

Regulator Gene Controlling Enzymes Concerned in Tyrosine Biosynthesis in *Escherichia coli*

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Mutants of *Escherichia coli* K-12 have been isolated in which several enzymes concerned with tyrosine biosynthesis are derepressed. These mutants were obtained from a parent strain possessing only a single 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthetase isoenzyme, DAHP synthetase (tyr), by selecting for resistance to the tyrosine analogue, 4-aminophenylalanine. The mutation responsible for this derepression has been mapped and the gene, which is not closely linked to *aroF* and *tyrA*, has been designated *tyrR*.

The first reaction specific to the synthesis of the aromatic amino acids and vitamins in bacterial cells is the condensation of erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP) to 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP). In *Escherichia coli*, this conversion has been shown to be carried out by three DAHP synthetase isoenzymes, the activity and formation of which are controlled by the aromatic amino acid end products (5, 8, 14, 16, 17).

In some cases, repression of enzyme synthesis and inhibition of enzyme activity can be caused not only by the normal amino acid end product of a biosynthetic sequence but also by analogues of that particular amino acid. As a result, these analogues may inhibit the growth of bacterial cells if added to the culture medium. In other cases, certain analogues have been described which can cause repression of enzyme synthesis but which have little or no ability to act as feedback inhibitors. The tyrosine analogue, 4-aminophenylalanine (APA), is such a compound; it is as efficient as tyrosine in repressing the formation of the enzyme DAHP synthetase (tyr) in *E. coli*, but it is an extremely weak feedback inhibitor of this enzyme (15).

It is our purpose to describe the isolation and characteristics of mutants of *E. coli* which are derepressed for certain enzymes involved in tyrosine biosynthesis. These mutants have been derived from a strain of *E. coli* containing only DAHP synthetase (tyr) by selecting for growth in the presence of 4-aminophenylalanine.

MATERIALS AND METHODS

Organisms. Strains used in this work are all derivatives of *E. coli* K-12, and are shown in Table 1.

Media and culture methods. Media and culture methods used in this work were described by Adelberg and Burns (1).

Buffers. Sodium phosphate buffers used were prepared by the method of Dawson and Elliott (7).

Chemicals. Chemicals used were obtained commercially and not further purified. Reagent grade Selectacel (DEAE cellulose), obtained from the Brown Co., Berlin, N.H., was used for chromatography. D-Erythrose-4-phosphate dimethylacetal dicyclohexylammonium salt (A grade) was obtained from Calbiochem, Los Angeles, Calif. Free erythrose-4-phosphate was prepared by the method of Ballou, Fischer, and MacDonald (3).

Mating procedures. Conditions under which the conjugation experiments were carried out have been described (11).

Transduction tests. Transductions involving phage P1 were carried out as previously described (11).

Isolation of mutants. Conditions under which cells were treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) were those described by Adelberg, Mandel, and Chen (2). After a 2-hr period of phenotypic expression in L-broth, the cells were plated to minimal medium supplemented with 10^{-4} M 4-aminophenylalanine and incubated at 37 C for 2 days.

Growth of cells and preparation of cell-free extracts. Cells were grown in either minimal medium or minimal medium supplemented with 5×10^{-4} M DL-phenylalanine, 2×10^{-4} M DL-tryptophan, 10^{-3} M L-tyrosine, and 10^{-6} M shikimic acid, and harvested at the mid-exponential phase of growth. The cells were washed with chilled 0.9% NaCl, suspended in 0.1 M sodium phosphate buffer (pH 7.6), and smashed by being forced through a French press at a pressure of 20,000 psi. Cell-free extracts, obtained by centrifugation at $21,600 \times g$ for 20 min, were dialyzed for 2 hr at 2 to 4 C against 0.01 M sodium phosphate buffer (pH 7.0).

Growth of cells for growth rate experiments. The growth of cells in T-tubes shaken at 37 C was fol-

TABLE 1. Description of strains used

Strain	Genotype ^a									Sex
	<i>aroF</i>	<i>aroG</i>	<i>aroH</i>	<i>tyrR</i>	<i>thi</i>	<i>his</i>	<i>proA</i>	<i>argE</i>	<i>ilv</i>	
AB3253 ^b	+	365 ^c	367	+	1	4	2	3	7	F ⁻
AB3271 ^d	+	365	367	352	1	4	2	3	7	F ⁻
AB3272	+	365	367	353	1	4	2	3	7	F ⁻
AB3273	+	365	367	354	1	4	2	3	7	F ⁻
AB3274	+	365	367	355	1	4	2	3	7	F ⁻
AB3248	363	365	367	+	1	4	2	3	7	F ⁻
AB3280	+	365	367	+	1	+	2	3	7	F ⁻
AB3276	+	365	367	352	1	+	2	3	7	F ₁₈
AB259	+	+	+	+	1	+	+	+	+	Hfr
AB3278	+	365	367	352	1	4	+	+	+	Hfr
AB2825	+	+	+	+	1	4	2	3	7	F ⁻

^a The following symbols stand for structural genes concerned with various biosynthetic pathways: *aro*, the biosynthesis of chorismic acid; *tyr*, tyrosine biosynthesis; *thi*, thiamine biosynthesis; *his*, histidine biosynthesis; *pro*, proline biosynthesis; *arg*, arginine biosynthesis; *ilv*, isoleucine and valine biosynthesis. *aroF* is the structural gene for DAHP synthetase (*tyr*); *aroG* is the structural gene for DAHP synthetase (*phe*); *aroH* is the structural gene for DAHP synthetase (*trp*).

^b Strain AB3253 was prepared by introducing the wild-type *aroF* allele into strain AB3248 (*aroF*363, *aroG*365, *aroH*367) by conjugation. It was selected by its ability to grow on minimal medium but not on minimal medium supplemented with tyrosine.

^c Numbers refer to allele numbers allotted to mutant strains in this laboratory.

^d The possibility that strains AB3271, AB3272, AB3273, and AB3274 are siblings has not yet been excluded.

lowed with time by measuring the optical density of the cell suspension using a Spekker photoelectric absorptiometer (Hilger and Watts, Ltd., London, England). Minimal medium unsupplemented, or supplemented as described in the text, was used.

Column chromatography of enzymes. Chromatography was carried out using DEAE cellulose by the method previously described (16), except that neither ethylenediaminetetraacetic acid nor mercaptoethanol was included in the buffers.

Assay of DAHP synthetase. The method described by Doy and Brown (8) was used.

Protein estimation. Protein was estimated by the method of Lowry et al. (9).

Assay of chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase. The method described by Cotton and Gibson (6) was used for all of these assays.

Transaminase A assay. Transaminase A is the enzyme which catalyses the reversible conversion of 4-hydroxyphenylpyruvate to tyrosine (13). This activity was measured by estimating the conversion of tyrosine to 4-hydroxyphenylpyruvate. A 1.0 ml amount of reaction mixture contained 0.5 μ mole of tyrosine, 5 μ moles of α -ketoglutarate, 0.06 μ mole of pyridoxal-5-phosphate, 5 μ moles of $MgCl_2$, 50 μ moles of sodium phosphate buffer (pH 7.6), and an appropriate amount of cell-free extract. The reaction mixture was incubated at 37 C for 30 min, and then 3 ml of 1 M NaOH was added. The alkaline solution was incubated at 37 C for 30 min before the absorbance at 330 nm was measured. The molar extinction coefficient in alkali was taken as 24,300 at 330 nm.

Specific activities. The specific activity of each enzyme preparation is expressed as the number of units of 0.1 μ mole of substrate used or product formed per 20 min per mg of protein at 37 C.

RESULTS

The growth of wild-type *E. coli* is only slightly affected by the addition of APA or tyrosine to minimal medium at a final concentration of 10^{-4} M. On the other hand, the growth of AB3253, a mutant strain of *E. coli* which possesses only a single DAHP synthetase isoenzyme [DAHP synthetase (*tyr*)], is almost completely inhibited in minimal medium supplemented with either APA or tyrosine (10^{-4} M). These results are shown in Fig. 1 (a and b). Since APA is a very weak feedback inhibitor of DAHP synthetase (*tyr*), inhibition of the growth of strain AB3253 by APA (10^{-4} M) is almost certainly due entirely to the repression of the formation of DAHP synthetase (*tyr*).

Isolation of APA-resistant mutants. Strain AB3253 was treated with mutagen as described in Materials and Methods, and the survivors were plated to minimal medium containing APA (10^{-4} M). Sixty APA-resistant colonies were isolated and purified. These mutants were screened for the presence of mutations restoring activity to either DAHP synthetase (*phe*) or DAHP synthetase (*trp*), since restoration of either of

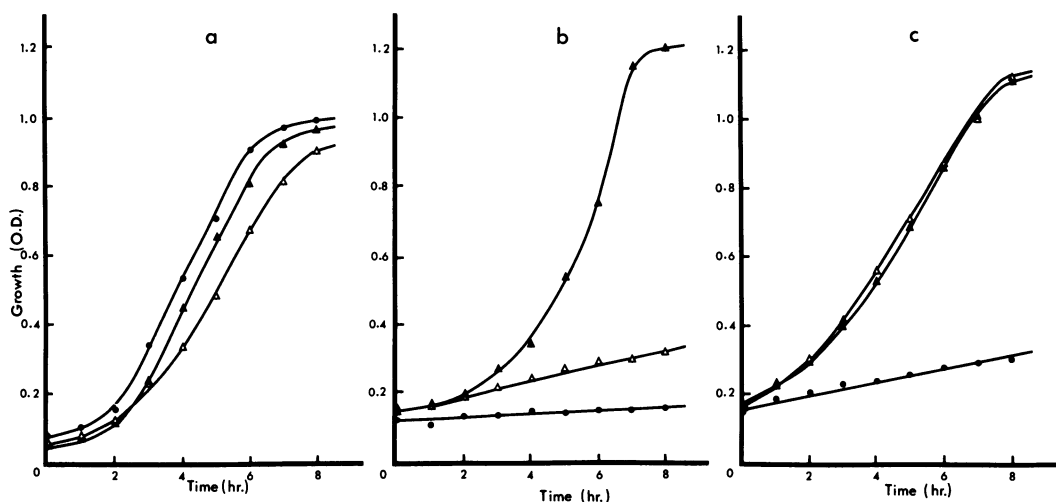


FIG. 1. Growth curves: (a) AB2825, a wild-type strain of *E. coli*; (b) AB3253, a mutant strain of *E. coli* possessing only DAHP synthetase (*tyr*); and (c) AB3271, an APA-resistant mutant derived from AB3253. Symbols: (▲), minimal medium; (△), minimal medium supplemented with 10^{-4} M APA; (●), minimal medium supplemented with 10^{-4} M L-tyrosine.

these activities would be expected to allow growth on APA. Fortunately, these isoenzymes are controlled by phenylalanine and tryptophan, respectively, and such revertants can readily be detected by simple nutritional tests. For example, a mutant strain which has regained functional activity for DAHP synthetase (*phe*) in addition to DAHP synthetase (*tyr*), will grow in minimal medium supplemented with tyrosine and tryptophan, but will give a very poor growth response in minimal medium supplemented with tyrosine and phenylalanine. In a similar fashion, revertants for DAHP synthetase (*trp*) will not grow in minimal medium supplemented with tyrosine and tryptophan but will grow in minimal medium supplemented with tyrosine and phenylalanine. When the 60 strains were tested for their ability to grow on minimal medium supplemented with either tyrosine and tryptophan or tyrosine and phenylalanine, 42 of the strains were able to grow on one of the two media, but not on both. These were presumed to be revertants that had regained functional DAHP synthetase (*phe*) or functional DAHP synthetase (*trp*) and were not retained. The remaining 18 strains grew slowly on both media and were kept for further study. An investigation of each of these strains indicated that they could be divided into four groups on the basis of the level of DAHP synthetase (*tyr*) produced by the cells when grown in the presence and in the absence of the aromatic amino acids. We intend to deal with only one of these groups, one in which DAHP synthetase (*tyr*) is produced constitutively. Four strains

comprise this group, AB3271, AB3272, AB3273, and AB3274.

Growth curves for APA-resistant mutants. Although these mutant strains are able to grow on minimal medium supplemented with APA (mean generation time, 130 min at 10^{-4} M), the addition of L-tyrosine (10^{-3} M) to minimal medium severely inhibits their growth rate (mean generation time, 9 hr). Growth curves of one of these strains, strain AB3271, is shown in Fig. 1c. It should be remembered that although tyrosine severely inhibits the growth rate, it does not completely inhibit the growth of these mutant strains as it does the parent strain AB3253 (Fig. 1b).

Inhibition by tyrosine of DAHP synthetase activity in APA-resistant mutants. An examination of dialyzed cell-free extracts prepared from strain AB3253 and each of the four mutant strains showed that the DAHP synthetase (*tyr*) made by AB3253 and by each of the mutants was equally sensitive to feedback inhibition by L-tyrosine, since the addition of L-tyrosine (10^{-4} M) to the reaction mixture caused approximately 95% inhibition of enzyme activity in each case. The observed changes in growth patterns for the mutant strains do not, therefore, reflect alterations to the feedback sensitivity of the enzyme DAHP synthetase (*tyr*).

Repression of DAHP synthetase (*tyr*) in mutant strains. Each of the four mutant strains and the parent strain AB3253 were grown in minimal medium and in minimal medium supplemented with L-tyrosine (10^{-3} M), DL-phenylalanine ($5 \times$

10^{-6} M), DL-tryptophan (2×10^{-4} M), and shikimic acid (10^{-6} M). Cells were harvested in mid-exponential phase, smashed in the French Press, dialyzed, and assayed for DAHP synthetase (tyr) activity. The results of these assays are shown in Table 2, where it can be seen that the level of DAHP synthetase (tyr) activity in the mutant strains was the same in extracts prepared from cells grown either in minimal medium or in medium supplemented with the aromatic amino acids. On the other hand, no DAHP synthetase (tyr) activity was detectable in extracts prepared from strain AB3253 grown in the presence of the aromatic amino acids. Table 2 also shows that the level of DAHP synthetase (tyr) activity in extracts prepared from cells grown in minimal medium was higher for the mutant strains than in the parent strain AB3253.

Repression of enzymes of the terminal pathway of tyrosine biosynthesis. There are two enzymes involved in the conversion of chorismate to tyrosine. The first enzyme has two activities, one concerned with the conversion of chorismate to prephenate and referred to as chorismate mutase T activity, and the other concerned with the conversion of prephenate to 4-hydroxyphenylpyruvate and referred to as prephenate dehydrogenase activity (6). The second enzyme, transaminase A, carries out the conversion of 4-hydroxyphenylpyruvate to tyrosine. These reactions are shown in Fig. 2. There is a second chorismate mutase activity, chorismate mutase P, which is concerned with phenylalanine biosynthesis (Fig. 2). Consequently, measurement of total chorismate mutase activity in cell-free extracts reflects the combined activity of chorismate mutase P and chorismate mutase T. Since both chorismate mutase P and prephenate dehy-

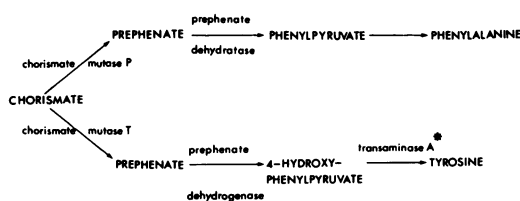


FIG. 2. Outline of reactions involved in the conversion of chorismate to phenylalanine and tyrosine. Transaminase A (*) also possesses a limited capacity to convert phenylpyruvate to phenylalanine (13).

dratase activity exist on a single protein, the level of prephenate dehydratase in cell-free extracts gives an indication of the level of chorismate mutase P in these same extracts.

In wild-type cells, tyrosine is able to repress the formation of transaminase A (13) and chorismate mutase T and its associated prephenate dehydrogenase. In order to test the repression of these enzymes in the mutant strains, cells were grown in minimal medium and in minimal medium supplemented with all aromatic end products. Cell-free extracts were prepared and assayed for chorismate mutase, prephenate dehydratase, and transaminase A activity (Table 3). Although the level of prephenate dehydratase does not vary significantly in extracts of parent and mutant strains, the total chorismate mutase activity is much higher in extracts prepared from the APA-resistant mutants than in extracts prepared from strain AB3253. This suggests that the increase in chorismate mutase activity in the mutants is due to an increase in the level of chorismate mutase T. Furthermore, the increased level of mutase activity is not decreased in extracts of the mutant strains when cells are grown in the presence of the aromatic end products. Table 3 also shows that the level of transaminase A activity in cell-free extracts prepared from both parent and mutants is the same when cells are grown in minimal medium. However, the transaminase A activity in extracts prepared from strain AB3253 when grown in medium supplemented with the aromatic amino acids is repressed threefold to fourfold compared to the activity in extracts prepared from the mutant strains when grown under identical conditions.

Separation of enzymes by chromatography on DEAE cellulose. To confirm the results obtained by assaying cell-free extracts, different enzymatic activities were assayed separately after chromatographic separation was achieved on DEAE cellulose. Cell-free extracts prepared from two mutant strains, AB3271 and AB3272, and from the parent strain AB3253 were chromatographed as described in Materials and Methods; they were

TABLE 2. Repressibility of DAHP synthetase (tyr) in parent and mutant strains

Strain	Specific activities of extracts prepared from cells grown in	
	Minimal medium	Minimal medium supplemented with aromatic amino acids and shikimic acid ^a
AB3253 (parent)	15	<0.1
AB3271	82	64.0
AB3272	126	82.0
AB3273	75	107.0
AB3274	176	150.0

^a Growth supplements were used in the following concentrations: 10^{-3} M L-tyrosine, 5×10^{-4} M DL-phenylalanine, 2×10^{-4} M DL-tryptophan, and 10^{-6} M shikimic acid.

TABLE 3. *Repressibility of chorismate mutase, prephenate dehydratase, and transaminase A in parent and mutant strains^a*

Strain	Cell-free extracts prepared from cells grown in	Specific activity of		
		Prephenate dehydratase	Chorismate mutase (P and T)	Transaminase A
AB3253 (parent)	Minimal medium	2.5	9.6	7.8
	Minimal medium plus aromatic amino acids plus shikimic acid ^b	2.0	2.2	2.3
AB3271	Minimal medium	1.9	34.0	7.5
	Minimal medium plus aromatic amino acids plus shikimic acid	1.7	22.0	6.7
AB3272	Minimal medium	1.5	26.5	9.5
	Minimal medium plus aromatic amino acids plus shikimic acid	1.3	41.0	9.5
AB3273	Minimal medium	2.9	42.0	4.0
	Minimal medium plus aromatic amino acids plus shikimic acid	2.3	42.0	6.7
AB3274	Minimal medium	2.2	40.0	7.6
	Minimal medium plus aromatic amino acids plus shikimic acid	1.4	38.0	7.0

^a Since prephenate dehydrogenase activity in cell-free extracts could not be determined by the usual fluorimetric estimation of $\text{NADH}^+\cdot\text{H}$ production, this activity was estimated as described in the text.

^b Growth supplements were used in the following concentrations: 10^{-3} M L-tyrosine, 5×10^{-4} M DL-phenylalanine, 2×10^{-4} M DL-tryptophan, and 10^{-6} M shikimic acid.

assayed for DAHP synthetase, chorismate mutase, prephenate dehydratase, and prephenate dehydrogenase activities. Since the results obtained with extracts from AB3271 and AB3272 were essentially the same, only the results using one of these extracts, AB3272, is included in the Figures.

Figure 3 shows the chromatographic pattern of DAHP synthetase activities in the parent

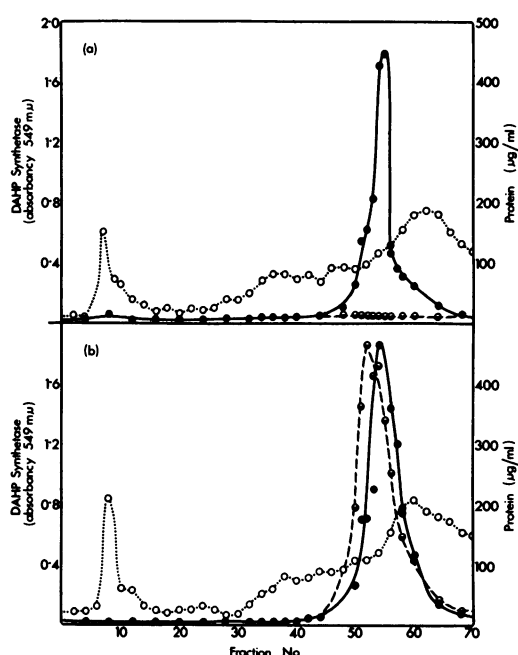


FIG. 3. DAHP synthetase (tyr) activity after chromatography on DEAE cellulose of cell-free extracts of strains (a) AB3253 and (b) AB3272. (In the case of AB3253, 0.4 ml of column fraction was used for the DAHP synthetase assay, whereas for AB3272 only 0.1-ml volumes were used.) Symbols: ●, cell-free extracts prepared from cells grown in minimal medium; ●, cells grown in minimal medium supplemented with the aromatic amino acids (see text); and ○, protein. Since the elution profile for protein was identical for both columns in Fig. 3(a) and in Fig. 3(b), only one protein elution pattern has been included in each case.

(AB3253) and mutant (AB3272) strains. In each case, this activity occurred as a single peak in fractions 48 to 52 and was completely inhibited (95%) by the presence of L-tyrosine (10^{-3} M) in the reaction mixture.

In the case of the parent strain, the formation of the enzyme was totally repressed by supplementing the culture medium with tyrosine, phenylalanine, and tryptophan. On the other hand, the level of DAHP synthetase activity in strain AB3272 was unaffected by the presence of the three aromatic amino acids in the culture medium.

Figure 4 shows the relative levels and the repressibility by the three aromatic amino acids of chorismate mutase P and T activities. It is evident in strain AB3272 that whereas the chorismate mutase P parallels the behavior of that in the parent strain, the level of chorismate mutase T activity shows a fourfold increase (peak fractions), and the formation of this activity is no

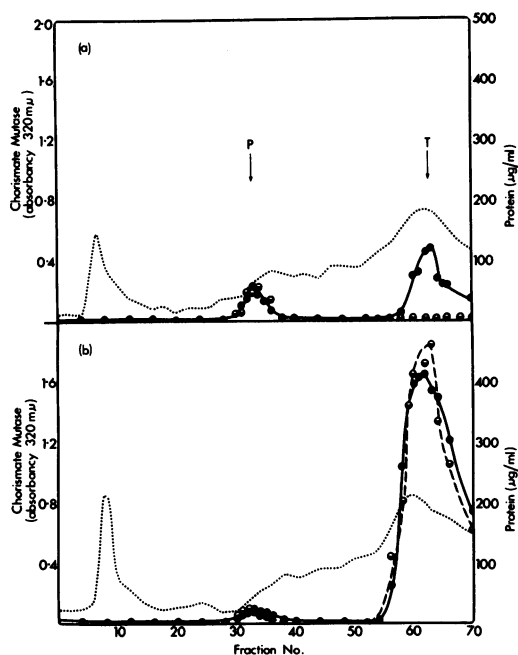


FIG. 4. Chorismate mutase activity after chromatography on DEAE cellulose of cell-free extracts of strains (a) AB3253 and (b) AB3272. Symbols: ●, cell-free extracts prepared from cells grown in minimal medium; and ○, cells grown in minimal medium supplemented with the three aromatic amino acids (see text). The protein curves (dotted lines) are identical to those in Fig. 3. P indicates chorismate mutase P activity; T indicates chorismate mutase T activity.

longer repressed by the inclusion of the three aromatic amino acids in the culture medium.

Figure 5 shows the pattern of enzymatic activity obtained when column fractions were assayed for prephenate dehydrogenase activity. As expected from the results obtained in the chorismate mutase assays, the prephenate dehydrogenase activity in the mutant strain AB3272 was no longer repressed by the addition of the three aromatic amino acids to the culture medium, whereas the enzymatic activity present in the parent strain showed, under the same conditions, a sixfold reduction.

Both the level and repressibility of prephenate dehydratase were the same for both parent and mutant strains (Fig. 6). This behavior was to be expected from the results of assays on chorismate mutase P, since both chorismate mutase P and prephenate dehydratase activities are associated with the same protein molecule.

Genetic analyses of mutant strains: transduction. The structural genes for two of the three enzymes that are derepressed in the mutant strains are cotransducible, and the location of

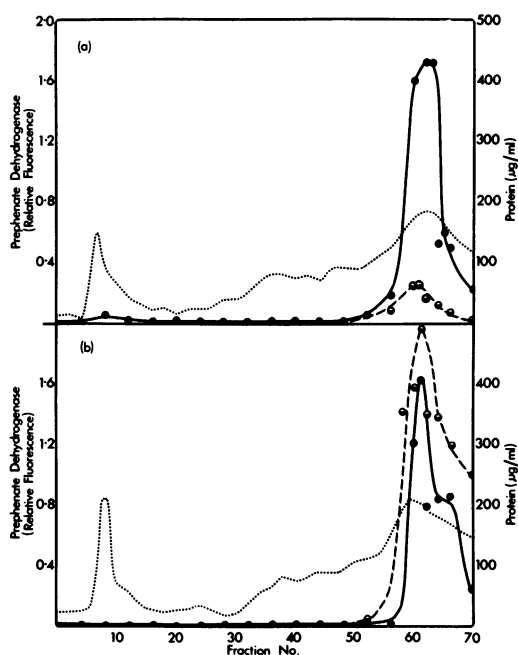


FIG. 5. Prephenate dehydrogenase activity after chromatography on DEAE cellulose of cell-free extracts of strains (a) AB3253 and (b) AB3272. Symbols: ●, cell-free extracts prepared from cells grown in minimal medium; and ○, cells grown in minimal medium supplemented with the aromatic amino acids (see text). The protein curves (dotted lines) are identical with those in Fig. 3.

the structural gene for the third enzyme, transaminase A, has not yet been determined. It seemed possible, therefore, that if these three genes constituted an operon, the mutation causing their derepression might be affecting a single operator locus which necessarily would be closely linked to these structural genes. This was tested in the following manner. Phage P1 was propagated on strains AB3271, AB3272, AB3273, and AB3274 and used to transduce *aroF*⁺ into the recipient AB3248, which possesses mutant alleles for the three DAHP synthetase isoenzymes and is unable to grow on minimal medium unless supplemented with the aromatic amino acids and vitamins. *AroF*⁺ transductants were selected by their ability to grow on minimal medium not supplemented with the aromatic amino acids and vitamins; 40 transductants from each cross were tested for their ability to grow on minimal medium supplemented with APA (10⁻⁴ M). Not one of the transductants grew, indicating that the mutations causing resistance to APA are not closely linked to the *aroF tyrA* genes.

Conjugation. One of the problems encountered

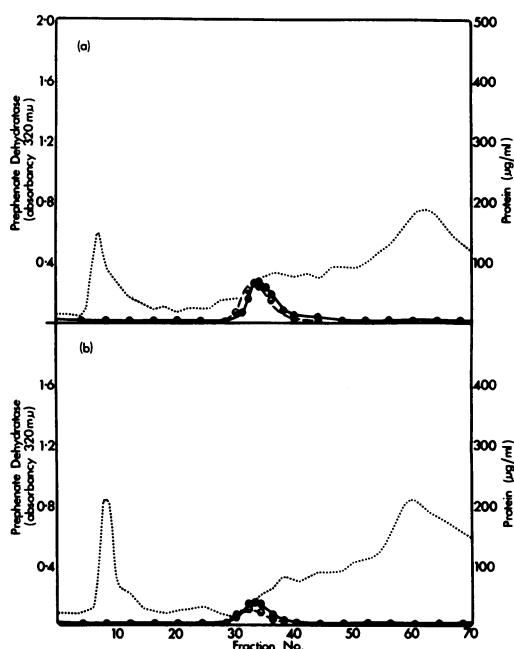


FIG. 6. Prephenate dehydratase activity after chromatography on DEAE cellulose of cell-free extracts of strains (a) AB3253 and (b) AB3272. Symbols: ●, cell-free extracts prepared from cells grown in minimal medium; and ○, cells grown in minimal medium supplemented with the aromatic amino acids (see text). The protein curves (dotted lines) are identical with those in Fig. 3.

in mapping these mutations depends on the fact that selection for the transfer of these mutations is made by selecting those recombinants that can grow on minimal medium containing APA. As was mentioned previously, the restoration of either DAHP synthetase (*phe*) or DAHP synthetase (*trp*) activity will also confer ability to grow on medium supplemented with APA. To carry out an unambiguous selection, therefore, both male and female strains must possess mutant alleles for both *aroH* and *aroG* genes, the structural genes for DAHP synthetase (*trp*) and DAHP synthetase (*phe*). Because of these limitations, this mapping has been carried out so far using only one mutant strain, AB3271. The mutation carried by this strain has been designated *tyr-352*.

In the first attempt to map *tyr-352*, an F' male strain was prepared by transferring the small F-merogenote [F₁₆ (10)] into an *his*⁺ recombinant of strain AB3271. The new strain, AB3276, is able to transfer chromosomal markers in the order *ilv*, *xyl*, *mal*, *his*, *pro*, *leu*.

Strain AB3276 was crossed with strain AB3253 in an uninterrupted cross and selection was made

for *his*⁺ and for *tyr-352* recombinants. A comparison of the numbers obtained indicated that *tyr-352* was probably in the region of the chromosome between *his* and *pro* and, consequently, too distant from the origin of AB3276 for time-of-entry experiments. Therefore, to obtain a more accurate estimation of the location of *tyr-352*, a new Hfr male was prepared by crossing Hfr AB259 (Hayes Hfr) with the recipient AB 3271 and by selecting a recombinant that had received and integrated the sex factor but which had retained the *aroH367*, *aroG365*, and *tyr-352* mutations of AB3271. This strain, AB3278, which transfers chromosomal markers in the same order as Hfr AB259 (i.e., *leu*, *proA*, *trp*, *his*, *ilv*, *sex factor*), was crossed with AB3280 (*his*⁺ transductant of AB3253). The mating was interrupted at 5-min intervals, as described in Materials and Methods, and selection was made for *pro*⁺ and for *pro*⁺ *tyr-352* recombinants. The double selection for *pro*⁺ and *tyr-352* was made to overcome the difficulty caused by the high frequency of revertants that are obtained in single *tyr-352* selection. Whereas the *pro*⁺ allele enters the recipient at 15 min, the *tyr-352* mutation does not begin to enter until approximately 34 min (Fig. 7). The gene in which this mutation occurs has been designated *tyrR*, and attempts are currently being made by transduction to confirm its exact chromosomal location. When this has been done, the location of mutations

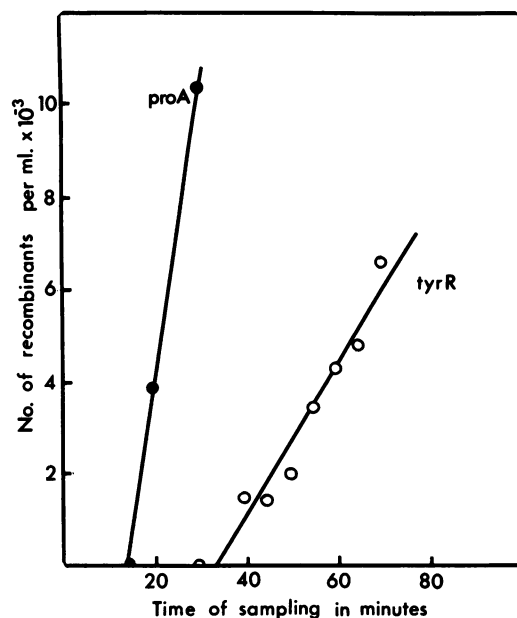


FIG. 7. Kinetics of zygote formation when the Hfr AB3278 is mated with the female AB3280.

tyr-353, *tyr-354*, and *tyr-355* will also be determined.

DISCUSSION

A mutation in a gene which has been designated *tyrR* can cause constitutive synthesis of at least three proteins concerned with the biosynthesis of tyrosine. These are DAHP synthetase (*tyr*), chorismate mutase T and its associated prephenate dehydrogenase activity, and transaminase A. The structural genes for two of these enzymes are situated at some distance from *tyrR* on the chromosome, whereas the structural gene for the third enzyme, transaminase A, has yet to be mapped.

Ravel, White, and Shive (12) suggested that tyrosine itself, rather than charged tyrosyl-transfer ribonucleic acid (tRNA), is the true corepressor for DAHP synthetase (*tyr*), because APA can repress the formation of this enzyme although it is not activated by the tyrosine-specific t-RNA synthetase. If this hypothesis is correct, the simplest explanation for the pleiotropic effects of the mutation in the *tyrR* gene is that this gene normally makes an aporepressor which can combine with tyrosine to repress the formation of the above-mentioned enzymes. In *tyrR*⁻ strains, this aporepressor is presumably nonfunctional. Partial diploid strains of *E. coli* are currently being prepared to examine the dominance or recessiveness of the *tyrR*⁻ mutations.

In tryptophan biosynthesis, the structural gene for DAHP synthetase (*trp*) and the genes of the tryptophan operon are situated at some distance from each other on the chromosome, but the expression of each is controlled by a mutation in a gene (*trpR*) which is not closely linked to either (4; J. Pittard and J. Camakaris, *in preparation*). In the case of tyrosine biosynthesis, the situation is very similar in that the expression of both the gene for DAHP synthetase (*tyr*), *aroF*, and the gene for chorismate mutase T and prephenate dehydrogenase, *tyrA*, is controlled by a mutation in a gene, *tyrR*. In this case, however, transduction tests have shown that *tyrA* and *aroF* are closely linked to each other (16), and further investigations are being carried out to determine whether these two genes constitute an operon.

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