Genetic Mapping of a Locus for Triosephosphate Isomerase on the Genome of *Escherichia coli* K12

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SUMMARY

A gene specifying triosephosphate isomerase (*tpi*) has been located between *rha* and *glpK* on the *Escherichia coli* chromosome. Very recently a gene specifying fructose-6-phosphate kinase (*pfk*) has been located in this same area and we have found that the *tpi* and *pfk* are highly cotransducible. This finding provides the first indication of any close chromosomal association between genes specifying glycolytic enzymes.

INTRODUCTION

The selection of mutants of *Escherichia coli* K12 deficient in triosephosphate isomerase activity (TPI) has been described previously (Anderson & Cooper, 1969). The pleiotropic characteristics of such mutants showed that only one species of TPI was involved in both glycolysis and gluconeogenesis. In this paper the position of the *tpi* locus on the *E. coli* genome has been determined to see how it relates to the known genes which specify other glycolytic or gluconeogenic enzymes (Böck & Neidhart, 1966; Brice & Kornberg, 1967; Fraenkel, 1967; Morrissey & Fraenkel, 1969).

METHODS

Bacterial strains. Table 1 lists the strains used. The symbol *tpi* has been chosen for the gene specifying triosephosphate isomerase.

Media. Bacteria were maintained on Oxoid nutrient agar slopes supplemented with L-methionine and thymine (40 µg./ml.). Selective plates contained minimal medium (Ashworth & Kornberg, 1966) solidified with 1.5% (w/v) Oxoid no. 1 agar and the appropriate carbon source, at a final concentration of 50 mM (D,L-sodium lactate) or 25 mM (D-glucose, sodium D-gluconate and mannitol). Where required, amino acids were added at a final concentration of 80 µg./ml. The medium used for phage propagation and transduction (Z-medium) consisted of Oxoid nutrient broth or nutrient agar supplemented with MgCl₂ (10 mM) and CaCl₂ (2.5 mM). Soft minimal agar was minimal medium containing 0.6% (w/v) agar. The eluting fluid used for harvesting the phage was 0.8% NaCl + 0.8% Oxoid bacteriological peptone in chloroform-saturated water.

Bacteria were grown in liquid minimal medium or nutrient broth as described by Ashworth & Kornberg (1966).

Bacterial crosses. Log-phase cells growing in nutrient broth were mixed at 37° to give approximately 1×10^8 Hfr cells and 5×10^8 F⁻ cells/ml. After 5 min. gentle

agitation the mating mixture was diluted into minimal medium containing gluconate and the appropriate amino acids (de Haan & Gross, 1962). Conjugation was interrupted by 1 min. agitation of a 2 ml. sample using a Vortex Jun. Mixer (Scientific Industries, Inc., Springfield, Mass., U.S.A.). Samples were spread on to the appropriate selective plates containing streptomycin sulphate (100 µg./ml.) to select against the Hfr cells.

Table 1. *Characteristics of Escherichia coli K12 strains*

Strain	Sex	Genotype*	Source or reference
K 10	HfrC	<i>thi, str-s</i>	F. C. Neidhardt
AA 200	HfrC	<i>tpi-1, thi, str-s</i>	From K 10 by mutagenesis (Anderson & Cooper, 1969)
AA 201	HfrC	<i>tpi-2, thi, str-s</i>	From K 10 by mutagenesis (Anderson & Cooper, 1969)
AA 202	HfrC	<i>tpi-3, thi, str-s</i>	From K 10 by mutagenesis (Anderson & Cooper, 1969)
PA 309	F ⁻	<i>thi, argHBCE, his, leu, thr, trp, str-r (gal, xyl, man, mal)</i>	R. H. Walmsley
AA 210	F ⁻	<i>thi, tpi-1, argHBCE, leu, thr, trp, str-r</i>	AA 200 × PA 309 by selection for His ⁺ (this paper)
P 10	HfrJ ₄	<i>thr, leu, str-s (lac, mal)</i>	F. Jacob
AB 2297	Hfr	<i>ilv, ade, str-s (xyl)</i>	E. A. Adelberg
AT 2444	HfrH	<i>metE, thi, str-s</i>	A. L. Taylor
K 1	HfrH	<i>metBF, thy, str-s</i>	H. L. Kornberg
K 1.17	HfrH	<i>metBF, thy, glpK, str-s</i>	From K 1 by mutagenesis (this paper)
K 1.17.18	HfrH	<i>metBF, thy, glpK, rha, str-s</i>	From K 1.17 by mutagenesis (this paper)
K 1.1.5°.16	HfrH	<i>metBF, thy, pfk, str-s (pps, icl^o)</i>	H. L. Kornberg
PA 374	F ⁻	<i>thr, leu, thi, his, metE, argH, str-r</i>	N. Glansdorff
AB 1911	F ⁻	<i>metBF, argHBCE, str-r</i>	H. L. Kornberg

Abbreviations used: *thi* = thiamin; *arg* = arginine; *his* = histidine; *leu* = leucine; *thr* = threonine; *trp* = tryptophan; *ilv* = isoleucine and valine; *met* = methionine; *thy* = thymine; *ade* = adenosine; *gal* = galactose; *xyl* = xylose; *man* = mannose; *mal* = maltose; *lac* = lactose; *rha* = rhamnose; *str-r* = resistance to streptomycin; *str-s* = sensitivity to streptomycin; *tpi* = triose-phosphate isomerase; *glpK* = glycerol kinase; *pfk* = fructose 6-phosphate kinase; *pps* = phosphoenolpyruvate synthetase; *icl^o* = constitutive formation of isocitrate lyase; *fda* = fructose diphosphate aldolase.

* Non-relevant carbohydrate markers are given in parentheses.

Transduction. Phage P1_{kc} lysates were prepared by confluent lysis of donor bacteria on Z-agar plates. About 2 × 10⁹ donor bacteria in Z-broth were mixed with 2 to 4 × 10⁸ P1_{kc} particles. After 20 min. at 37° the mixture was diluted sixfold into soft minimal agar at 46° and 3 ml. samples were plated as an overlay on to Z-agar plates. This surface layer was collected after 6 h. incubation at 37°, homogenized in 2 ml. of

eluting fluid and the debris was removed by centrifugation at 12,000g for 5 min. at 2°. The lysates were stored at 4° over chloroform and generally contained about 10^{10} plaque-forming units/ml. when titrated with K10 as the indicator strain.

Recipient bacteria were grown on 20 ml. nutrient broth to about 1×10^8 bacteria/ml. then harvested and resuspended in 2 ml. of Z-broth. The phage were added (multiplicity = 1) and adsorption was allowed to proceed for 30 min. at 37° without aeration. The mixture was centrifuged, the bacterium pellet resuspended in 1 ml. of nutrient broth and suitable dilutions were plated on to the appropriate selection media. The colonies obtained after incubation for about 48 h. at 37° were streaked on to fresh plates and then restreaked on to the appropriate plates to score the inheritance of unselected markers. In each experiment, controls were run to check reversion of the recipient strain and sterility of the lysate.

Isolation of mutants. Glycerol kinase negative (*glpK*) mutants were isolated as described by Cozarelli & Lin (1966). Rhamnose negative (*rha*) mutants were isolated after ethylmethane sulphonate mutagenesis (Lin, Lerner & Jorgensen, 1962) and penicillin treatment (Gorini & Kaufman, 1960). Rhamnose non-fermenters were identified on rhamnose-eosin-methylene blue plates.

Assay of enzymes. Triosephosphate isomerase was assayed as described by Anderson & Cooper (1969).

RESULTS AND DISCUSSION

The approximate map position of the *tpi* gene was determined by mating AA 200 and PA 309. After 120 min. incubation, streptomycin-resistant recombinants which had inherited the ability to grow in the absence of a particular amino acid were selected on appropriately supplemented gluconate minimal plates. A large proportion (30 to 40%) of the *arg*⁺ recombinants and a smaller proportion (5%) of the *his*⁺ recombinants were unable to grow on lactate, suggesting that they had inherited the *tpi*⁻ allele. Such lactate negative recombinants had no detectable TPI activity and one, AA 210, was selected for use in further experiments.

Interrupted mating experiments between the Hfr donor strain P10 (Schwartz, 1966) and AA 210 showed that the *tpi*⁺ allele entered about 1 min. after the *argHBCE* cluster, thus placing *tpi* at about 76 min. on the *Escherichia coli* linkage map (Taylor & Trotter, 1967). Thus *tpi* should be cotransducible with one or more of the amino acid markers *argHBCE*, *metBF*, *metE* and *ilv*. The results of various cotransduction studies are shown in Fig. 1 and Table 2. When AA 210 was the recipient there was no detectable cotransduction between *tpi* and *ilv*. There was no linkage of *tpi* to *metE* but *tpi* was readily cotransducible with both *argHBCE* (8 to 15%) and *metBF* (38%). In the reciprocal cross (Table 2, Expt. 4, 5) the *tpi* lesion from the donor showed extremely low linkage of *tpi* to *metE* (< 0.3%) but significant, albeit reduced, linkage to both *argHBCE* (3%) and *metBF* (20%) was again observed.

From the values deduced by Taylor & Trotter (1967) to relate cotransduction frequency with P1 to map distance, these linkage values suggest that *tpi* is 0.5 min. from *metBF*, 1 min. from *argHBCE* and 2 min. from *metE*. Each of these values places *tpi* at 76.3 min. on the *Escherichia coli* map (Taylor & Trotter, 1967), a region which contains markers for the metabolism of rhamnose (*rha*) (Power, 1967) and glycerol (*glpK*) (Cozzarelli & Lin, 1966).

To locate *tpi* more precisely a *glpK* mutant, K1.17, was isolated and subsequently

further mutated to yield a *rha* mutant, K1.17.18 (see Methods). Both were used in cotransduction studies with *tpi*. Since the absence of TPI renders organisms unable to grow on rhamnose or glycerol (Anderson & Cooper, 1969) the selection in such three-factor crosses must be for *tpi*⁺ to allow unambiguous determination of the various phenotypes. As is shown in Fig. 1 and Table 2, Expt. 6, 7, there was a very high linkage of *tpi* to both *glpK* (81%) and *rha* (55%). Essentially identical linkage values were found for two further *tpi* mutants, AA201 and AA202 (Table 2, Expt 8, 9).

Fig. 1. Genes at 76 to 77 min. on the *Escherichia coli* linkage map (modified from Taylor & Trotter, 1967). Cotransduction frequencies from Table 2 are given as the fraction of the selected recombinants which have inherited the unselected marker. The selected marker is at the head of the arrow and the unselected marker at the tail.

If we assume that transductants requiring a minimum of four crossovers will occur significantly less frequently than those requiring a minimum of two crossovers, the frequencies of the various classes of recombinants shown in Table 2 are consistent with a gene order *rha tpi glpK*. In particular in Expt. 6, the least frequent of the four possible phenotypes is that expected for the order *tpi glpK metBF*, whilst in Expt. 7 the failure to find a single phenotype of especially low frequency is consistent with the gene order *rha tpi glpK*.

When the location of a *pfk* gene was reported very recently (Morrissey & Fraenkel, 1969) its position between *rha* and *glpK* indicated that it should be readily cotransducible with *tpi*. Since a similar *pfk* mutant was available in this laboratory, we have measured the cotransduction of *tpi* and *pfk* and the anticipated high linkage (82%) was found. Thus the *tpi* and *pfk* genes appear to be very close together on the *Escherichia coli* chromosome. The frequencies of the various phenotypes observed in this experiment are shown in Table 2, Expt. 10, and are consistent with the gene order *tpi pfk metBF*. Since the properties of the Pfk mutant used in these studies (H. L. Kornberg, personal communication) appear to be identical to those described

Table 2. Transduction of *tpi* and *pfk*

The selection and scoring of nutritional markers was on the appropriately supplemented gluconate minimal plates since both *tpi* and *pfk* mutants grow normally on gluconate. The selection and scoring of the other markers involved the use of the following appropriately supplemented minimal plates: GlpK⁺ on glycerol, Rha⁺ on rhamnose, Tpi⁺ on lactate and Pfk⁺ on mannitol.

Expt.	Donor	Recipient	Selected transductants	No. scored	Distribution of unselected markers in the transductants (%)			
					Tpi ⁺ Ilv ⁺	Tpi ⁺ Ilv ⁻	Tpi ⁺ Tpi ⁻	Tpi ⁺ Tpi ⁺
1	AB2297 (<i>ilv</i>)	AA210 (<i>tpi-1</i>)	Tpi ⁺	305	100	0	0	Met ⁺ Tpi ⁺ 90
2	AT2444 (<i>metE</i>)	AA210 (<i>tpi-1, argHBC</i>)	Arg ⁺	330	0	0	0	Met ⁺ Arg ⁺ 8
3	K1 (<i>metBF</i>)	AA210 (<i>tpi-2, argHBC</i>)	Tpi ⁺	148	0	0	0	Met ⁺ Arg ⁺ 92
4	AA200 (<i>tpi-1</i>)	PA374 (<i>metE, argH</i>)	Arg ⁺	518	0	0	0	Met ⁺ Tpi ⁺ 67
5	AA200 (<i>tpi-1</i>)	AB1911 (<i>metBF, argHBC</i>)	Tpi ⁺	364	11	27	24	Met ⁺ Arg ⁺ 58
6	K1.17 (<i>glpK, metBF</i>)	AA210 (<i>tpi-1</i>)	Arg ⁺	327	96	4	0	Met ⁺ Tpi ⁺ 0
7	K1.17.18 (<i>rha, glpK</i>)	AA200 (<i>tpi-1</i>)	Met ⁺	288	>99	<1	0	Arg ⁺ Tpi ⁺ 0
8	K1.17.18 (<i>rha, glpK</i>)	AA201 (<i>tpi-2</i>)	Arg ⁺	576	64	0	0	Met ⁺ Tpi ⁺ 33
9	K1.17.18 (<i>rha, glpK</i>)	AA202 (<i>tpi-3</i>)	Met ⁺	506	32	10	10	Arg ⁺ Tpi ⁺ 48
10	K1.1.5 ^c .16 (<i>pfk, metBF</i>)	AA200 (<i>tpi-1</i>)	Tpi ⁺	182	1	43	46	Met ⁺ Glp ⁺ 18
11	K1.1.5 ^c .16 (<i>pfk, metBF</i>)	K1.17.18 (<i>rha, glpK</i>)	Tpi ⁺	234	9	46	49	Rha ⁺ Glp ⁺ 11
			Tpi ⁺	335	10	29	29	Rha ⁺ Glp ⁺ 12
			Tpi ⁺	231	10	39	40	Rha ⁺ Glp ⁺ 11
			Tpi ⁺	313	56	26	26	Pfk ⁺ Met ⁺ 14
			Rha ⁺	180	32	21	43	Pfk ⁺ Glp ⁺ 4

by Morrissey & Fraenkel (1969) and the *pfk* allele is located in the same region (see Table 2, Expt. 11), the overall results suggest the order *rha tpi pfk glpK metBF*, as shown in Fig. 1.

The results reported in this paper mean that the chromosomal locations of four genes, *pgi*, *pfk*, *fda* and *tpi* (Böck & Neidhart, 1966; Fraenkel, 1967; Morrissey & Fraenkel, 1969; this paper), specifying four enzymes which act in sequence in the glycolytic pathway, are now known and of these only *tpi* and *pfk* are closely linked. The significance of this close linkage is not yet known.

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