JOURNAL OF BACTERIOLOGY, Feb. 1987, p. 894–896
0021-9193/87/020894-03$02.00/0

Vol. 169, No. 2

Genetic Expression of Enzyme I* Activity of the Phosphoenolpyruvate:Sugar Phosphotransferase System in *ptsHI* Deletion Strains of *Salmonella typhimurium*

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Received 30 May 1986/Accepted 13 November 1986

Mutants expressing a novel enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system, termed enzyme I*, were isolated from strains of *Salmonella typhimurium* which were deleted for the HPr and enzyme I structural genes. The mutations lay in a newly defined gene, termed *ptsJ*, which mapped on the *S. typhimurium* chromosome between the *ptsHI* operon and the *cysA* gene.

The phosphoenolpyruvate:sugar phosphotransferase system (PTS) is a complex, multicomponent enzyme system whose primary functions include sugar reception, transport, and phosphorylation (5, 6). Secondary functions of the PTS include regulation of the activities of carbohydrate permeases, catabolic enzymes, and adenylate cyclase. As a consequence of these regulatory interactions, the PTS controls transcription of several carbohydrate catabolic enzyme systems.

In the enteric bacteria *Escherichia coli* and *Salmonella typhimurium*, the PTS consists of about 20 known proteins, but new proteins of the system are continually being discovered. It is now known that *E. coli* can utilize at least 15 different sugars for growth via the PTS provided that the bacteria possess the general energy-coupling proteins of the PTS, enzyme I and HPr, as well as the sugar-specific enzyme II and enzyme II-III pairs which serve as the receptor group translocators (6, 10; F. C. Grenier, E. B. Waygood,and M. H. Saier, Jr., J. Cell. Biochem., in press).

A novel *pts* gene is described here. Mutations in this gene allowed the expression of a cryptic enzyme I (enzyme I*), whose expression had regulatory consequences. This gene, termed *ptsJ*, mapped between the *pts* operon and *cysA* at min 49 of the *S. typhimurium* chromosome.

(Preliminary results were presented at a meeting of the Royal Netherlands Academy of Arts and Science in September 1985 as well as at the annual meeting of the American Society for Microbiology in March 1986.)

Representative bacterial strains used in this study are listed in Table 1. All phenotypes were shown by transfer of the mutant genes into a single isogenic background to be due to the mutations indicated. LJ701 (9) was subjected to Tn*10* mutagenesis, and mutants were selected for growth on minimal-mannitol plates as described previously (7, 8). The *fruR51*::Tn*10* mutation (1, 2) was transduced into strain LJ703 (3) with bacteriophage P22 as the carrier and strain LJ702 (4) as the donor to give strain LJ704. Strain LJ704, when plated on eosin-methylene blue-fructose agar, spontaneously gave rise to mutants which had gained the ability to ferment fructose. These strains were clonally isolated. Several such mutants, one of which was LJ705, were characterized. Other such mutants were isolated similarly but with the Δ*cysK ptsHI41* mutation replaced by the Δ*cysK ptsHI crrA49* mutation (3). These different mutants fermented PTS sugars to differing degrees and exhibited various levels of enzyme I* activity. Finally, LJ706 (*cysA1151*) was transduced to cysteine positivity by using strain LJ705 as the donor. About 40% of the cysteine-positive transductants were fructose positive and mannitol negative and were shown to have the growth and fermentation characteristics of strain LJ707 (Table 1).

Bacteria were harvested during the late exponential phase of growth by centrifugation, washed three times with medium 63 (8), and resuspended to a cell density appropriate for uptake studies or for rupture by passage through a French pressure cell. Enzymes of the PTS were assayed as described previously (7). Assays for the uptake of radioactive substrates by intact cells (7) have also been described previously.

The growth and fermentation properties of an isogenic series of bacteria on a variety of carbohydrates are summarized in Table 2. When the *fruR* mutation was crossed into strain LJ707, the bacteria grew on all PTS substrates. Since *fruR* led to constitutive expression of the fructose regulon, including the HPr-related FPr, the presence of both FPr and enzyme I* allowed growth on all PTS sugars. Both wild-type strain LJ709 and mutant strain LJ710 (*ptsJ52*) could grow on all PTS sugars. Strain LJ703 (Δ*cysK ptsHI41*) was incapable of growth on PTS sugars, but the *ptsJ52* mutation (strain LJ707) restored fructose utilization and slow utilization of glucose and mannose as well as 2-deoxyglucose-resistant glycerol utilization. The hexose fermentation correlates with the induction specificity of FPr (8). Introduction of a *crp* or *cya* mutation into strain LJ707 resulted in depressed fructose and mannose utilization but did not alter the rate of glucose utilization. The *fruR51*::Tn*10* mutation was epistatic over the *ptsJ* allele with respect to 2-deoxyglucose resistance.

As revealed by the transduction experiment in which strain LJ707 was constructed from strains LJ706 and LJ705, the *ptsJ* mutation appeared to map near the *pts* operon. To establish this fact and determine more precisely the map position, a three-point transductional cross was performed. Tetracycline-resistant transductants were selected on eosin-methylene blue-mannitol plates containing 15 μg of tetracycline per ml. A total of 157 transductants were clonally isolated, shown to be free of phage, and characterized. The relevant gene order in the *ptsJ* region, consistent with the data summarized in Table 3, was *cysA ptsJ ptsHI cysK*. Also, the presence of class 3 recombinants (Table 3) shows that *ptsJ* and *ptsI* were genetically separable. No double

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TABLE 1. Bacterial strains[a]

Strain	Genotype	Defect	Origin or reference
LJ701	*ptsH15 crrA6*	HPr; enzyme III^{glc}	7
LJ702	*ptsH15 crrA6 fruR51*::Tn*10*	HPr; enzyme III^{glc}; *fru* repressor	Tn*10*-induced Mtl$^+$ mutant of LJ701
LJ703	Δ*cysK ptsHI41*	Deletion for HPr and enzyme I	Cys$^+$ Fru$^-$ transductant of LJ706 obtained by using SB2309 (3) as donor
LJ704	Δ*cysK ptsHI41 fruR51*::Tn*10*	Deletion for HPr and enzyme I; *fru* repressor	Tet^r transductant of LJ703 obtained by using LJ702 as donor
LJ705	Δ*cysK ptsHI41 fruR51*::Tn*10 ptsJ52*	Same as for LJ704; enzyme I* expressed	Fru$^+$ mutant of LJ704
LJ706	*cysA1151*	Sulfate transport	Pts$^+$ *cysA1151* transductant of SB1477 (*ptsI18*) obtained by using LJ116 (*cysA1151 pgtA3 nal^r strA934* [M. H. Saier, unpublished data]) as donor
LJ707	Δ*cysK ptsHI41 ptsJ52*	Same as for LJ703; enzyme I* expressed	Cys$^+$ Fru$^+$ Mtl$^-$ transductant of LJ706 with LJ705 as donor
LJ708	*cysA1539*::Tn*10*	Sulfate transport (tetracycline resistant)	Tet^r transductant of LJ709 with NK136 as donor
LJ709	Wild type	None	Cys$^+$ Fru$^+$ transductant of SB1477 with LJ116 as donor
LJ710	*ptsJ52*	Same as for LJ709; enzyme I* expressed	2-Deoxyglucose-resistant Mtl$^+$ transductant of LJ707 with LJ709 as donor
LJ711	Δ*cysK ptsHI41 ptsJ52 crp-773*::Tn*10*	Same as for LJ707; cyclic AMP receptor protein inactive	Tet^r transductant of LJ707 with PP1037 as donor

[a] Preferred genetic and biochemical nomenclature for the PTS has been summarized elsewhere (6). Relevant gene-enzyme relationships are as follows: *ptsH* codes for HPr; *ptsI* codes for enzyme I; *crrA* codes for enzyme III^{glc}; *ptsJ* when mutated allows expression of enzyme I*; *fruH* codes for FPr.

crossovers (*ptsJ52 cysA1539*::Tn*10*) were observed. Our data suggest that *cysA* and *ptsJ* were about 47% cotransducible and that less than 0.1 min of the *S. typhimurium* chromosome separated the *ptsI* and *ptsJ* loci.

The specific activities of enzyme I plus enzyme I* and of HPr plus FPr in crude extracts derived from several isogenic *S. typhimurium* strains are given in Table 4. Several important features should be noted. (i) Whereas strain LJ703 exhibited detectable FPr activity, enzyme I* activity was not observed. (ii) Inclusion of the *fruR51*::Tn*10* mutation rendered FPr expression constitutive and allowed the detection of enzyme I* activity. (iii) Introduction of the *ptsJ52* mutation into the LJ704 genetic background led to a 100-fold increase in enzyme I* activity without altering FPr activity. (iv) Elimination of the *fruR* mutation reduced enzyme I* activity sevenfold and reduced FPr activity back to its basal level (compare strains LJ707 and LJ703). (v) Introduction of the *crp-773*::Tn*10* mutation in the genetic background of LJ707 reduced FPr activity without altering enzyme I* activity. The results establish that (i) both enzyme I* and FPr were subject to negative control by the product of the *fruR* gene; (ii) the two activities were not coordinately regulated; (iii) expression of enzyme I* was not under cyclic AMP-CRP control; and (iv) although enzyme I* activity could be detected in the absence of the *ptsJ* mutation, the level of its expression was insufficient to allow utilization of PTS carbohydrates. It is not yet known if *ptsJ* encodes enzyme I* or is regulatory in nature.

Enzyme I* from strain LJ705 was examined in vitro. The enzyme could replace enzyme I for the rapid phosphoenolpyruvate-dependent phosphorylation of both HPr and FPr. It had a monomeric molecular weight of about 60,000, and it associated to an active dimer. Its thermal denaturation curve resembled that of wild-type enzyme I. Moreover, enzyme I* and enzyme I had similar mobilities on ion-exchange columns of DEAE cellulose. Polyclonal antibodies as well as a monoclonal antibody prepared against enzyme I were active against enzyme I*. The results establish that enzyme I* closely resembled the wild-type enzyme.

The results of this study establish the existence of a gene located between *cysA* and the *ptsHI* operon on the *S. typhimurium* chromosome, which when mutated allowed expression of enzyme I*, an apparently novel but closely related form of enzyme I. In view of the conclusion that the

TABLE 2. Growth and fermentation of bacterial strains on a variety of carbon compounds[a]

Carbon compound	Growth and fermentation of strains with indicated genetic background						
	LJ709 (wild type)	LJ710 (*ptsJ*)	LJ703 (Δ*ptsHI*)	LJ707 (Δ*ptsHI ptsJ*)	LJ711 (Δ*ptsHI ptsJ crp* or *cya*)	LJ704 (Δ*ptsHI fruR*)	LJ705 (Δ*ptsHI ptsJ fruR*)
Fructose	+	+	−	+	−	−	+
Glucose	+	+	−	±	±	−	+
Mannose	+	+	−	±	−	−	+
N-Acetylglucosamine	+	+	−	−	−	−	+
Mannitol	+	+	−	−	−	−	+
Glucitol	+	+	−	−	−	−	+
Galactitol	+	+	−	−	−	−	+
Glycerol	+	+	−	+	−	−	+
Glycerol + 2-deoxyglucose	−	+	−	+	−	−	−

[a] +, Utilization; −, no utilization; ±, intermediate response.

TABLE 3. Three-point transductional analysis of the *ptsJ52* mutation with *cysA1539*::Tn*10* and Δ*cysK ptsHI41* as flanking markers[a]

Class	Mannitol fermentation	Growth on glycerol + 2-deoxyglucose	Fructose fermentation	No. of transductants
1	+	−	+	63
2	−	+	±	83
3	−	−	−	11
4	+	+	+	0

[a] The donor was LJ708, and the recipient was LJ707 (Table 1). Fermentation responses were determined on eosin-methylene blue medium, and glycerol utilization was measured on minimal-agar medium containing 0.2% glycerol and 0.2% 2-deoxyglucose. +, Fermentation or growth; −, no fermentation or growth; ±, intermediate response.

Δ*cysK ptsHI41* mutant lacks the N-terminal third of the wild-type enzyme I gene and that the Δ*cysK ptsHI crrA49* mutant lacks the entire structural gene for enzyme I (3), it must be concluded that enteric bacteria possess two genes encoding enzyme I-like proteins. This fact correlates with the presence of two genes encoding HPr-like proteins in these bacteria. Because enzyme I* activity (like that of FPr) appeared to be under *fruR* control, the structural genes for FPr and enzyme I* may be considered to be part of the *fru* regulon. Consequently, *fruI* may be the most appropriate designation for the enzyme I* structural gene, and *fruH* would be the corresponding designation for the FPr structural gene. Further studies are required to establish the nature of the *ptsJ* gene and to identify the structural gene for enzyme I*.

TABLE 4. Specific activities of the energy-coupling enzymes of the PTS in an isogenic series of *S. typhimurium* strains[a]

Strain	Genotype	Sp act of enzyme I + enzyme I* (cpm/protein)	HPr + FPr (cpm/protein)
LJ709	Wild type	72	390
LJ703	Δ*ptsHI41*	<0.03	4.6
LJ704	Δ*ptsHI41 fruR51*::Tn*10*	0.78	360
LJ705	Δ*ptsHI41 fruR51*::Tn*10 ptsJ52*	85	490
LJ707	Δ*ptsHI41 ptsJ52*	12	3.9
LJ711	Δ*ptsHI41 ptsJ52 crp-773*::Tn*10*	11	1.1

[a] The isogenic strains were grown to early stationary phase in LB medium without the addition of sugar. Extracts were prepared and assayed as described in the text. Specific activities are expressed in terms of counts per minute per protein per 20 min at 37°C. The specific activity of the [^{14}C]mannitol used for the assay was 5 μCi/μmol.

This work was supported by Public Health Service grants 5 RO1 AI 21702 and 2 RO1 AI 14176-09A1 from the National Institute of Allergy and Infectious Diseases.

We gratefully acknowledge receipt of bacterial strains from K. E. Sanderson, J. R. Roth, and P. Postma.

LITERATURE CITED

1. **Calvo, J. M., M. Goodman, M. Salgo, and N. Capes.** 1971. *Salmonella* locus affecting phosphoenolpyruvate synthase activity identified by a deletion analysis. J. Bacteriol. **106**:286–288.
2. **Chin, A. M., B. U. Feucht, and M. H. Saier, Jr.** 1987. Evidence for regulation of gluconeogenesis by the fructose phosphotransferase system in *Salmonella typhimurium*. J. Bacteriol. **169**:897-899.
3. **Cordaro, J. C., and S. Roseman.** 1972. Deletion mapping of the genes coding for HPr and enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system in *Salmonella typhimurium*. J. Bacteriol. **112**:17–29.
4. **Ely, R., R. M. Weppleman, H. C. Massey, Jr., and P. E. Hartman.** 1974. Some improved methods in P22 transduction. Genetics **76**:625–631.
5. **Postma, P. W., and J. W. Lengeler.** 1985. Phosphoenolpyruvate:carbohydrate phosphotransferase system of bacteria. Microbiol. Rev. **49**:232–269.
6. **Saier, M. H., Jr.** 1985. Mechanisms and regulation of carbohydrate transport in bacteria. Academic Press, Inc., New York.
7. **Saier, M. H., Jr., and S. Roseman.** 1976. Sugar transport. The *crr* mutation: its effect on repression of enzyme synthesis. J. Biol. Chem. **251**:6598–6605.
8. **Saier, M. H., Jr., R. D. Simoni, and S. Roseman.** 1970. The physiological behavior of enzyme I and heat-stable protein mutants of a bacterial phosphotransferase system. J. Biol. Chem. **245**:5870–5873.
9. **Saier, M. H., Jr., R. D. Simoni, and S. Roseman.** 1976. Sugar transport. Properties of mutant bacteria defective in proteins of the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6584–6597.
10. **Waygood, E. B., R. L. Mattoo, and K. G. Peri.** 1984. Phosphoproteins and the phosphoenolpyruvate:sugar phosphotransferase system in *Salmonella typhimurium* and *Escherichia coli*: evidence for III^{Mannose}, III^{Fructose}, III^{Glucitol}, and the phosphorylation of Enzyme II^{Mannitol} and Enzyme II$^{N\text{-}Acetylglucosamine}$. J. Cell. Biochem. **25**:139–159.