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Transketolase Mutants of *Escherichia coli*

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Transketolase mutants have been selected after ethyl methane sulfonate mutagenesis of *Escherichia coli*. These strains are unable to grow on any pentose and, in addition, require a supplement of aromatic amino acids or shikimic acid for normal growth on any other carbon source. Revertants are normal in both respects and also contain transketolase. Transketolase mutants do not require exogenous pentose for growth. Preliminary genetic mapping of the locus is presented.

The hexose monophosphate shunt is a pathway with two branches. The oxidative branch is a series of reactions leading from glucose-6-phosphate to ribulose-5-phosphate (Fig. 5); *Escherichia coli* mutants for these reactions have been described (4, 8). The nonoxidative branch (Fig. 2) is a series of reversible reactions catalyzing the interconversion of pentose phosphates with fructose-6-phosphate and glyceraldehyde-3-phosphate. We now describe *E. coli* mutants affected in one of these enzymes, transketolase.

MATERIALS AND METHODS

Chemicals. Glucose was purchased from Merck & Co., Inc., Rahway, N.J., glycerol from Fisher Scientific Co., Pittsburgh, Pa., and all other carbon sources from Pfanstiehl Chemical Co., Waukegan, Ill. Amino acids, shikimic acid, and sugar phosphates were either Sigma Chemical Co. (St. Louis, Mo.) or Calbiochem (Los Angeles, Calif.) products. Ribulose-1, 5-diphosphate and ribulose-*o*-phenylhydrazone were gifts from B. L. Horecker. *p*-Aminobenzoic acid was a product of Mann Research Laboratories, New York, N.Y. *p*-Hydroxybenzoic acid was a gift from B. D. Davis. Auxiliary enzymes used in assays were purchased from Boehringer Mannheim Corp.

D-Erythrose-4-phosphate was prepared as follows. A 25-mg amount of D-erythrose-4-phosphate dimethylacetal-dicyclohexylammonium salt (Calbiochem) was dissolved in 1.3 ml of water. The solution was equilibrated with 0.5 ml of water-washed Dowex 50-H^+ and filtered, and the resin was rinsed with 0.5 ml of water. The filtrate was incubated at 37 C for 18 hr and then neutralized with 1 N $NaHCO_3$. This preparation was stored at 2 C and was used for 2 weeks.

Hydroxypyruvate was synthesized from bromopyruvic acid (Calbiochem) by the method of Sprinson and Chargaff (15); the preparation was stored at 2 C and was used for two days.

Enzyme assays. Extracts for transketolase and transaldolase assays were prepared from stationary-phase cultures (250 ml) in minimal medium containing glucose and the aromatic supplement. For other assays extracts were prepared from stationary-phase broth cultures (250 ml). All procedures up to assay were done at 2 C. The cells were harvested by centrifugation, washed with 0.9% NaCl, and suspended in 2 ml of buffer (*p*H 7.8) containing 0.01 M tris (hydroxymethyl)aminomethane (Tris)-hydrochloride, 0.01 M $MgCl_2$, and 0.001 M dithiothreitol. The suspensions were treated for 2 min with an ultrasonicator (Measuring & Scientific Equipment, Ltd., London, England), and then centrifuged at 17,000 × *g* for 30 min. The supernatant fraction was assayed for pentose phosphate isomerase, phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, gluconate-6-phosphate dehydrogenase, and transketolase (assay 3).

For transaldolase and the other transketolase assays, the above supernatant fraction was first centrifuged at 165,000 × *g* for 130 min to pellet particulate reduced nicotinamide adenine dinucleotide (NADH) oxidase activity. Details of most of the enzyme assays are in the legend to Table 2. Pentose phosphate isomerase was assayed by the cysteine-carbazole procedure of Axelrod (2). Color was read at 520 nm. D-Ribulose-1, 5-diphosphate was used as a standard. Its ribulose content was determined by an orcinol reaction (1) by using as a standard pure L-ribulose-*o*-phenylhydrazone which has the same specific absorbance as ribulose-1, 5-diphosphate in this reaction (B. L. Horecker, *personal communication*).

Protein was measured by the Folin method (11) using bovine serum albumin corrected for moisture content as a standard.

Organisms and media. *E. coli* strain K-10, the HfrC derivative of K-12, was the parent strain. It is a *str-s* prototroph. Hfr derivatives KL16 and KL98 (9) are also *str-s* prototrophs and were obtained from B. Low. Origins and direction of injection of these males, as well as positions of other markers used, are shown in Fig. 1. Strain AB2826 (F^-, *aroB*$^-$; 12) was from L. Gorini, AT1359 (F^-, *aroD*$^-$, *arg*$^-$, *gal*$^-$, *his*$^-$, *lac*$^-$, *pro*$^-$, *str-s*; 16) and AT713 (F^-, *argA8*, *cysC39*, *lysA10*; 17) from A. L. Taylor, I7006 (F^-, *aroG*$^-$, *str-s*) and X'121 (F^-, *gal*$^-$, *his*$^-$, *pyrD*$^-$, *str-r*, *trp*$^-$, *tyrA*$^-$, *xyl*$^-$) from J. R. Beckwith, and KL F16/KL110 (F', *thy*$^+$/*argG*$^-$, *his*$^-$, *leu*$^-$, *metB*$^-$, *recA1*, *thy*$^-$)

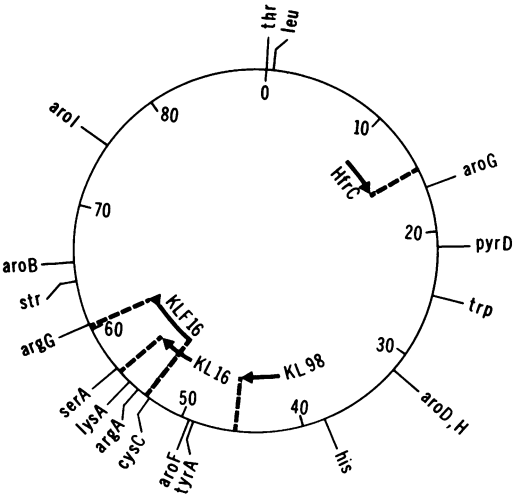

FIG. 1. *Genetic map of E. coli, adapted from Taylor and Trotter (17), showing origins of male strains and positions of some genes referred to in this paper. Positions of aro genes are according to Gibson and Pittard (5).*

from B. Low. Minimal medium 63 (14) was always supplemented with thiamine-hydrochloride (1 μg/ml) and a carbon source (4 mg/ml). The aromatic amino acid supplement was L-phenylalanine, L-tryptophan, L-tyrosine (each 25 μg/ml), and *p*-aminobenzoic acid and *p*-hydroxybenzoic acid (each 1 nmole/ml). Shikimic acid (25 μg/ml) was used, where indicated, instead of the aromatic supplement. Broth was medium 63 with 1% tryptic digest (BBL) and 0.4% yeast extract (Difco). Plates also contained 2% agar. Soft agar tops were 0.7% agar in medium 63. All growth incubations were aerobic and at 37 C.

Genetic techniques. In Hfr × F^- crosses, overnight cultures were diluted 1:10 in fresh broth and incubated 1 hr. The cultures were then mixed to give Hfr:F^- ratios of approximately 1:5 in a final volume of 2.5 ml, half of which was always fresh broth. Mating mixtures were incubated in 125-ml flasks without shaking. Interruption before plating was by 1 min of agitation of 2-ml portions in tubes (22 mm) with a SP Deluxe Mixer (Scientific Products, Evanston, Ill.). Dilutions in medium 63 were spread on selective plates usually containing streptomycin sulfate (100 μg/ml) for counter-selection of the males. F′ × F^- crosses were like Hfr × F^- crosses, except that the ratio of F′:F^- was about 1:1. In interrupted matings, the mating mixtures were diluted 1:100 in fresh broth after 3 min, and samples were taken at 3-min intervals and interrupted (as above) before plating. Phage P1 transduction was as previously described (3).

Recombinants were scored by patching to the selective plate and replicating, usually after single colony reisolation. The transketolase character was scored according to ability to grow on L-arabinose as sole carbon source. [For this, it is necessary that the strains do not contain a lesion in the specific arabinose pathway.]

RESULTS

Isolation of transketolase mutants. Figure 2 shows the nonoxidative pathway of pentose metabolism. It is generally believed that this pathway has three functions. (i) It is a degradative pathway for pentoses. Figure 3 shows how *E. coli* converts D-xylose, L-arabinose, and D-ribose to D-xylulose-5-phosphate, which is further metabolized by the common pathway. (ii) It produces an essential precursor, D-erythrose-4-phosphate, for the synthesis of the aromatic amino acids and vitamins (Fig. 4). (iii) It might catalyze the synthesis of pentose phosphate from glycolytic intermediates since all the reactions are reversible. Radioactive labeling experiments with *E. coli* have been interpreted (13) as showing that ribose originates from both the oxidative pentose phosphate pathway (Fig. 5) and the nonoxidative pathway. Accordingly, a mutant lacking transketolase ought to require products of the aromatic pathway and be unable to grow on any pentose. It should not require ribose since this could be made by the oxidative pentose phosphate pathway. Mutants of this phenotype were isolated as follows. A 0.1-ml amount of *E. coli* strain K-10, grown to stationary phase in minimal medium 63 containing glucose as sole carbon source, was added to 0.9 ml of medium 63 containing 4% ethyl methane sulfonate at 37 C (10). After 20 min (50% survival), the treated cells were recovered by centrifugation and grown in 6 ml of

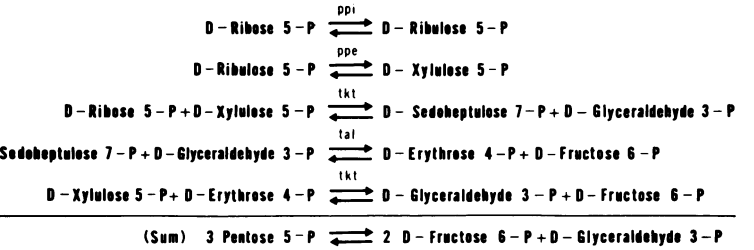

FIG. 2. *The nonoxidative pentose phosphate pathway. Gene abbreviations are ppi, pentose phosphate isomerase, ppe, pentose phosphate epimerase, tkt, transketolase, and tal, transaldolase.*

glucose minimal medium supplemented with aromatic amino acids. A subculture of these cells was inoculated to minimal medium containing 0.2% L-arabinose, 0.2% D-xylose, and aromatic amino acids. In this medium, mutants affected in one of the specific pentose degradation pathways (Fig. 3) or in the aromatic pathway after erythrose-4-phosphate (Fig. 4) ought to grow, whereas mutants in the nonoxidative pentose phosphate pathway should not. In early log phase (6), penicillin G (2,000 units/ml) was added. Gross lysis was observed after about 3 hr, and the survivors (1%) were plated on minimal glucose medium supplemented with aromatic amino acids. After 48 hr at 37 C, the colonies were screened for an aromatic requirement (on minimal plates with glucose) and for pentose utilization (on minimal plates containing a mixture of 0.2% L-arabinose, 0.2% D-xylose, and the aromatic supplement). Of 1,000 clones thus screened, three showed the desired phenotype of pentose negativity and an aromatic requirement. Two of these mutants, BJ501 and BJ502, were derived from the same experiment (but have slightly different characteristics). The third mutant, BJ503, was obtained in another experiment.

Growth characteristics of the mutants. Table 1 shows that BJ502 and BJ503 failed to grow on arabinose, xylose, or ribose, even in the presence of shikimic acid. On glucose and other carbon sources, normal growth was seen only with an aromatic supplement or shikimic acid included in the medium; however, BJ503 was notably leaky in this respect, and even BJ502 formed small colonies on unsupplemented plates after prolonged incubation. The third mutant, BJ501 (not shown), was similar to BJ503.

Arabinose-positive revertants of all three mutants and aromatic-independent revertants of the least leaky mutant (BJ502) were selected (*see* legend to Table 1). The arabinose revertants grew normally on xylose and no longer had an aromatic requirement (Table 1). The aromatic revertant of BJ502 likewise grew normally on both pentoses. Thus, the mutants contain single gene mutations affecting both pentose utilization and aromatic biosynthesis.

Table 1 also shows that *E. coli* strains carrying known mutations (*aroB*, *D*, and *G*) in the early aromatic pathway (Fig. 4) differ from the mutants described here for they grew on arabinose and on ribose when supplemented with shikimic acid.

Enzyme deficiency. The loss of ability to grow on several pentoses might be caused by a lesion in any step of the nonoxidative pentose phosphate pathway. However, the simultaneous requirement for an aromatic supplement is consistent

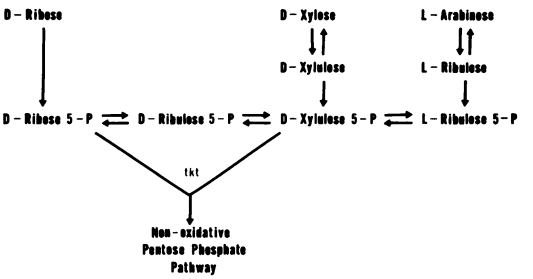

FIG. 3. *Specific pentose pathways in E. coli.*

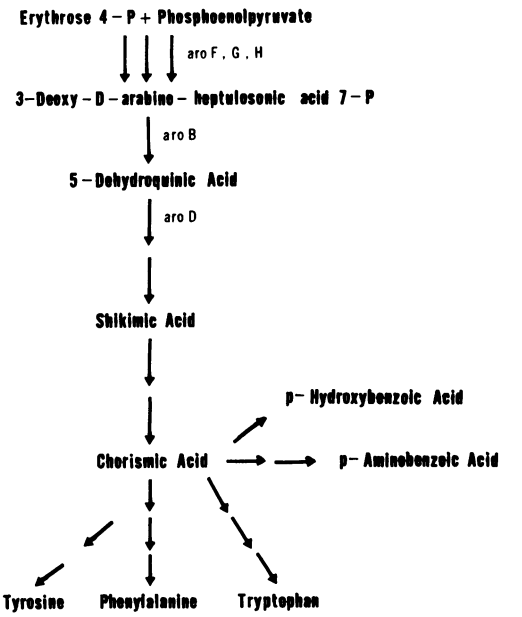

FIG. 4. *Aromatic biosynthesis in E. coli (adapted from Gibson and Pittard; 5).*

FIG. 5. *The oxidative branch of the hexose monophosphate shunt.*

only with lack of transketolase. Table 2 shows that the parent has transketolase activity in vitro, as assayed in each of four ways; the mutants have, at most, marginal activities of this enzyme, and the revertants have the parental level. Table 2 also shows that two other enzymes of the nonoxidative pentose pathway (transaldolase and pentose phosphate isomerase), two enzymes of the oxidative pentose phosphate pathway (glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenase), and an enzyme of glycolysis (phosphoglucose isomerase) all occur at normal levels in the mutants.

TABLE 1. *Growth of mutants*[a]

Minimal medium	K-10 (parent)	Strains							
		BJ502 (mutant)	BJ520 (arabinose revertant[b] of BJ502)	BJ523 (aromatic revertant of BJ502)	BJ503 (mutant)	BJ530 (arabinose revertant of BJ503)	AB 2826 (*aroB*⁻)	AT1359[c] (*aroD*⁻)	I7006[d] (*aroG*⁻)
Glucose	>2.0	0.0	>2.0	>2.0	0.8	>2.0	0.0	0.0	1.2
Glucose + SA[e]	>2.0	>2.0	>2.0	>2.0	>2.0	>2.0	0.8	0.9	1.2
Glucose + AROS[f]	>2.0	>2.0	>2.0	>2.0	>2.0	>2.0	ND[g]	ND	ND
L-Arabinose	1.7	0.0	2.0	>2.0	0.0	1.5	0.0	0.0	1.0
L-Arabinose + SA	1.5	0.0	2.0	>2.0	0.0	1.2	0.9	0.7	1.0
Xylose	1.5	0.0	1.7	1.8	0.0	1.0	0.0	0.0	1.0
Xylose + SA	1.5	0.0	2.0	2.0	0.0	1.3	0.8	0.0	1.0
Ribose	0.9	0.0	ND	ND	0.0	ND	0.0	0.0	ND
Ribose + SA	1.0	0.0	0.9	0.9	0.0	0.9	0.5	0.4	ND
Mannitol	2.0	0.0	1.4	1.8	1.1	1.0	ND	ND	ND
Mannitol + SA	1.7	>2.0	>2.0	>2.0	1.5	1.6	ND	ND	ND
Glycerol	1.0	0.0	1.0	0.8	0.2	1.0	ND	ND	ND
Glycerol + SA	0.9	0.4	0.9	1.0	0.8	1.0	ND	ND	ND
Gluconate	>2.0	0.2	>2.0	>2.0	1.0	>2.0	ND	ND	ND
Gluconate + SA	>2.0	0.4	>2.0	>2.0	1.5	>2.0	ND	ND	ND

[a] The various strains were grown to stationary phase in broth, and 0.1-ml portions of 10^{-6} dilutions in 63 medium were spread on each of the plates specified. All sugars, with the exception of L-arabinose, were of the D configuration. After 48 hr of incubation at 37 C, average colony sizes were estimated and are reported in millimeters.

[b] Revertants were selected as follows. The mutants were grown to stationary phase in 5 ml of broth, washed, and suspended in 1 ml of 63 medium, and 0.1-ml portions were spread on glucose minimal plates (for aromatic-independent revertants), and on arabinose plates supplemented with aros (for arabinose-positive revertants).

[c] Plates for AT1359 also contained L-arginine, L-histidine, and L-proline, each 50 μg/ml.

[d] This strain does not require an aromatic supplement because it lacks only one of the isozymes for the first reaction (*see* Fig. 4).

[e] Shikimic acid.

[f] Aromatic amino acid supplement.

[g] Not done.

Genetic mapping. In Fig. 2, we propose genetic abbreviations for the enzymes of the nonoxidative pentose phosphate pathway. Accordingly, strains BJ501, 502, 503 are derivatives of strain K-10 (HfrC) carrying the *tkt-1*, *tkt-2*, and *tkt-3* mutations, respectively. To map this locus, first a female strain carrying a *tkt* mutation was selected. BJ502 (HfrC, *str-s*, *tkt-2*) was mated for 3 hr with X'121 (F^-, *his*⁻, *pyrD*⁻, *str-r*, *trp*⁻, *tyrA*⁻) and streptomycin-resistant recombinants which had inherited one of the wild-type nutritional alleles selected on appropriately supplemented minimal glucose plates (also containing shikimic acid). Rare recombinants among them (2/100 of the *his*⁺, 2/100 of *pyrD*⁺, and 0/100 of the *tyr*⁺ and *trp*⁺) were found, both by plate scoring and enzyme assay, to have also inherited *tkt*⁻. One such recombinant, selected as *pyrD*⁺, strain BJ562 (F^-, *str-r*, *tkt-2*, *trp*⁻, *tyrA*⁻), was used in further experiments. Several Hfr derivatives (*see* Fig. 1) were mated with BJ562 and *tkt*⁺, *str-r* recombinants selected on arabinose plates. Such recombinants appeared in 30-min matings with KL16, but not with K-10 or KL98. In an interrupted mating (Fig. 6) between KL16 and BJ562, the gene order, according to times of entry, was origin KL16-*tkt-tyr-trp*. This order was confirmed by the results of scoring the selected recombinants: early *tkt*⁺ recombinants had not inherited *tyr*⁺, whereas about 70% of *tyr*⁺ recombinants were *tkt*⁺, regardless of time of selection.

In a 30-min F-duction between KLF16/KL110 (F', *thy*⁺) and BJ562 (*tkt-2*, *tyrA*⁻), *tkt*⁺ recombinants appeared, but *tyr*⁺ recombinants did not. [Counterselection of the males in this cross was by omission of methionine, histidine, and arginine.] Thus *tkt* must be on the KLF16 episome. Therefore, *tkt* should be between the origin of KL16 and the end of KLF16 (*see* Fig. 1). KLF16 is known (B. Low, *personal communication*) to carry *thy* but not *recA*. We find, crossing

TABLE 2. *Specific activity of enzymes*[a]

Enzyme	Strain					
	K-10 (parent)	BJ502 (mutant)	BJ520 (arabinose revertant of BJ502)	BJ523 (aromatic revertant of BJ502)	BJ503 (mutant)	BJ530 (arabinose revertant of BJ503)
Transketolase (assay 1)	148	3	101	ND	3	148
Transketolase (assay 2a)	175	2	ND[b]	ND	ND	159
Transketolase (assay 2b)	120	2	49	32	ND	51
Transketolase (assay 3)	13	0	14	12	0.5	17
Pentose phosphate isomerase	3,200	4,800	ND	ND	3,900	ND
Transaldolase	240	150	ND	ND	219	ND
Glucose-6-phosphate dehydrogenase	232	223	ND	ND	134	ND
Gluconate-6-phosphate dehydrogenase	134	131	ND	ND	89	ND
Phosphoglucose isomerase	950	516	ND	ND	612	ND

[a] Values are expressed as nanomoles per minute per milligram of protein. Assay mixtures contained 0.05 M Tris-hydrochloride and 0.01 M $MgCl_2$, *p*H 7.6, in a final volume of 1 ml. Transketolase assay 1 measured glyceraldehyde-3-P formation in the reaction ribose-5-P + xylulose-5-P $\rightleftarrows$ sedoheptulose-7-P + glyceraldehyde-3-P. The mixture contained 0.5 mM ribose-5-P, 0.1 mM NADH, and 10 μg of a mixture of α-glycerophosphate dehydrogenase and triose phosphate isomerase (Boehringer Mannheim Corp.). Note that the formation of one of the substrates, xylulose-5-P, depends on the presence of pentose phosphate isomerase and pentose phosphate epimerase in the extract. Transketolase assay 2a contained assay 1 ingredients and also 0.3 mM D-erythrose-4-P so as to also follow the reaction erythrose-4-P so as to also follow the reaction erythrose-4-P + xylulose-5-P $\rightleftarrows$ fructose-6-P + glyceraldehyde-3-P. Transketolase assay 2b measured fructose-6-P. Transketolase assay 2b measured fructose-6-P formation in the above reaction. The mixture contained 0.5 mM ribose-5-P, 0.3 mM erythrose-4-P, 0.2 mM nicotinamide adenine dinucleotide phosphate (NADP), 4 μg of phosphoglucose isomerase, and 2 μg of glucose-6-P dehydrogenase (Boehringer Mannheim Corp.). Thiamine pyrophosphate, a cofactor of transketolase, did not, at 10^{-5} or 10^{-4} M, alter observed activities in parent or mutants. Transketolase assay 3 measured fructose-6-P formation in the reaction erythrose-4-P + hydroxypyruvate $\rightleftarrows$ fructose-6-P + CO_2 (a reaction known to be catalyzed by transketolase from other sources; 7). This was done as a two-step assay because of reduced NADP-dependent hydroxypyruvate reductase activity in extracts. The primary incubation mixture contained 1 mM hydroxypyruvate, 0.4 mM erythrose-4-P, and approximately 200 μg of extract protein. After 15 min at 25 C, the reaction was stopped by boiling for 1 min in a boiling bath; the mixture was then centrifuged and the supernatant fraction was assayed spectrophotometrically for fructose-6-P. Transaldolase assay contained 0.3 mM erythrose-4-P, 0.4 mM fructose-6-P, 0.1 mM NADH, and 10 μg of the α-glycerophosphate dehydrogenase-triose phosphate isomerase mixture. The phosphoglucose isomerase assay contained 0.4 mM fructose-6-P, 0.2 mM NADP, and 2 μg of glucose-6-phosphate dehydrogenase. The glucose-6-P dehydrogenase assay contained 0.5 mM glucose-6-P and 0.2 mM NADP, and the gluconate-6-P dehydrogenase assay 1.0 mM gluconate-6-P and 0.2 mM NADP. Preparation of extracts is described in Materials and Methods. All reactions (except transketolase 3) were initiated by addition of 50 to 100 μg of extract protein, and oxidation of NADH or reduction of NADP was followed at 340 nm in a Gilford model 2000 spectrophotometer with the cell chamber at 25 C. For pentose phosphate isomerase assay, see Materials and Methods.

[b] Not done.

KLF16/KL110 with AT713 (*argA*$^-$, *cysC*$^-$, *lysA*$^-$), that this episome transfers *argA* but not *cysC*. KL16 transfers *serA* as a late marker (9). Accordingly, *tkt* should lie between *serA* and *cysC* (e.g., 53 to 56.5 min on the map of Taylor and Trotter; 17).

Nonetheless, the assignment of the transketolase gene to a position between *serA* and *cysC* must be regarded as tentative, for we have been unable to demonstrate cotransduction of *tkt* with *serA*, *lysA*, *argA*, or *cysC*, even in experiments which confirmed the reported (17) cotransduction frequencies for the latter three markers.

DISCUSSION

The rationale for the isolation of transketolase mutants was that if *E. coli* contained a single transketolase and this enzyme were necessary for both pentose degradation and aromatic biosynthesis, then a mutant lacking transketolase should fail to grow on pentose and also should require an aromatic supplement. The successful isolation of such mutants, whose revertants are

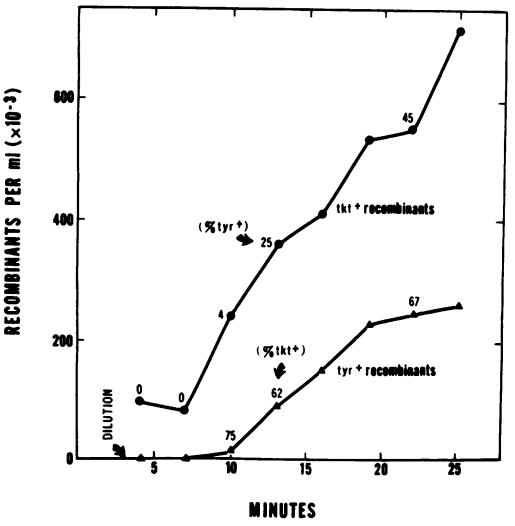

FIG. 6. *Ordering of tkt and tyrA in an interrupted mating. Tkt⁺, tyr⁺, and trp⁺ recombinants were selected in an interrupted mating between KL16 and BJ502 (tkt⁻, tyrA⁻, trp⁻). Selective plates for tkt⁺ contained arabinose, tyrosine, tryptophan, and shikimic acid; for tyr⁺, glucose, tryptophan, and shikimic acid; for trp⁺, glucose, tyrosine, and shikimic acid. Counter-selection was with streptomycin. Trp⁺ recombinants appeared only after 30 min. Tkt⁺ and tyr⁺ recombinants, for each time of interruption, were scored for inheritance of tyr and tkt, respectively. These data are shown in the figures over each of the curves.*

normal in both respects, lends support to the original presumption.

Besides showing that *E. coli* has a single transketolase, these results also are in accord with the usual assumption that this enzyme catalyzes two different reactions in the cell, shown in lines 3 and 5 of Fig. 2. For several organisms, this is assumed from the properties of the purified enzyme (7). The *E. coli* enzyme has not yet been purified, but the fact that the mutants lack transketolase activity according to the several different assays (Table 2) makes it likely that the *E. coli* enzyme, too, is multifunctional in this sense.

Another assumption which might be made about the role of transketolase is that it not be required for ribose synthesis. Again, our results support this assumption, for the mutants do not require ribose. We are now determining whether ribose is derived in these mutants exclusively by the oxidative branch of the shunt.

Aside from their pentose negativity, transketolase mutants might be regarded as a new class of multiple aromatic requirers. They seem to map elsewhere from previously described mutants of this type (*aro* markers, Fig. 1) which include mutants for each step between erythrose-4-phosphate and shikimic acid (Fig. 4), three of which we showed (Table 2) to be pentose-positive. It is not clear why (assuming they were not routinely screened for growth on pentoses) *tkt* mutants were not found in earlier searches for multiple aromatic requirers. We wonder whether this might be related to the fact that all three of our isolates, considered as aromatic mutants, are somewhat leaky. One might speculate that were erythrose-4-phosphate or sedoheptulose 7-phosphate precursors of some compound (other than an aromatic) essential in *E. coli*, then the selection procedure would not have revealed non-leaky mutants. To clarify this, it would be useful to have deletion or nonsense mutants in *tkt*.

Whatever causes the "leakiness"—residual activity of a single mutated enzyme, or a second transketolase of low activity—it is not surprising to see the leakiness reflected in the aromatic requirement but not in pentose growth, since the requirement of transketolase activity for aromatic biosynthesis is much less than for pentose degradation, for which it is a major catabolic enzyme. Furthermore, even an incomplete transketolase deficiency might cause accumulation of some compounds (e.g., pentose phosphates) inhibitory to growth. Such accumulation should be greater in cells given pentoses than in cells given other carbon sources, such as glucose or glycerol, whose degradations do not use transketolase as an obligatory step; this is under study.

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