Genetic Separability of the Chorismate Mutase and Prephenate Dehydrogenase Components of the *Escherichia coli* tyrA Gene Product

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Fragments of the tyrA gene of *Escherichia coli*, when suitably engineered, can express either the chorismate mutase activity or the prephenate dehydrogenase activity without the other.

The conversion of chorismic acid to tyrosine or phenylalanine involves one of two bifunctional enzymes in enteric bacteria: chorismate mutase-prephenate dehydrogenase, encoded by tyrA, is necessary for tyrosine synthesis; and chorismate mutase-prephenate dehydratase, encoded by pheA, is required for phenylalanine synthesis. Coincidentally, tyrA and pheA occur immediately adjacent to each other on the chromosome of *Escherichia coli*, although they do not constitute an operon and are not coregulated (8). Enzymological studies (6, 7, 9) of chorismate mutase-prephenate dehydrogenase have suggested that it has a single substrate-binding site which subjects chorismate to sequential enzymatic action. However, mutations in tyrA which result in the loss of prephenate dehydrogenase activity without the loss of chorismate mutase activity have been described (11). Mutations in tyrA which eliminate chorismate mutase activity while preserving prephenate dehydrogenase activity have not been reported previously. In the case of chorismate mutase-prephenate dehydratase, each of the two encoded activities can be independently lost by mutations in pheA (2).

The nucleotide sequences of tyrA and pheA have significant similarities in their 5' portions, suggesting that this region determines chorismate mutase, the activity common to the two enzymes (8). Support for this view comes from the sequence of a monofunctional prephenate dehydratase from *Corynebacterium glutamicum* which has homology only to the portion of pheA which is not homologous to tyrA (5).

We report here that certain deletions within tyrA allow the independent expression of chorismate mutase or prephenate dehydrogenase. Our observations consist of growth phenotypes conferred by plasmids carrying various portions of tyrA or pheA or both on a pBR322 (4) backbone. It is therefore important that the host strain carries no vestige of either tyrA or pheA. Strain KB357 is a derivative of YMC9 (1) in which pheA and tyrA are precisely removed by recombination into the chromosome of a deletion constructed in vitro (manuscript in preparation). Plasmids carrying various portions of tyrA and the adjacent genes aroF and pheA were prepared by standard recombinant DNA techniques (3). Growth experiments were performed on agar plates containing M9 minimal salts medium (10) supplemented with glucose (2 g/liter), thiamine (1 mg/liter), and phenylalanine or tyrosine or both when needed (40 mg/liter). The medium pH was adjusted with 6 N HCl.

Chorismate mutase portion of tyrA. Strain KB357 was separately transformed with pKB641, pKB644, or pKB934 (Fig. 1). In this way, strains which carried no pheA gene and various portions of the tyrA gene were obtained. Such strains required both phenylalanine and tyrosine for growth on ordinary M9 minimal salts medium.

If we postulate that the portion of tyrA in the various strains determines an active chorismate mutase, then two statements are true: the strains are capable of making prephenate (by definition), and the strains are incapable of further metabolizing prephenate (as shown by double phenylalanine-tyrosine auxotrophy). Such strains may release some prephenate into the external medium. Prephenate can be converted to phenylpyruvate (the reaction normally catalyzed by the prephenate dehydratase component of the pheA gene product) by general acid catalysis (15, 16). We have previously observed that phenylpyruvate permits the growth of pheA mutants of *E. coli* (unpublished data), indicating that *E. coli* can utilize exogenous phenylpyruvate for the synthesis of phenylalanine.

We tested the ability of these strains to grow on media of decreasing pH and containing tyrosine but lacking phenylalanine. Although none of the strains grew at pH 7.0 or pH 6.0, two strains which carried all of the portion of tyrA homologous to pheA grew at pH 5.5 (Fig. 1). The strain which was unable to grow, even at pH 5.5, lacked a portion of tyrA homologous to pheA. This evidence of a tyrA-determined chorismate mutase activity independent of prephenate dehydrogenase is in agreement with earlier reports (11). The use of a truncated tyrA gene in our work suggests that the chorismate mutase activity determined by tyrA is not dependent on the structure provided by the prephenate dehydrogenase portion of the native enzyme. Since growth of the strains requires each step of our hypothetical scheme to be true, we note that *E. coli* apparently can release prephenate into the external medium.

Prephenate dehydrogenase portion of tyrA. Plasmid pKB663 (Fig. 2) was not expected to confer a Tyr* phenotype on Tyr* cells because a portion of tyrA was intentionally deleted during its construction. To our surprise, KB357 (pKB663) grew without tyrosine supplementation. Since the chorismate mutase portion of tyrA was absent, the ability to grow without tyrosine should have depended on the chorismate mutase activity of pheA. pKB1036, an analog of pKB663 that lacks pheA, was not able to confer tyrosine prototrophy on KB357 (Fig. 2). Attempts to supply prephenate in the medium did not permit the growth of this strain,

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suggesting that the prephenate release noticed above is not readily reversible.

The fortuitous creation of a tetA-tyrA fusion protein does not account for the expression of prephenate dehydrogenase in pKB663. The genes are joined at the BamHI site in tetA and at the BglII site in tyrA. The reading frame of tetA across the BamHI site (14) is not the same as that of tyrA across the BglII site (8). Moreover, insertion of an 8-base-pair Sall linker at the EcoRV site of pKB663 (pKB952; Fig. 2) did not abolish prephenate dehydrogenase expression. This result strongly argues that the reading frame is not important for that expression.

Elements necessary for translational reinitiation occur just past the tetA-tyrA junction. These include an in-frame stop codon to terminate tetA translation, a ribosome-binding site

FIG. 1. Diagram showing the aroF and tyrA genes as thickened segments on a line representing a portion of the E. coli chromosome. The region of tyrA which is homologous to the E. coli pheA gene is indicated by cross hatching. aroF and tyrA are expressed by transcription from a promoter located to the right of aroF (indicated by an arrow labeled p). Above the genes, bars indicate the extent of material contained on the plasmids indicated at the right. The precise endpoints are the restriction endonuclease cleavage sites indicated at the top. When the various plasmids were transformed into strain KB357, the resulting transformants were unable to grow on M9 minimal salts medium containing glucose and tyrosine (measured pH, 7.0). The ability of such strains to grow on media adjusted in various ways is tabulated at the right (+, good growth; −, no growth). +phe, Addition of phenylalanine.

FIG. 2. Diagram of plasmid pKB663 indicating (by arrows) the expression of pheA from a lac promoter and the production of a tetA-tyrA fusion from the tet promoter. Cross hatching indicates the portion of pheA which is homologous to a portion of wild-type tyrA: that portion of tyrA is not present in these constructions. Below the diagram of pKB663 are indicated modifications which are found in the derivatives indicated at the right. Plasmid pKB952 differs from pKB663 by the insertion of a Sall linker in the EcoRV site located in tetA. Plasmid pKB1036 differs from pKB663 by the deletion of the lac promoter and the pheA gene. The ability of pKB663 and the plasmids derived from it to permit strain KB357 to grow on M9 minimal salts medium with various additions is tabulated at the right (+, good growth; −, no growth). phe, Phenylalanine; tyr, tyrosine.

(5'GAGGA3') (12, 13), and potential in-frame start codons (TTG, GTG, and ATG). However, the spacing between the Shine-Dalgarno sequence and the putative start codon is not optimal (4, 13, and 16 base pairs, respectively). Also, constructions (not shown) which used promoters other than tetP and which did not provide for translation of a fragment of tetA did not result in the expression of prephenate dehydrogenase. We do not know what specific components of pKB663 are particularly necessary for prephenate dehydrogenase expression from the portion of tyrA present; rather we show that under at least some conditions, such expression can occur.

LITERATURE CITED


