JOURNAL OF BACTERIOLOGY, Nov. 1970, p. 793-797
Vol. 104, No. 2

Printed in U.S.A.

Isolation and Mapping of Phosphotransferase Mutants in *Escherichia coli*

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Received for publication 17 July 1970

Mutants of *Escherichia coli* K-12 defective in enzyme I or Hpr, the two common components of the phosphoenolpyruvate-dependent phosphotransferase system, were isolated by a simple, direct method. The *ptsI* locus, the structural gene for enzyme I, and the *ptsH* locus, the site of mutations leading to loss of Hpr activity, are adjacent genes and could be part of a single operon. These two genes lie between the *purC* and *supN* markers in the order: *strA* . . . *guaB-purC-ptsI-ptsH-supN-dsdA* . . . *his*.

In 1964 Kundig, Ghosh, and Roseman described a bacterial system capable of phosphorylating a number of carbohydrates (5). This novel phosphotransferase system uses phosphoenolpyruvate as the primary phosphate donor in a two-step reaction:

$$\text{Phosphoenolpyruvate} + \text{Hpr} \xrightarrow{\text{enzyme I}} \text{P-Hpr} + \text{pyruvate}$$

$$\text{Sugar} + \text{P-Hpr} \xrightarrow{\text{enzyme II}} \text{P-sugar} + \text{Hpr}$$

In the first step, enzyme I catalyzes the transfer of phosphate from phosphoenolpyruvate to the protein cofactor, Hpr. In the second step, a family of membrane-bound enzymes (enzymes II), each with relatively high induction and substrate specificity, transfer phosphate from P-Hpr to the carbohydrate. Mutant strains defective in either Hpr or enzyme I are unable to grow on a large number of carbon sources (for review, see reference 1). We report here a simple method for the isolation of *pts* mutants in *Escherichia coli* and data on the mapping of *pts* mutations. We use the symbols suggested by Lin: *ptsH* for mutations affecting Hpr, and *ptsI* for those affecting enzyme I (Annu. Rev. Genet., *in press*).

MATERIALS AND METHODS

Media. TP medium contains, per liter: mannitol, 8 g; sorbitol, 8 g; peptone, 17 g; proteose-peptone, 3 g; 2,3,5-triphenyltetrazolium chloride, 50 mg; and agar, 15 g. These ingredients are mixed with distilled water and autoclaved together without prior boiling to hydrate the agar. K10 medium, used as minimal medium in this work, and ML medium have been described elsewhere (3).

Strains. The strains used in this work are listed in Table 1.

Isolation of mutants. Bacteria in the exponential phase of growth in minimal medium were irradiated with ultraviolet light, or treated with HNO_2 as described by Schwartz and Beckwith (7). After this treatment, cells were either plated directly on TP medium or plated after a 1-hr incubation in ML medium. The nonfermenting bright red colonies were repurified on TP medium, and then tested for growth properties on minimal medium plates containing different carbon sources.

Mapping. The methods used in mating, transduction, and the preparation of P1kc lysates have been described (3). Growth on mannitol was used to score or select for Pts^+. Although all Pts^- mutants in all strains grow on galactose, pyruvate, and glucose-6-phosphate, the Pts^- recombinants tend to form smaller colonies than the Pts^+ recombinants when galactose or pyruvate are used; only glucose-6-phosphate seemed to be free of any discriminatory effect. Therefore, glucose-6-phosphate (sodium salt, 1.5 g/liter) was used as carbon source in plating whenever *pts* was an unselected marker in a cross. Scoring and selecting for *supN* was done by use of the tryptophan requirement of strains carrying *trp827*, an amber mutation that is suppressed by *supN*. When *supN* was selected, the donor genotype was *supN trp827*. Since Pts^- strains do not grow on D-serine, the linkage of *ptsI* and *dsdA* was measured by using a lysate of a strain carrying the temperature-sensitive mutation *ptsI101R* (*see* Results) to transduce a pts^+ *dsdA* recipient to growth on D-serine at 30 C. At this temperature the *ptsI101R* product is functional. Inheritance of the donor *pts* marker was scored by testing for growth on mannitol at 42 C.

The mapping of *ptsI* was carried out largely with *ptsI211*, a mutation isolated in CHE9 and transferred into derivatives of FF8005 or X9137 by mating. In the X9137 background the Pts^- recombinants are usually not red on TP medium, but are nonetheless easily identified on this medium by the small, smooth colonies that they form.

Enzyme assays. Enzyme I and Hpr were assayed

TABLE 1. *Strains*

Strain	Mating type	Pertinent markers[a]	Origin
FF8005	F⁻	*proC bgl*⁺ *strA*ʳ	From MO of S. Brenner
X9137	F⁻	*nadB trp827 lacZ*$_{X82}$*strA*ʳ	From FRAG-5 (3).
EM3003	F⁻	*dsdA aroC purF*	E. McFall
0150	F⁻	*purC*	H. J. J. Nijkamp
H724	F⁻	*purC guaB*	H. J. J. Nijkamp
CHE9	Hfr KL16	*bgl*⁺	From KL16 of B. Low
KL98	Hfr KL98		B. Low
AB2547	Hfr AB313	*supN*	E. A. Adelberg

[a] Only markers used in this study are listed. The symbol *bgl*⁺, for the ability to ferment aryl-β-glucosides, is listed because most strains of *E. coli* cannot utilize these compounds as a carbon source.

by measuring the rate of phosphorylation of methyl-α-D-glucoside as described elsewhere (4). When precise quantitation was desired, the amount of test extract added was within the range over which a plot of the amount of extract versus activity is linear. In screening assays used to classify mutants, the assay was usually performed near the end of the linear range.

RESULTS

Isolation of mutants. Our screening method for *pts* mutants is based on the fact that the phosphotransferase system seems to be the only catabolic pathway for mannitol and sorbitol in *E. coli*. Using several different types of indicator media containing these two hexitols, we tested different strains and found that the peptone-tetrazolium medium (TP medium) differentiated two known *pts* mutants [1101, defective in Hpr (4); and X-19, an X-ray-induced mutant defective in enzyme I] most clearly from a number of *pts*⁺ strains in our collection. The *pts* mutants from most strains give rise to colonies with a distinctive dark-red color on these plates, whereas *pts*⁺ strains form colonies that either remain buff colored or turn purple. The color of *pts* mutants on TP medium is strain dependent: all mutants in strain FF8005 give red colonies, those in CHE9 tend to form smaller colonies that turn red only after more prolonged incubation, and *pts* mutants in the X9137 background give rise to small smooth colonies which often do not turn red at all. These differences are correlated with differences in the growth of the strains on various carbon sources, as summarized elsewhere (W. Epstein, S. Jewett, and R. H. Winter. Fed. Proc., p. 601, 1970).

A total of 97 red colony mutants of strain FF8005 were purified and tested for the ability to grow on different carbon sources by streaking onto minimal medium plates. Seventy-seven of the mutants failed to grow on mannitol, sorbitol, or arbutin (*p*-hydroquinonyl-β-D-glucoside), grew slowly on glucose, lactose, and glycerol, and grew very well on galactose. Of these, 35 were subjected to assay and found lacking in enzyme I activity. These are therefore *ptsI* mutants. Most of the other mutants grew very slowly on mannitol and sorbitol, and grew almost as well as the parental strain on glucose, lactose, and glycerol. These mutants were found to be defective in Hpr activity, and are therefore *ptsH* mutants. A few had measurable traces of Hpr activity. The only exceptions noted were: five strains which did not grow on any minimal media and were found to carry both a *pts* mutation and an unlinked auxotropic mutation, and a few mutants that had the phenotype characteristic of Hpr mutants but were leaky enzyme I mutants with between 1 and 5% of wild-type enzyme I activity.

Mapping of the ptsI locus. Matings of a F⁻ Smʳ *ptsI* strain with a number of different Hfr strains showed that the *ptsI* locus was transferred as an early marker by KL16 but not by KL98, indicating that *ptsI* is between minute 44 and 55 on the map of Taylor and Trotter (8). More precise localization is based on the P1kc cotransduction frequencies with a number of markers in this part of the map (Table 2). The *ptsI* was found to be cotransduced at high frequency with *supN*, at modest frequency with *purC* and *dsdA*, but not with any of the other markers tested. No cotransduction of *nadB* with *ptsI* was found among 300 *ptsI*⁺ selected transductants scored for inheritance of the donor *nadB* marker. This finding is expected in view of the actual location of *nadB*, several minutes from the location indicated on the 1967 map of the *E. coli* chromosome (9).

The order of *ptsI* and *supN* was established in a transductional cross in which the donor was *purC*⁺ *ptsI*⁺ *supN*, the recipient *purC ptsI101R*, and *purC*⁺ transductants were selected. Out of 976 *purC*⁺ transductants, 32 inherited donor markers for both *ptsI* and *supN*, 22 inherited

only the donor marker for *ptsI*, and 1 received only the donor *supN*. Both the lower linkage of *supN* to *purC* as compared with the linkage of *ptsI* to *purC*, and the low frequency of transductants carrying the donor marker for *supN* but not for *ptsI*, show that *ptsI* is closer to *purC* than is *supN*. The order of markers in the vicinity of *ptsI* and the observed cotransduction frequencies are shown in Fig. 1.

Structural gene for enzyme I. A mutation causing temperature sensitivity in enzyme I was isolated by screening revertants of *ptsI* mutants for temperature sensitivity of growth on mannitol. The mutation so isolated, *ptsI101R*, allows normal growth on mannitol and sorbitol at 30 C, but at 42 C growth on these compounds is very slow. Extracts of the mutant strain grown at 30 C have wild-type enzyme I activity when assayed at 28 C but showed rapid loss of activity on incubation at 45 C, a temperature which causes only a slow rate of inactivation of wild-type enzyme I (Fig. 2). The curve for a mixture of mutant and wild-type extracts is that expected for a mixture of two components with different rates of heat inactivation. Therefore, the temperature sensitivity of the mutant enzyme is due neither to the absence of a stabilizing factor nor to the presence of a labilizing factor, but must reside in the properties of the mutant enzyme itself.

TABLE 2. *P1 cotransduction frequencies*

Selected donor marker	Inheritance of unselected donor marker (No. inheriting marker/total colonies scored)				
	purC	*ptsI*[a]	*supN*	*dsdA*	*aroC*
guaB+	72/227	0/227			
purC+		54/976	33/976		
ptsI+[a]	10/228		88/108	15/128	0/304
supN		183/212			
dsdA+		22/240			

[a] The mutation used in most of these measurements was *ptsI211*. In a few cases the results are combined ones for *ptsI211* and *ptsI219*. Where *dsdA*+ was selected, the *ptsI101R* mutation was used. The other markers are from the strains listed in Table 1.

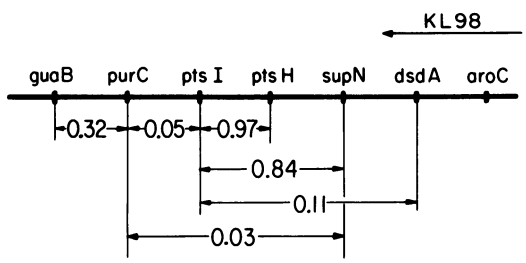

FIG. 1. *Order of markers in the vicinity of the pts genes. The numbers are average P1kc cotransduction frequencies between the markers and are based on data from Table 2 and the text. The origin and direction of transfer of Hfr strain KL98 is also shown.*

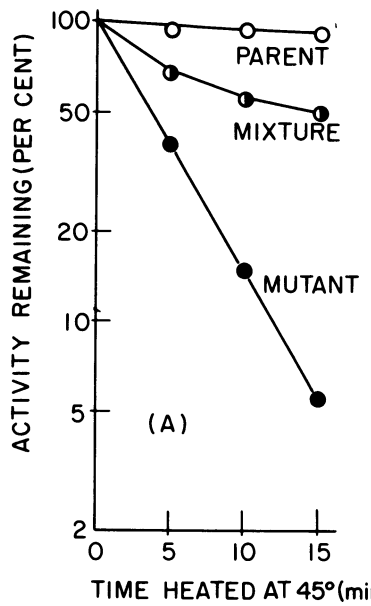

FIG. 2. *Heat inactivation of temperature-sensitive and wild-type enzyme I in extracts. Cultures of a strain carrying the ptsI101R mutation and of its pts*+ *parent were grown at 30 C in minimal medium containing 10 g of Casamino Acids per liter as carbon source. Sonic extracts were prepared from the cells, and enzyme I assays were performed at 28 C (4) on control samples, and on samples heated at 45 C for the times indicated. Also shown is the effect of heating on the activity of a mixture containing approximately equal initial activities of the two extracts.*

The mutation responsible for temperature sensitivity is very closely linked to other *ptsI* mutations. When a P1kc lysate of a *ptsI101R* strain was used to transduce other *ptsI* mutants to growth on mannitol at 30 C, almost all of the transductants were temperature-sensitive for growth on mannitol. In scoring 302 transductants of *ptsI211* and *ptsI219* obtained in this way, 4 and 6, respectively, were wild type, and all the rest were temperature-sensitive. This recombination frequency of up to 2% is consistent with expectations for recombination between two mutations in the same gene.

Mapping the ptsH locus. Preliminary mapping by Hfr × F^- crosses showed that the *ptsH* mutations were in the same general region of the chromosome as the *ptsI* mutations. Attempts to map the locus by transduction in which Pts+ was

selected were complicated by the fact that all of the *ptsH* mutants are somewhat leaky and have rather high reversion rates. The leakiness was found useful, however, in mapping by using a *ptsH* lysate to transduce *ptsI* strains to growth on mannitol. In addition to a few colonies that grew at normal rates on mannitol, a much larger number appeared which grew rather slowly on mannitol. Several of these slow-growing colonies were purified; they were found to have the mutant red phenotype on TP plates, and on assay had normal levels of enzyme I but were defective in Hpr activity. Since the total number of colonies growing on mannitol in such crosses is equal to the number of pts^+ colonies obtained with a wild-type lysate (both numbers being normalized to the number of $proC^+$ transductants obtained in each cross), the linkage of *ptsH* with *ptsI* can be expressed simply as the number of slowly growing transductants to the total number obtained. The linkage between several *ptsI* mutations and *ptsH111* ranged from 97 to 98%; expressed in another way, the recombination frequency between these pairs of mutations is between 2 and 3%. However, for *ptsI211* and *ptsH111* the recombination frequency is only 0.2%, showing that the *ptsH* and *ptsI* genes are very closely linked indeed, and are most likely adjacent genes on the chromosome.

The order of *ptsI* and *ptsH* was established by reciprocal three-factor transductional crosses in which *supN* was the selected marker, and the frequency of recombination between the *ptsH111* and *ptsI101R* mutations was measured (Table 3). These results show that the order is as drawn in Fig. 2: *ptsI-ptsH-supN*.

DISCUSSION

The use of TP indicator medium is a very convenient way to isolate *pts* mutants in *E. coli*. The only drawback to the use of this medium is that in some strains the *pts* mutants form small colorless colonies which, although readily distinguished from the parental colonies, can be produced by a number of types of mutation in addition to those affecting either Hpr or enzyme I.

We were somewhat surprised to find that all of the Hpr mutants isolated are phenotypically leaky. This trait could be due to an unusual resistance to total loss of Hpr activity by most amino acid substitutions. Since missense mutations usually reduce enzyme activity by altering tertiary structure and thereby affecting the active site, proteins with a very stable tertiary structure might be less affected by a change in a single amino acid. This might be the case for Hpr, because it is a very stable protein as shown by its resistance to denaturation by boiling (5).

The map position reported here for *ptsI* is generally consistent with the data of others for enzyme I mutants in *E. coli* (2, 10). The only inconsistency is that we find close linkage (84%) between *ptsI* and *supN* by P1kc transduction, whereas Wang, Morse, and Morse (10) found slight cotransduction (1.9%) when measured in one direction and no cotransduction when measured in the opposite direction. There is good agreement between our data and the most recent mapping of these loci in *Salmonella typhimurium*. The location of mutations affecting Hpr (*carB*, reference 6) corresponds to our mapping of *ptsH*, and, contrary to an earlier report (6), enzyme I mutants in *S. typhimurium* also map near the *purC* locus (D. Berkowitz, *personal communication*). The very close linkage between the *ptsH* and *ptsI* genes found in our work suggests that these two genes may be part of an operon.

TABLE 3. *Ordering of ptsH, ptsI, and supN by three-factor crosses*

P1 donor	Recipient	Incidence of pts^+ among $supN$ transductants[a]
ptsI101R supN	*ptsH111*	3.4
ptsH111 supN	*ptsI101R*	0.4

[a] In each of these two transductional crosses, 500 *supN* transductants were selected on glucose-6-phosphate minimal medium and scored for *pts* by testing for growth on mannitol at 42 C.

ACKNOWLEDGMENTS

We thank E. A. Adelberg, B. Low, E. McFall, and H. J. J. Nijkamp for the gift of strains. B. S. Kim and J. Stevens provided technical assistance.

This investigation was supported by Public Health Service grants GM 15766 from the Institute of General Medical Sciences, AM 10987 from the Institute for Arthritis and Metabolic Diseases, and General Research Support grant FR5367. Two of the authors are supported by Research Career Development Awards from the Institute of General Medical Sciences of the Public Health Service: GM10725 to W. Epstein, and GM42359 to C. F. Fox.

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