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# Promoter-Like Mutation Affecting HPr and Enzyme I of the Phosphoenolpyruvate:Sugar Phosphotransferase System in *Salmonella typhimurium*[1]

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A promoter-like mutation, *ptsP160*, has been identified which drastically reduces expression of the genes specifying two proteins, HPr and enzyme I, of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) in *Salmonella typhimurium*. This mutation lies between *trzA*, a gene specifying susceptibility to 1,2,4-triazole, and *ptsH*, the structural gene for HPr. It leads to a loss of active transport of those sugars that require the PTS for entry into the cell. Pseudorevertants of strains carrying this promoter-like mutation have additional lesions very closely linked to *ptsP160* by transduction analysis and are noninducible for HPr and enzyme I above a basal level. Presumably, strains carrying *ptsP160* are defective in the normal induction mechanism for HPr and enzyme I, and the pseudorevertants derived from them result from second-site initiation signals within or near this promoter-like element. The induction of HPr and enzyme I above their noninduced levels apparently is not required for transport of at least one PTS sugar, methyl α-D-glucopyranoside, since this sugar is taken up by the pseudorevertants at the same rate as by the wild type. The existence of a promoter-like element governing the coordinate inducibility of both HPr and enzyme I suggests that *ptsH* and *ptsI* constitute an operon. Wild-type levels of a sugar-specific PTS protein, factor III, are synthesized in response to the $crr^+$ gene in both a *ptsP160* strain and its pseudorevertants; this suggests that the $crr^+$ gene has its own promoter distinct from *ptsP*.

In *Salmonella typhimurium*, a group of D-sugars including glucose, mannose, fructose, hexitols, *N*-acetylglucosamine, glucosamine, and β-glucosides are translocated across the cell membrane concomitant with phosphorylation. This process is catalyzed by an enzyme complex designated phosphoenolpyruvate:sugar phosphotransferase system (PTS) (1, 6-8, 11, 15).

The phosphorylation of a given PTS sugar requires four proteins, lipid, and divalent cation (1, 6, 7, 8, 11). Two of the proteins, HPr and enzyme I, are cytoplasmic constituents and are required for the phosphorylation of all PTS sugars. In addition to HPr and enzyme I, the phosphorylation of a given PTS sugar requires a pair of proteins specific for that sugar. In every case thus far investigated, at least one member of the sugar-specific protein pair is a membrane component. The phosphoryl group is transferred sequentially from phosphoenolpyruvate to enzyme I to HPr to one of the sugar-specific proteins and then finally to the sugar. The last step is catalyzed by the second member of the sugar-specific protein pair which is invariably a membrane protein.

In addition to its defined role in the group translocation of PTS sugars, the PTS apparently affects the regulation of physiological processes such as the uptake of non-PTS sugars (12), the intracellular concentration of cyclic 3',5'-adenosine monophosphate (M. H. Saier and S. Roseman, manuscript in preparation), and the induction of some catabolic enzymes (12). Evidence for the regulatory role of the PTS in these diverse processes has been obtained by isolating a series of mutants designated *crr* (carbohydrate repression resistant [12]). The only known biochemical defect associated with

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the *crr* mutation is the absence of one class of soluble sugar-specific PTS proteins, factor III. The *crr* gene and the structural genes for HPr (*ptsH*) and enzyme I (*ptsI*) are co-transducible with *cysA* and *trzA* (a gene specifying sensitivity to 1,2,4-triazole) in *Salmonella typhimurium*. These genes are arranged in the following sequence: *cysA-trzA-ptsH-ptsI-crr* (4) with the direction of transcription and translation of the *pts* gene cluster proceeding from left to right as represented on the genetic map (Fig. 1). Biochemical analyses of a variety of *pts* point mutations and deletions indicated that HPr and enzyme I are coordinately regulated independently of *crr*, which leads to the suggestion that the *crr* gene has a promoter-like element distinct from the one governing the expression of the HPr and enzyme I structural genes (4). Mutations in the promoter-like element for *ptsH* and *ptsI*, designated *ptsP*, would be expected to have the following characteristics: (i) to occur between *trzA* and *ptsH*, leaving the HPr and enzyme I structural genes intact; (ii) to severely reduce the levels of both HPr and enzyme I, but to allow production of normal factor III levels; and (iii) to result in the inability of the cell to transport and accumulate those sugars requiring a functional PTS.

This report describes the identification and characterization of such a promoter-like mutation, *ptsP160*, and the pseudorevertants derived from it. The data to be presented suggest that all revertant mutations suppressing *ptsP160* are second-site initiation signals near the original mutation; the pseudorevertants lack the normal promoter configuration, thereby preventing HPr and enzyme I induction above a basal level of synthesis. The uninduced levels of HPr and enzyme I produced by the pseudorevertants of *ptsP160* strains are sufficient for normal transport of at least one PTS sugar, methyl α-glucoside. The existence of a promoter-like element affecting the coordinate regulation of HPr and enzyme I supports the idea that *ptsH* and *ptsI* constitute an operon.

## MATERIALS AND METHODS

**Bacterial strains and media.** Table 1 lists the phenotypes and derivations of the strains used in this study. The double mutant SB2729 (*trzA225 ptsP160*) was isolated, after nitrous acid mutagenesis, by the induction and selection procedures previously employed for the isolation of *trzApts* deletion mutants (4,14). Growth and indicator media were prepared as described (4, 5) as were methods for induction, selection, and isolation of the *ptsP160* pseudorevertants; transduction crosses with phage P22 (mutant L7) (16) and phage KB1 (2) and the selection and analysis of *cysA*$^+$ and ptsI$^+$ recombinants were performed as described (4). Phenotypic expression of each unselected marker was scored after streaking for single colonies (3, 4) and incubating them at 37 C for 72 h.

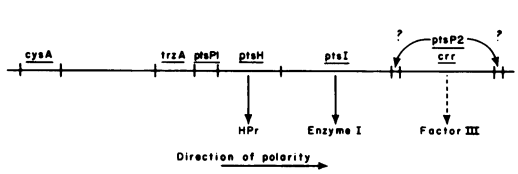



FIG. 1. *The PTS gene region in Salmonella typhimurium.*

**Enzyme and protein assays.** The general assay methods in crude extracts for the PTS proteins have been described (1, 4, 7, 8). In the present studies, the protein being assayed was made the rate-limiting component in incubation mixtures containing the other three protein constituents; the rate was determined by measuring the quantity of labeled sugar substrate converted to the corresponding labeled sugar phosphate using the ion-exchange procedure (1, 7, 8). The source of the membrane proteins (enzyme II) were membrane preparations from *S. typhimurium* SB2950, a strain containing a complete deletion of *ptsH*, *ptsI*, and *crr* (4). Uninduced levels of enzyme I, HPr, and factor III were obtained by analyzing extracts from cells grown on 0.2% lactate, whereas enzyme I and HPr were induced by growth of the cells on 0.2% D-fructose (13). Protein was determined by a biuret procedure (9) with bovine serum albumin as standard. Specific activity is expressed as micromoles of sugar phosphate formed per milligram of protein in 30 min at 37 C.

**Transport studies.** Bacterial cultures (500 ml of medium A containing 0.2% lactate and 20 μg of L-tryptophan per ml) in midexponential growth were harvested, washed twice in cold medium A minus supplements and carbon source, concentrated 100-fold by centrifugation at 4 C for 10 min at 16,300 × *g*, and diluted 2-fold prior to the transport experiments. The stop-flow transport apparatus used in these experiments, permitting measurement of initial transport rates, was designed by Jeffry Stock (17). Transport experiments were initiated at room temperature by rapid mixing of equal volumes of the cell suspension in medium A (lacking supplements and carbon source) with a solution containing methyl α-D-[U-$^{14}$C]glucopyranoside (Amersham/Searle) in the same medium. The final mixture of cells and isotope contained the labeled glycoside at a 0.1 mM concentration (specific activity, 5.6 × $10^6$ counts per min per μmol) and 3 mg of cells (dry weight) per ml. Samples (0.1 ml) were removed at the indicated times and rapidly diluted with a 100-fold volume of medium A at room temperature (effectively stopping glycoside uptake), and the mixture was filtered through Reeve Angel glass-fiber filters (984H) premoistened with medium A and mounted on a manifold (Millipore Corp.). The time from the initial mixing of the cell suspension with the labeled glycoside to dilution of each sample was recorded automatically and represents the time for uptake of the

TABLE 1. *Strains of Salmonella typhimurium used in the analysis of a PTS promoter-like mutation*

| SB no. | Relevant genotype[a] | Method of isolation[b] | PTS phenotype[c] |
|---|---|---|---|
| | *trpB223*, wild-type | E. Balbinder strain collection | A |
| 2729 | *trpB223, trzA225 ptsP160* | NA | B |
| 2758 | *trpB223, trzA225 ptsP160* | Recombination[d] | B |
| 2759 | *trpB223, trzA⁺ptsP160* | Recombination[d] | B |
| 2737 | *trpB223, trzA225 ptsP160 PR-1* | NG | A |
| 2761 | *trpB223, trzA⁺ptsP160* | Recombination[d] | B |
| 2762 | *trpB223, trzA225 ptsP160* | Recombination[d] | B |
| 2738 | *trpB223, trzA225 ptsP160 PR-2* | NG | A |
| 2763 | *trpB223, trzA225 ptsP160* | Recombination[d] | B |
| 2764 | *trpB223, trzA⁺ptsP160* | Recombination[d] | B |
| 2748 | *trpB223, trzA225 ptsP160 PR-3* | DES | A |
| 2774 | *trpB223, trzA225 ptsP160* | Recombination[d] | B |
| 2749 | *trpB223, trzA225 ptsP160 PR-4* | DES | A |
| 2780 | *trpB223, trzA225 ptsP160* | Recombination[d] | B |

[a] In strains SB2737, SB2738, SB2748, and SB2749, PR is defined as the promoter-restart mutation, the presence of which restores the strains containing the *ptsP160* mutation to *pts*⁺.

[b] Mutagens used are: NA, nitrous acid; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DES, diethyl sulfate.

[c] PTS phenotype is defined as the ability of a strain to grow on and ferment the following PTS sugars after 48 h at 37 C: glucose, mannose, fructose, and mannitol. A, Positive; B, negative. All strains were positive on 0.2% lactate (for growth) and 0.2% D-galactose (for growth and fermentation). All strains carrying the wild-type *trzA*⁺ allele were susceptible to 1,2,4-triazole in that no growth occurred on medium A agar plates containing 10 mM triazole plus 0.2% lactate after 72 h at 37 C, whereas with strains containing the *trzA225* mutation growth did occur under the above conditions.

[d] SB2758 and SB2759 were derived from a cross using KB1 phage grown on SB2729 as donor and *cysA20* as recipient; *cysA*⁺ recombinants were selected on medium A plates containing 0.2% lactate as the sole carbon source. The same procedure was used to derive the following recombinants from the indicated strains: SB2761 and SB2762 from SB2737; SB2763 and SB2764 from SB2738; SB2774 from SB2748; and SB2780 from SB2749.

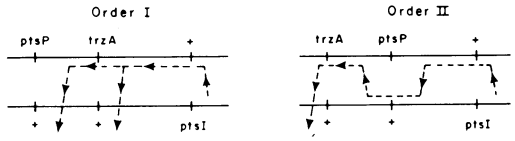
glycoside. The time between dilution and complete filtration of each sample was less than 3 s. The filters were dried and counted in a toluene-based scintillation fluid containing Triton X-100 in a mixture recommended by Packard Instrument Co. The dry weight of each sample was determined for each culture after desiccation of a sample for 72 h at room temperature. Transport data are expressed as micromoles of sugar taken up per gram of cells (dry weight) at 22 C.

## RESULTS

**Separation of ptsP160 from trzA225 and map position.** The following transduction experiments show that the *ptsP160* and *trzA225* mutations in strain SB2729 are separable from each other and do not result from an extensive *trzAptsHI* deletion. Transduction with donor phage propagated on strain SB2729 (*trzA225 ptsP160*) and strain SB1690 (*ptsI34*) as recipient demonstrated the separability of the *trzA* and *ptsP* mutations and discriminated between order I and order II represented in Fig. 2. If order I were correct, the selection of *ptsI*⁺ recombinants would yield approximately equal numbers of triazole-sensitive (SP⁺) and triazole-resistant (RP⁺) prototrophs; since only one triazole-resistant prototroph (RP⁺) occurred in 333 recombinants tested, the inferred gene sequence is order II, i.e., *trzA-ptsP-ptsI*. Transduction performed with donor phage grown on strain SB2729 and with strain *cysA20* (10) as recipient substantiates the results obtained from the previous cross. If order I were correct (Fig. 3), the minority recombinant class should be triazole-resistant (RP⁺) prototrophs. The results presented in Fig. 3 show that the minority recombinant class consists of triazole-sensitive auxotrophs (SP⁻). The results of this three-point test suggest order II, i.e., *cysA-trzA-ptsP*.

Table 2 contains the results of transduction experiments that demonstrate that all *ptsH* and *ptsI* point mutations tested gave prototrophic recombinants when crossed into strain SB2729 recipient. These results show that the genes coding for HPr (*ptsH*) and enzyme I (*ptsI*) are structurally intact in strain SB2729 within the limits of our tests. The cumulative gene order of this region of the *Salmonella* chromosome is *cysA-trzA-ptsP-ptsH-ptsI-crr*.

**Isolation and genetic analysis of ptsP160 pseudorevertants.** Either diethyl sulfate or nitrosoguanidine was used as mutagen to revert SB2729 to the *pts*⁺ phenotype on medium A agar plates containing 20 μg of L-tryptophan




Donor: trzA225 ptsP160
Recipient: ptsI34

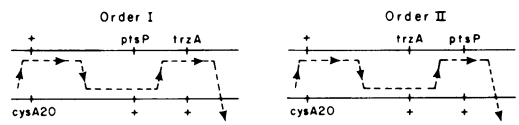
Recombinant classes

| SP+ | RP+ | Total |
|---|---|---|
| 332 | 1 | 333 |

FIG. 2. *Transduction with donor phage grown on SB2729 with ptsI34 as recipient. Transduction was performed under standard conditions, with medium A agar plates containing 0.2% D-mannitol and 20 μg of L-tryptophan per ml, for the selection of ptsI+ recombinants with P22 (mutant L-7) phage grown on SB2729 (trzA225 ptsP160) and with ptsI34 as recipient. L-Tryptophan was added because of the trpB223 mutation in the ptsI34 genetic background. The pts+ recombinants were streaked for single-colony isolation. The colonies were scored for growth after 72 h of incubation at 37 C on the following medium A agar plates for examination of segregation of the trzA225 mutation among the pts+ recombinants: (i) 0.2% D-mannitol plus 20 μg of L-tryptophan per ml, and (ii) 0.2% lactate plus 20 μg of L-tryptophan per ml + 10 mM 1, 2, 4-triazole. SP+ (trzA+ ptsP+) refers to recombinants showing the ptsP+ phenotype, but which were susceptible to triazole: (i) +, (ii) −; RP+ (trzA225 ptsP+) refers to recombinants showing the ptsP+ phenotype, but which were resistant to triazole: (i) +; (ii) +.*

per ml and 0.2% D-mannitol as the sole carbon source. Thirty-three independently isolated pseudorevertants were tested with results similar to the four examples given in Fig. 4. For these analyses, phage were grown on each pseudorevertant containing both the *trzA225* mutation and the *pts*+ phenotype. Donor phage were crossed into strain *cysA20* as recipient, and *cysA*+ recombinants were selected. Each of the *pts*+ pseudorevertants gave rise to the *ptsP*− (RP−) recombinant class (Fig.4), indicating that none of the pseudorevertants was derived from true back-mutation at the primary site and that the original *ptsP160* mutation was still present in each pseudorevertant. The results in Fig. 4 also show that the second-site pseudorevertant allele (or promoter initiation signal) in each strain probably is located distally (to the right) of the original *ptsP160* mutation. If order I in Fig. 4 were correct, then the number of RP− and SP− recombinants should be approximately equal; if order II is correct, then the SP− recombinant class should be in the minority. In each case examined, the SP− recombinants constituted the minority class, suggesting that the initiation signals in each pseudorevertant strain are located to the right of *ptsP160* with the following order: *cysA-trzA-ptsP-initiation-ptsH-ptsI-crr*.

**Biochemical characterization of the ptsP160 mutant and its pseudorevertants.** Table 3 contains the results of assays for the relevant, soluble PTS proteins, i.e., HPr, enzyme I, and factor III, produced by the wild type (*trpB223*), the promoter-like mutant (*ptsP160*), its four pseudorevertants, and the promoter-like (*ptsP*−) recombinants derived from each pseudorevertant. The strains were grown on lactate as sole carbon source to determine the constitutive levels of the PTS proteins and on D-fructose to determine their levels upon induction. Table 3 shows that: (i) strain SB2729 (*trzA225 ptsP160*) produces about 2% of the wild-type (lactate grown) uninduced levels of both HPr and enzyme I; (ii) unlike *ptsH* and *ptsI* point mutants (13), strain SB2729 was not induced by fructose to produce more enzyme I or HPr; (iii) the synthesis of factor III is not regulated coordinately with HPr and enzyme I, as the levels of the latter two proteins vary independently of factor III; (iv) the pseudorevertants of strain SB2729 exhibited from one-third to normal levels of HPr and enzyme I under uninduced conditions of synthesis, and similar to strain SB2729 itself, produced normal levels of




Donor: trzA225 ptsP160
Recipient: cysA20

Recombinant classes

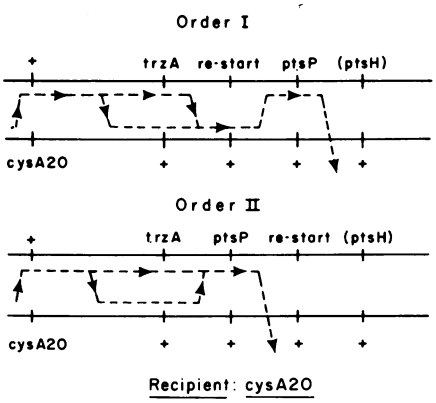
| SP+ | RP− | RP+ | SP− | Total |
|---|---|---|---|---|
| 242 | 231 | 59 | 8 | 540 |

FIG. 3. *Transduction was performed with donor phage P22 (mutant L7) grown on SB2729 (trzA225 ptsP160) and cysA20 as recipient; cysA+ recombinants were selected on medium A agar plates containing 0.2% lactate as the sole carbon source. The cysA+ recombinants were streaked for single-colony isolation on the following medium A agar plates: (i) 0.2% lactate, (ii) 0.2% D-mannitol, and (iii) 0.2% lactate plus 10 mM 1,2,4-triazole. The following phenotypes among the recombinants were recorded after incubation for 72 h at 37 C. SP+ (trzA+ ptsP+) were susceptible to triazole but able to grow on mannitol; SP− (trzA+ ptsP160) were susceptible to triazole and unable to grow on mannitol. RP+ (trzA225ptsP+) were resistant to triazole and utilized mannitol. RP− (trzA225ptsP160) were resistant to triazole and unable to utilize mannitol.*

TABLE 2. *Transduction with pts point mutations as donors using SB2729 (trzA225 ptsP160) as recipient*[a]

| Recipient | *pts* point mutations as donors | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *H15* | *H28* | *H38* | *I18* | *I19* | *I33* | *I34* | *I39* | *I26* | *I29* | *I20* | *I21* | *I23* | *I30* | *I40* |
| *trzA225 ptsP160* | 2 | 26 | 88 | 37 | 177 | 33 | 88 | 185 | 38 | 47 | 147 | 222 | 98 | 67 | 2,000 |

[a] Transduction was performed under standard conditions using KB1 phage grown on *pts* point mutations as donors and SB2729 (*trzA225 ptsP160*) as recipient. Prototrophic (*pts*$^+$) recombinants were selected on medium A agar plates containing 0.2% D-mannitol plus 20 $\mu$g of L-tryptophan per ml, and their growth was scored after incubation for 5 days at 37 C. The values represent the number of recombinants which appeared per $4 \times 10^8$ recipient bacteria. The donor *pts* point mutations are given in the order in which they occur on the *Salmonella* chromosome as determined by deletion mapping (4).




Recipient: cysA20

| Donors | Recombinant classes | | | | |
|---|---|---|---|---|---|
| | SP$^+$ | RP$^+$ | RP$^-$ | SP$^-$ | Total |
| trzA225 ptsP160 PR-1 | 403 | 285 | 11 | 2 | 701 |
| trzA225 ptsP160 PR-2 | 263 | 146 | 14 | 3 | 416 |
| trzA225 ptsP160 PR-3 | 311 | 312 | 14 | 0 | 637 |
| trzA225 ptsP160 PR-4 | 334 | 304 | 14 | 0 | 652 |

FIG. 4. *Recovery of the ptsP160 mutation from four independently isolated pseudorevertants (PR) with cysA20 as the recipient. Transduction and isolation of cysA$^+$ recombinants was performed on medium A agar plates containing 0.2% lactate as the sole carbon source. KB1 phage was grown on the donor pseudorevertant strains, and cysA20 was used as the recipient in each case. Recombinants were streaked on the same series of agar plates, with the phenotypes of the recombinants appearing from each cross as described in the legend to Fig. 3.*

factor III; (v) the pseudorevertants were not inducible to produce higher levels of HPr and enzyme I; (vi) each of the *ptsP*$^-$ recombinants derived from the pseudorevertants contained negligible levels of HPr and enzyme I and normal levels of factor III.

The biochemical results, therefore, are in complete accord with the phenotypic behavior of the mutants and with the genetic results described above. The induction data also provide evidence for the conclusion that each pseudorevertant contains an initiation signal for transcription (and/or translation) of *ptsH* and *ptsI* which alters the ability of the promoter to respond to the normal induction mechanism for HPr and enzyme I.

**Transport studies.** Figure 5 shows that the kinetics of methyl $\alpha$-glucoside uptake in the promoter-like mutant SB2729 (*trzA225 ptsP160*) is drastically reduced when compared with both its wild-type progenitor (*trpB223*) and the *ptsP160* pseudorevertants. Whereas the parent and the pseudorevertants derived from strain SB2729 transport the glycoside normally, representative recombinants derived from each pseudorevertant (those showing the *ptsP*$^-$ phenotype) behaved like *ptsP160* strains. Three *ptsP160* pseudorevertants contained 30 to 50% of the normal, uninduced levels of both HPr and enzyme I, but were able to take up the glycoside at the wild-type rate. These results suggest that, at least for the uptake of methyl $\alpha$-glucoside, the sugar-specific PTS proteins are probably the rate-limiting components of this transport system and not the general proteins, HPr and enzyme I.

## DISCUSSION

Previous studies from this laboratory (4, 14) have suggested the possibility that the structural genes for HPr and enzyme I of the PTS constitute an operon. This conclusion is based on the observations that the above two proteins (but not factor III) are coordinately inducible (14), that *trzAptsHI* deletions produce normal levels of factor III from the *crr*$^+$ gene, and that some point mutations in the HPr structural gene (*ptsH*) exert a severe polar effect on enzyme I synthesis from *ptsI* (4).

In a study concerned with the selection of *trzApts* deletions (4), SB2729 was isolated. The present results show that the genetic lesion in this strain can recombine with all known *pts* point mutations and can be separated into *trzA* and *pts* components. We therefore conclude

TABLE 3. *Comparison between parent, promoter mutant, promoter restarts (PR), and recombinants with respect to induction by D-fructose*[a]

| SB no. | Type of mutation | Method of isolation[b] | Sp act of PTS components | | | | |
|---|---|---|---|---|---|---|---|
| | | | HPr | | Enzyme I | | Factor III |
| | | | Lactate[c] | Fructose | Lactate | Fructose | Lactate |
| | *trpB223, wild-type* | | *0.36* | *1.5* | *0.53* | *1.9* | 0.32 |
| 2729 | *ptsP160* | NA | 0.02 | 0.02 | 0.02 | 0.02 | 0.28 |
| 2758 | *ptsP*⁻ | Rec | 0.02 | 0.02 | 0.01 | 0.01 | 0.26 |
| 2759 | *ptsP*⁻ | Rec | 0.01 | 0.01 | 0.01 | 0.01 | 0.25 |
| 2737 | *ptsP160 PR-1* | NG | *0.12* | *0.21* | *0.31* | *0.45* | 0.28 |
| 2761 | *ptsP*⁻ | Rec | 0.01 | 0.01 | 0.02 | 0.02 | 0.26 |
| 2762 | *ptsP*⁻ | Rec | 0.01 | 0.01 | 0.01 | 0.01 | 0.24 |
| 2738 | *ptsP160 PR-2* | NG | *0.30* | *0.33* | *0.69* | *0.53* | 0.22 |
| 2763 | *ptsP*⁻ | Rec | 0.01 | 0.03 | 0.02 | 0.02 | 0.20 |
| 2764 | *ptsP*⁻ | Rec | 0.02 | 0.03 | 0.01 | 0.01 | 0.24 |
| 2748 | *ptsP160 PR-3* | DES | *0.10* | *0.13* | *0.30* | *0.35* | 0.27 |
| 2774 | *ptsP*⁻ | Rec | 0.01 | 0.03 | 0.02 | 0.02 | 0.25 |
| 2749 | *ptsP160 PR-4* | DES | *0.10* | *0.15* | *0.28* | *0.32* | 0.30 |
| 2780 | *ptsP*⁻ | Rec | 0.02 | 0.02 | 0.02 | 0.02 | 0.26 |

[a] One-liter quantities of each culture were grown to saturation in medium A containing 20 μg of L-tryptophan per ml plus either 0.2% lactate for uninduced, constitutive conditions or 0.2% D-fructose for induction conditions. Since the *ptsP*⁻ recombinants do not grow on D-fructose as the sole carbon source, induction experiments with these strains were performed by growth in the presence of both 0.2% lactate and 0.2% D-fructose. Wild-type (*trpB223*) has the same induced levels of HPr and enzyme I when grown on either lactate plus fructose or on fructose alone. Cell-free extracts were prepared and assayed as previously described (4) and as indicated in the text. Specific activity of each PTS component is expressed as micromoles of sugar phosphate formed in 30 min at 37 C per mg of protein. Methyl α-D-[$^{14}$C]glucoside (specific activity, $2.5 \times 10^5$ counts per min per μmol) was the substrate used in the HPr and enzyme I determinations, whereas thiomethyl-β-D-[$^{14}$C]galactoside (specific activity, $3.0 \times 10^5$ counts per min per μmol) was used to determine factor III levels.

[b] NA, Nitric acid; Rec, recombination; NG, nitrosoguanidine; DES, diethyl sulfate.

[c] Growth medium.

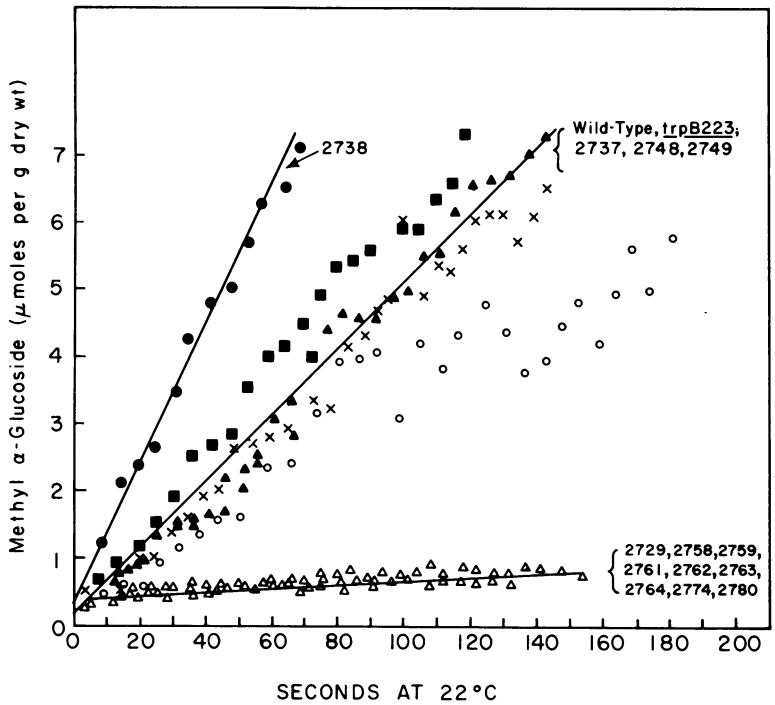



FIG. 5. *Methyl α-glucoside transport in strains used in the analysis of a PTS promoter-like mutation. Uptake studies with methyl α-D-[$^{14}C$]glucopyranoside as substrate were conducted with a glycoside concentration of 0.1 mM (specific activity, 5.6 × $10^5$ counts per min per μmol). The following strains were examined for initial rates of glycoside uptake: ptsP$^+$ (x), trpB223, wild-type; ptsP160 and ptsP$^-$ recombinants (Δ), which include strains SB2729, 2758, 2759, 2761, 2762, 2763, 2764, 2774, and 2780; and ptsP160 PR (promoter-restarts), which include (▲) SB2737, (●) SB2738, (■) SB2748, and (○) SB2749.*

that strain SB2729 is a double mutant.

The *ptsP160* mutation almost completely prevents the synthesis of HPr and enzyme I. This mutation is suggested to be a microdeletion because of (i) the selection method used for its induction and isolation, nitrous acid mutagenesis, (ii) the fact that the phenotype of *pts*$^+$ was always restored by the occurrence of pseudorevertants and never by true primary site back-mutation as shown by the recovery of the original *ptsP160* mutation from each of 33 independently isolated pseudorevertants, and (iii) the observation that the wild-type (*pts*$^+$) response to induction by fructose is missing in each pseudorevertant.

The present results suggest that the *ptsP160* mutation is a lesion in the promoter or promoter-like element that regulates expression of the structural genes for HPr and enzyme I of the PTS. This lesion probably is not an extremely polar mutation in the proximal end of the HPr (*ptsH*) structural gene since fructose is unable to induce both HPr and enzyme I activities; similarly, if the initiation mutations in the pseudorevertant strains were in the HPr structural gene, restoration of both HPr and enzyme I levels would not be affected equally. The alternatives can be examined by determining the amino acid sequence of the HPr proteins isolated from the pseudorevertant strains. The possibility that the pseudorevertants result from operator-constitutive mutations in "*ptsO*" awaits a selection procedure that would allow the separation and identification of the pseudorevertant alleles apart from the *ptsP160* mutation to see whether, by themselves, they can respond to the normal induction mechanism for HPr and enzyme I, or whether they produce high levels of these two proteins in the absence of inducer.

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