3-Deoxy-d-arabino-heptulosonate 7-Phosphate Synthase
PURIFICATION AND MOLECULAR CHARACTERIZATION OF THE PHENYLALANINE-SENSITIVE ISOENZYME FROM ESCHERICHIA COLI

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Russell J. McCandliss,‡ Michael D. Poling, and Klaus M. Herrmann
From the Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907

The phenylalanine-sensitive 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase (7-phospho-2-keto-3-deoxy-d-arabino-heptonate 7-erythro-4-phosphate lyase (pyruvate phosphorylating), EC 4.2.1.15) was purified to apparent homogeneity from extracts of Escherichia coli K12. The enzyme has a molecular weight of 140,000 as judged by gel filtration and sedimentation equilibrium analysis. The enzyme has a subunit molecular weight of 35,000 as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, suggesting that the native form of the enzyme is a tetramer. This was confirmed by cross-linking the enzyme with dimethylsuberimidate and by analyzing the cross-linked material by gel electrophoresis in the presence of sodium dodecyl sulfate, suggesting that the native form of the enzyme is a tetramer. This was confirmed by cross-linking the enzyme with dimethylsuberimidate and by analyzing the cross-linked material by gel electrophoresis in the presence of sodium dodecyl sulfate, suggesting that the native form of the enzyme is a tetramer.

The enzyme shows inactivation of the enzyme. The enzyme is strongly inhibited by L-phenylalanine and to a lesser degree by sodium sulfate, suggesting that the native form of the enzyme is stable for several months when stored at -20°C in buffers containing phosphoenolpyruvate. Removal of phosphoenolpyruvate causes an irreversible inactivation of the enzyme. The enzyme is strongly inhibited by L-phenylalanine and in a lesser degree by dihydrophenylalanine. Molecular parameters of the previously isolated tyrosine-sensitive 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase from E. coli (Schoner, R., and Herrmann, K. M. (1976) J. Biol. Chem. 251, 5440-5447) are compared with those of the phenylalanine-sensitive isoenzyme from the same organism.

The first purified step in the biosynthesis of aromatic compounds is the condensation of P-enolpyruvate and erythro-4-P to give 3-deoxy-d-arabino-heptulosonate 7-phosphate and inorganic phosphate (1). This reaction is catalyzed by DAHP synthase (phosphoenolpyruvate-dependent phosphoketolase) (2) containing DAHP synthase (PHE) (3), tyrosine-sensitive DAHP synthase (TYR), and tryptophan-sensitive DAHP synthase (TRP). The ratio of the activities of the three isoenzymes in E. coli is about 75:25:<1. The control over the biosynthesis of the two major isoenzymes is achieved by different regulatory mechanisms (3).

The tyrosine-sensitive isoenzymes from E. coli (4) and Salmonella typhimurium (5) have recently been obtained in apparently homogeneous form, and their molecular properties were described. Although several purifications of DAHP synthases have been reported (6-8), no other DAHP synthase from enteric bacteria has been purified to apparent homogeneity thus far. We describe here the purification of the homogeneity of the phenylalanine-sensitive isoenzyme from E. coli. Furthermore, we report the molecular characterization of this enzyme and compare its structural parameters with those of DAHP synthase (TYR). This represents the first comparative structural data of DAHP synthase isoenzymes. A preliminary report of our results has appeared elsewhere (9). This preliminary description reported a subunit structure, but the subunit and molecular weights presented were somewhat larger than those reported here.

MATERIALS AND METHODS

Chemicals and Enzymes—P-enolpyruvate (20) and erythrose-4-P (21) were synthesized and assayed as described previously (4, 21). Dimethylsuberimidate was from Pierce Chemical Company. Dihydrophenylalanine was a gift of Dr. Heinz Plos, Department of Medicinal Chemistry, Purdue University. It was shown to be free of phenylalanine by NMR spectroscopy. All other chemicals were obtained commercially as the highest purity available and were used without further purification.

Pyruvate kinase was obtained from Calbiochem; lactate dehydrogenase, bovine serum albumin, and lysozyme were from Sigma; ovalbumin was from Schwarz/Mann; aldolase and diisopropyfluorophosphate-inactivated trypsin were from Worthington.

Growth of Cells—E. coli strain HS 401, isolated in our laboratory as a phase $0 vir-resistant derivative of wild type strain W 3110, was grown with aeration to late logarithmic growth phase in a 200-liter Fermacell Fermentor (New Brunswick Scientific) in minimal salts medium (22) containing 0.5% glucose. The cells were chilled to 8°C in the fermentor and then harvested by centrifugation in a Sharples centrifuge. Cells not used immediately could be stored at -20°C for up to one year with no detectable loss of activity.

Enzyme Assays and Unit of Activity—DAHP synthase (PHE) was routinely assayed by Method A of Schoner and Hermann (4) except that 1,3-propanediol was omitted from all buffers. Method A is based on the procedure of Weissbach and Hurwitz (24) as modified by Doy and Brown (2). In this assay the absorbance of the periodate degradation product complexed with thiobarbituric acid is measured at 549 nm. The kinetic data of Fig. 7 were obtained with assay Method B (4), based on the absorbance difference at 232 nm between P-enolpyruvate, $ \varepsilon = 2700$ (25), and DAHP, $ \varepsilon = 415$ (4). The unit of enzyme activity is defined as the amount of enzyme catalyzing the appearance of 1.0 $\mu$mol of DAHP (Method A) or the disappearance of 1.0 $\mu$mol of P-enolpyruvate (Method B) per min. The numerical values obtained from Method A and Method B assays are not identical because the assays are conducted at different temperatures.

Protein Determination—Protein was determined by the method of Lowry et al. (26) with bovine serum albumin as a standard. During
the later stages of purification and for the pure enzyme, protein was estimated with an $E_{\text{m}}$ at 280 nm of 10.67.

**RESULTS**

**Purification**

All manipulations were performed at 4°C. All buffers contained 2 mM P-enolpyruvate.

**Step I**—Cells were resuspended in Buffer A (0.1 M potassium phosphate, pH 6.5) containing $10^{-4}$ M phenylmethylsulfonyl fluoride; 1 ml of buffer were used for each gram of wet cells. The cells were disrupted in an Amino French pressure cell at 20,000 p.s.i., after which the cell debris was removed by centrifugation for 30 min at 25,000 $\times$ g.

**Step II**—To the supernatant of Step I was added dropwise a 2% solution of protamine sulfate in Buffer A to give a final concentration of 0.14 mg of protamine sulfate per mg of protein. After an additional 30 min of stirring, the suspension was centrifuged for 90 min at 25,000 $\times$ g.

**Step III**—The supernatant from Step II was run directly onto a hydroxylapatite column (Bio-Gel HTP; 5.0 $\times$ 100 cm) that had been equilibrated with Buffer A. The column was washed with 750 ml of Buffer A, and the enzyme was eluted with a linear gradient obtained from 1200 ml of Buffer A and 1200 ml of 0.4 M potassium phosphate, pH 6.5. Fractions of 15 ml were collected at a flow rate of 100 ml/h. Fractions containing DAHP synthase (PHE) activity were pooled.

**Step IV**—Finely ground (NH$_4$)$_2$SO$_4$ was added to the pooled fractions from Step III to give 50% saturation. The precipitated protein was collected by centrifugation and discarded. The precipitated protein was added to finely ground (NH$_4$)$_2$SO$_4$ to give 70% saturation. The precipitate was collected by centrifugation, dissolved in 0.6 M potassium phosphate, pH 6.5, and centrifuged to clarify the solution if necessary.

**Step V**—The resulting solution from Step IV was run onto a column of Phenyl-Sepharose CL-4B (Pharmacia; 2.5 $\times$ 25 cm) equilibrated with 0.05 M potassium phosphate, pH 6.5. After the buffer had been washed with 100 ml of equilibration buffer, the enzyme was eluted with a linear gradient obtained from 200 ml of equilibration buffer and 200 ml of 0.05 M potassium phosphate, pH 6.5. Fractions of 4 ml were collected at a flow rate of 80 ml/h. Fractions containing DAHP synthase (PHE) were pooled and diluted with an equal volume of 2 mM P-enolpyruvate, pH 6.5.

**Step VI**—The solution from Step V was run onto a DEAE-Sephadex A-50 column (2.6 $\times$ 100 cm) equilibrated with 0.05 M potassium phosphate, pH 6.5. The column was washed with 100 ml of 0.1 M potassium phosphate, pH 6.5, and was eluted with a linear gradient obtained from 200 ml of 0.05 M potassium phosphate, pH 6.5, and 200 ml of 0.3 M potassium phosphate, pH 6.5. Fractions of 4 ml were collected at a flow rate of 50 ml/h. In order to concentrate the protein, fractions containing DAHP synthase (PHE) were pooled, diluted, and centrifuged with an equal volume of 2 mM P-enolpyruvate, pH 6.5, run onto a small (0.9 $\times$ 15 cm) column of hydroxylapatite, and eluted with 0.4 M potassium phosphate, pH 6.5.

**Step VII**—The concentrated enzyme from Step VI was layered onto a Sephadex G-150 column (2.6 $\times$ 100 cm) which had been equilibrated with 0.05 M potassium phosphate, pH 6.5. The enzyme was eluted with the same buffer, and fractions of 2 ml were collected at a flow rate of 30 ml/h. Fractions containing DAHP synthase (PHE) were pooled.

**Step VIII**—The solution from Step VII was passed through a column (0.9 $\times$ 15 cm) of Blue Sepharose CL-6B (Pharmacia) equilibrated with 0.05 M potassium phosphate, pH 6.5. Fractions of 4 ml were collected at a flow rate of 50 ml/h. Under these conditions the enzyme does not bind to the column while the trace impurities do. The minor species could be eluted with 0.4 M potassium phosphate, pH 6.5. The fractions containing DAHP synthase (PHE) were pooled and were either used for further studies or concentrated as in Step VI and dialyzed against 0.05 M potassium phosphate, pH 6.5.

A summary of a representative purification run is given in Table I. On polyacrylamide gels under non-denaturing conditions the protein from Step VIII runs as a single band (Fig.
When the gel is sliced and assayed for enzyme activity, the activity coincides with the one protein band, as expected. The enzyme in Fig. 1 (I) is at least 97% pure. This value is calculated by integration of a densitometric scan of an SDS gel (Fig. 1B, inset). Two minor bands (II and III in Fig. 1) can be detected on SDS gels by applying a large amount of protein to the gels.

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total enzyme units</th>
<th>Specific enzyme activity $+2\text{mm}\text{Co}^{2+}$</th>
<th>Enrichment</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Crude extract</td>
<td>1720*</td>
<td>0.12</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>II. Protamine sulfate</td>
<td>1670*</td>
<td>0.18</td>
<td>0.13</td>
<td>1.5</td>
</tr>
<tr>
<td>III. Hydroxylapatite</td>
<td>1292</td>
<td>3.31</td>
<td>2.96</td>
<td>27.4</td>
</tr>
<tr>
<td>IV. Ammonium sulfate</td>
<td>1187</td>
<td>12.0</td>
<td>11.5</td>
<td>99</td>
</tr>
<tr>
<td>V. Phenyl-Sepharose CL-4B</td>
<td>1054</td>
<td>41.9</td>
<td>38.8</td>
<td>346</td>
</tr>
<tr>
<td>VI. DEAE-Sepharose A-50</td>
<td>846</td>
<td>65.2</td>
<td>67.9</td>
<td>547</td>
</tr>
<tr>
<td>VII. Sephadex G-150</td>
<td>519</td>
<td>82.6</td>
<td></td>
<td>683</td>
</tr>
<tr>
<td>VIII. Blue Sepharose CL-6B</td>
<td>369</td>
<td>65.6</td>
<td></td>
<td>65.6</td>
</tr>
</tbody>
</table>

*Enzyme activity is expressed in units measured in assay Method A.

These values represent DAHP synthase (PHE) only; preparations I and II also contain DAHP synthase (TYR) and DAHP synthase (TRP) activities.

### Ultraviolet Absorption Coefficient of DAHP synthase (PHE)

The enzyme has a maximum absorption at 279 nm. The ratio of the absorbances at 280 nm and 260 nm is 2.13. The value for $E_{280}^{	ext{cm}}$ at 280 nm is 10.87 with bovine serum albumin as a standard and the method of Lowry et al. (26) for protein determination.

### Molecular Weight and Subunit Structure

The molecular weight of the native enzyme was estimated to be $150,000 \pm 20,000$ by gel filtration on Sephadex G-200 with urease, catalase, lactate dehydrogenase, bovine serum albumin, and ovalbumin as standards (Fig. 2). From sedimentation equilibrium analysis of pure DAHP synthase (PHE), a molecular weight for the native enzyme of $136,000$ was calculated assuming a partial specific volume $\tilde{v}$ of 0.739 cm$^3$/g at 265°C (Fig. 3). The value for $\tilde{v}$ was calculated from the amino acid analysis according to the method of Cohn and Edsall (36).

The subunit molecular weight was determined by the method of Weber and Osborn (30). With bovine serum albumin, ovalbumin, aldolase, trypsin, and lysozyme as standards, a monomer molecular weight of 35,000 was obtained for DAHP synthase (PHE) (Fig. 4). This value, taken together with the value for the molecular weight of the native enzyme, suggests a tetrameric quaternary structure. This was confirmed by cross-linking experiments with dimethylsuberimidate. Pure DAHP synthase (PHE) was cross-linked and subjected to SDS gel electrophoresis. The resulting pattern (Fig. 5, inset) indicates the presence of cross-linked tetramers, trimers, and dimers, and uncross-linked monomers. The molecular weights were 140,000, 105,000, 70,000, 35,000, respectively, with cross-linked aldolase as a standard (Fig. 5). Thus native DAHP synthase (PHE) is a tetrameric protein with a molecular weight of 140,000.

### Amino Acid Analysis of DAHP Synthase (PHE)

The amino acid analysis of pure DAHP synthase (PHE) is given in Table II.

![Fig. 1](http://www.jbc.org/)  
![Fig. 2](http://www.jbc.org/)
Stability of DAHP Synthase (PHE)

The pure enzyme shows a rather narrow pH optimum for activity around pH 6.5 to 7.0 (Fig. 6A). Furthermore, the stability of the enzyme activity at the pH optimum is dependent upon protein concentration and upon the presence of P-enolpyruvate. The data in Fig. 6B show that the pure enzyme is quite stable in 2 mM P-enolpyruvate until the enzyme concentration is lowered to 1 μg/ml. In addition, when the P-enolpyruvate concentration is lowered to 2 μM, loss of enzyme activity is accelerated. This inactivation is not reversible by

![Graph](image)

**Fig. 3.** Molecular weight estimation of DAHP synthase (PHE) by sedimentation equilibrium analysis. The pure protein was dialyzed against 0.01 M potassium phosphate, pH 6.5, containing 2 mM P-enolpyruvate, and 0.2 M KCl. The In of the fringe displacement is plotted versus the square of the distance from the center of rotation.

![Graph](image)

**Fig. 4.** Subunit molecular weight determination of DAHP synthase (PHE) by SDS polyacrylamide gel electrophoresis. Ten micrograms of DAHP synthase (PHE) and of each of the indicated standards were denatured as described under “Materials and Methods,” and the resulting solution was subjected to SDS-polyacrylamide gel electrophoresis. Inset C, the noncross-linked enzyme. The gel shown in inset D was scanned and mobilities measured relative to bromphenol blue standard. Aldolase (40 μg) cross-linked in the same manner was the standard used for the plot of molecular weight versus relative mobility; O—O, DAHP synthase (PHE); •—•, aldolase.

**Table II**

<table>
<thead>
<tr>
<th>Amino acid composition of DAHP synthase (PHE)</th>
<th>Mol per 35,000 g</th>
<th>Nearest integer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>34.8</td>
<td>36</td>
</tr>
<tr>
<td>Arginine</td>
<td>13.2</td>
<td>13</td>
</tr>
<tr>
<td>Aspartic acid, asparagine</td>
<td>36.4</td>
<td>36</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5.3</td>
<td>5</td>
</tr>
<tr>
<td>Glutamic acid, glutamine</td>
<td>30.7</td>
<td>31</td>
</tr>
<tr>
<td>Glycine</td>
<td>27.5</td>
<td>28</td>
</tr>
<tr>
<td>Histidine</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>21.6</td>
<td>22</td>
</tr>
<tr>
<td>Leucine</td>
<td>30.9</td>
<td>31</td>
</tr>
<tr>
<td>Lysine</td>
<td>21.3</td>
<td>21</td>
</tr>
<tr>
<td>Methionine</td>
<td>7.8</td>
<td>8</td>
</tr>
<tr>
<td>Phenylyalanine</td>
<td>7.2</td>
<td>7</td>
</tr>
<tr>
<td>Proline</td>
<td>13.7</td>
<td>14</td>
</tr>
<tr>
<td>Serine</td>
<td>15.4</td>
<td>15</td>
</tr>
<tr>
<td>Threonine</td>
<td>13.0</td>
<td>13</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>5.4</td>
<td>5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.0</td>
<td>5</td>
</tr>
<tr>
<td>Valine</td>
<td>20.1</td>
<td>20</td>
</tr>
</tbody>
</table>
the addition of more P-enolpyruvate. The loss of activity is slower at lower temperatures. A number of other buffers were tested including 1,3-bis(tris(hydroxymethyl)methylamino)propane, N-2-hydroxethylpiperazine-N'-2-ethanesulfonic acid, 3-(N-morpholino)propanesulfonic acid, and piperazine-N,N'-bis(2-ethanesulfonic acid), and each gave the same result. Loss of enzyme activity in crude preparations is much faster than in highly purified ones.

**Stimulation of Enzyme Activity by Co^{2+}**

Crude preparations of DAHP synthase (PHE) are stimulated about 40% by 2 mM CoCl₂ (Table I). Most of the stimulatory effect is lost after the first chromatographic purification step, with the rest vanishing in the following steps. Enzyme purified through the DEAE-Sephadex step is totally independent of Co^{2+} for activity (Table I). Attempts to restore Co^{2+} activation by mixing fractions or adding fractions to the pure enzyme have thus far been unsuccessful.

**Inhibition of DAHP Synthase (PHE)**

Pure DAHP synthase (PHE) is inhibited by L-phenylalanine and by its two analogs β-2-thienyl-D,L-alanine and L-
dihydrophenylalanine (Fig. 7); 50% inhibition is obtained with 13, 25 (assuming only the L-isomer is inhibitory), and 175 μM inhibitor, respectively, when both substrates are 0.1 mM and assay Method B is used.

Neither L-tyrosine nor D-phenylalanine inhibit the pure enzyme.

**DISCUSSION**

DAHP synthase (PHE) purified through Step VIII of our procedure is at least 97% pure. Two polypeptides, recognizable in the later stages of the purification procedures as discrete bands on SDS-polyacrylamide gels, accompany the enzyme through seven purification steps and can be removed only by passing the preparation over a column of Blue Sepharose. This last “purification” step results in a 20% decrease in the specific activity of the enzyme. Attempts to reactivate homogeneous enzyme preparations through the addition of material removed in the last step have been unsuccessful. Therefore, it seems unlikely that the proteins removed in Step VIII interact directly with the DAHP synthase (PHE) activity, although this possibility cannot be excluded.

During the purification procedure the method of choice for concentrating the enzyme was adsorption to hydroxyapatite followed by elution with phosphate buffers of high ionic strength. More purified enzyme preparations can also be concentrated by precipitation with 80% (NH₄)₂SO₄ with little loss of activity (data not shown). In crude systems, however, (NH₄)₂SO₄ precipitations are rather unsuitable for use with the enzyme, since substantial losses of activity are encountered through irreversible inactivation.

The molecular weight of the native enzyme, determined by three different methods, is 140,000. This value differs somewhat from previously reported values of 160,000 (7) and 110,000 (9, 17), obtained by gel filtration alone. The subunit molecular weight of DAHP synthase (PHE) is 38,000, which is in fairly good agreement with the previously reported value of 33,000 (17). Although Simpson and Davidson (17) suggested that the native form of the enzyme is a trimer, our data conclusively prove that DAHP synthase (PHE) of *E. coli* has four subunits.

The amino acid analysis of pure DAHP synthase (PHE) shows no unusual features. However, it does not agree well with previously published data (17). Some of the discrepancies can be eliminated by recalculation of the data of Simpson and Davidson, since their values appear not to add up to a subunit molecular weight of 33,000. Some gross differences remain, e.g. the values for glycine and alanine. These differences might be best explained by impurities in previously available enzyme preparations, since Simpson and Davidson (17) estimate that their enzyme is about 85% pure.

Pure DAHP synthase (PHE) is 50% inhibited by 13 μM phenylalanine. This inhibition is specific, since up to 1 mM tyrosine does not inhibit the enzyme. Many phenylalanine analogs have been tested as inhibitors for this enzyme (data not shown; Ref. 37). In agreement with previous findings, analogs with substitutions at either the α-amino or the carboxyl group of phenylalanine do not inhibit this enzyme (37), and thienylalanine is an effective inhibitor (38). There have been no previous reports that dihydrophenylalanine, a non-aromatic compound, inhibits this enzyme. Dihydrophenylalanine is excreted into the growth medium of *Streptomyces* (39) and is assumed to be synthesized via the common aromatic pathway (40).

In comparing the previously purified tyrosine-sensitive DAHP synthase from *E. coli* (4) with pure phenylalanine-sensitive isoenzyme, one observes some common molecular parameters as well as some strikingly different ones (Table
Table III

<table>
<thead>
<tr>
<th>Molecular properties of DAHP synthase isoenzymes from E. coli</th>
<th>DAHP synthase (PHE)</th>
<th>DAHP synthase (TYR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% inhibition</td>
<td>13 µM phenylalanine</td>
<td>82 µM tyrosine</td>
</tr>
<tr>
<td>pH optimum</td>
<td>6.5–7.0</td>
<td>6.5–7.0</td>
</tr>
<tr>
<td>Percentage of activity</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>in wild type cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular weights</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native enzyme</td>
<td>140,000</td>
<td>78,000</td>
</tr>
<tr>
<td>Subunit</td>
<td>35,000</td>
<td>39,000</td>
</tr>
<tr>
<td>Quaternary structure</td>
<td>Tetramer</td>
<td>Dimer</td>
</tr>
</tbody>
</table>

III. First, both enzymes have a pH optimum between pH 6.5 and 7.0. Second, both enzymes can only be purified, and their activity maintained, with buffer systems containing P-enolpyruvate. Since extensive kinetic analysis (4) has shown P-enolpyruvate to be the first substrate to bind DAHP synthase (TYR), and since P-enolpyruvate is required to maintain enzyme activity for both isoenzymes, we suggest that both isoenzymes, as isolated, exist as enzyme-P-enolpyruvate adducts. The $K_a$ for P-enolpyruvate for DAHP synthase (TYR) is 5.8 µM (4), and for the DAHP synthase (PHE) is in the same range. Depending on growth conditions the P-enolpyruvate concentration in $E. coli$ cells varies about 10-fold (41). It is, however, never lower than 86 µM, which is approximately 15 times the $K_a$ for DAHP synthase. It seems likely that the growing polypeptide chains of DAHP synthase (TYR) and (PHE) may never encounter an environment containing less than saturating P-enolpyruvate concentrations. Therefore, we consider the enzyme-P-enolpyruvate complex to be the native form of these activities.

A third property observed for both isoenzymes is stimulation by Co$^{2+}$. Crude preparations of both enzymes are specifically activated by Co$^{2+}$. No other metal ion has a stimulatory effect. For both enzymes Co$^{2+}$ stimulation is lost during the early stages of purification; more highly purified enzyme preparations are not activated by Co$^{2+}$. The mechanism of the Co$^{2+}$ activation is not known. It is tempting to speculate that activation of the first enzyme in the common aromatic pathway involves some interaction with the second enzyme, dehydroquinase synthase, which is known to require Co$^{2+}$ for activity (22, 42).

Several differences between the two DAHP synthase isoenzymes are well documented. For example, they occur in dissimilar amounts in wild type cells of $E. coli$ (43). Also, the biosynthesis of the polypeptide chains is regulated by two conceptually different mechanisms (3). However, the most striking difference between the two DAHP synthase isoenzymes, other than their inhibition pattern, is their quaternary structure. Native DAHP synthase (TYR) is a dimer, while native DAHP synthase (PHE) is a tetramer. Furthermore, the subunit molecular weights of the two isoenzymes are different, 39,000 for the tyrosine-sensitive, and 35,000 for the phenylalanine-sensitive isoenzyme. The two isoenzymes can be clearly separated on SDS polyacrylamide gels.

The structural genes of true isoenzymes would lend support to the hypothesis (44) that multiple genes in $E. coli$ arose independently by convergent evolution, provided these genes are neither clustered nor separated by one half or one quarter of the genome length.

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