other bacterial species. Thus, the complex regulatory role ascribed to the PTS of other bacteria (3, 23, 26) apparently is not associated with the PTS of *P. aeruginosa*. This observation appears to be consistent with evidence against a clear relationship between cyclic adenosine 3',5'-monophosphate pools and the modulation of inducible enzyme synthesis in this species (22, 30).

Plasmid R68.45- and pR0271-mediated conjugational crosses showed that the *pts* mutations described in this study were located at approximately 55 min on the chromosomal map of *P. aeruginosa* and were linked to *argF10*. The *pts*

TABLE 6. Transductional analysis of *pts* linkage with *argF* and *lys-9015* in *P. aeruginosa*^a

Donor	Recipient	Selection	Cotransduction frequency with <i>pts</i>
PRP809	PRP818	<i>argF10</i> ⁺	0.13 (40/310) ^b
PRP810	PRP818	<i>argF10</i> ⁺	0.11 (27/255)
PRP902	PRP818	<i>argF10</i> ⁺	0.12 (8/69)
PRP905	PRP818	<i>argF10</i> ⁺	0.20 (13/64)
PRP809	PAO2196	<i>lys-9015</i> ⁺	0.02 (3/200)

^a Transductions were conducted with phage F116L as described in the text.

^b The numbers in parentheses are number unselected/number selected.

mutations exhibited transductional linkage to *argF10* in crosses mediated by phage F116L, and *pts-1* was also linked by transduction to *lys-9015*. Further analysis of the genetic markers near 55 min will be necessary to provide a definitive gene order in this region of the chromosome. The transductional linkage of *argF10* with the four independently isolated *pts* mutations varied between 11 and 20%, which may reflect mutations in separate but closely linked *pts* genes. Clustering of functionally related catabolic determinants is a common characteristic of *Pseudomonas* species (2, 15, 24, 33, 34).

Both soluble (enzyme I) and membrane-associated components of the fructose PTS are inducible in *P. aeruginosa* (4). A complementation analysis of the activities of PTS components in crude extracts provided some evidence that the mutations in strain PRP902 (*pts-3*) and strain PRP905 (*pts-6*) prevented the formation of specific PTS components and also appeared to preclude the formation of wild-type levels of the other PTS components. One interpretation of these results is that fructose 1-phosphate is the natural inducer for components of the PTS and a deficiency in any one component due to a *pts* mutation prevents inducer formation and precludes the induction of other structural components. However, the exact identification of the biochemical defects in these *pts* mutants will require further analysis of the PTS protein components and a more complete understanding of their regulation in *P. aeruginosa*.

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