Inhibition of 3-Deoxy-D-Arabinohexulosonic Acid-7-Phosphate Synthetase (trp) in Escherichia coli

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The growth of a strain of Escherichia coli K-12 which possesses only the single isoenzyme 3-deoxy-D-arabinohexulosonic acid-7-phosphate (DAHP) synthetase (trp), and which makes this enzyme constitutively, is inhibited by tryptophan. The accumulation of DAHP by a derivative of this strain unable to convert DAHP to dehydroquinate is also inhibited by tryptophan. The enzymic activity of DAHP synthetase (trp) in the presence of CO\(^2\)\(^+\) is sensitive to inhibition by tryptophan. Mutant strains of E. coli have been isolated in which DAHP synthetase (trp) is feedback-resistant.

The first reaction of aromatic biosynthesis, the conversion of erythrose-4-phosphate and phosphoenolpyruvate to 3-deoxy-D-arabinohexulosonic acid-7-phosphate (DAHP), is carried out in Escherichia coli by three isoenzymes (4, 10-12). The formation of each one of these isoenzymes is repressed by one of the three aromatic amino acids as follows. The formation of DAHP synthetase (tyr) is repressed by tyrosine, DAHP synthetase (phe) by phenylalanine, and DAHP synthetase (trp) by tryptophan. In addition, the activities of DAHP synthetase (phe) and DAHP synthetase (trp) are feedback-inhibited by phenylalanine and tyrosine, respectively. The failure to observe any significant inhibition of DAHP synthetase (trp) in E. coli by tryptophan has been reported from a number of laboratories (4, 8, 11). One exception to this is a recent report by Doy (6) of 32% inhibition of DAHP synthetase (trp) in extracts of E. coli W. There are reasons, however, why in vitro tests for inhibition of this enzyme have been difficult to carry out. In wild-type E. coli, DAHP synthetase (trp) is produced in much smaller amounts than either DAHP synthetase (tyr) or DAHP synthetase (phe), and it can only be identified as that activity not inhibited by either phenylalanine or tyrosine. Consequently, it is necessary to study the inhibition of a very small fraction of the total activity. Furthermore, tryptophan in concentrations of 5 \times 10^{-4} \text{M} or more interferes with the colorimetric test for the assay of DAHP. Although it is possible to measure the extent of this interference by carrying out appropriate controls, this adds an extra element of uncertainty to any small inhibitions that are observed. It is the purpose of this communication to describe in vivo and in vitro experiments which do, however, clearly demonstrate that DAHP synthetase (trp) is inhibited by tryptophan in E. coli K-12.

MATERIALS AND METHODS

Organisms. The strains used in this work (Table 1) are all derivatives of E. coli K-12.

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

Transduction. Transductions involving phage P1 were carried out as previously described (9).

Growth experiments. Cultures in T-tubes were incubated with shaking in a 37°C water bath. Increases in optical density were measured in a Spekker photoelectric absorptiometer (Hilger and Watts, Ltd., London, England).

Accumulation experiments. Cell suspensions were prepared as follows. Cells were grown overnight on minimal medium agar plates supplemented with phenylalanine, tyrosine, shikimic acid, and any additional amino acids required for growth. Cells were harvested from the plates in sterile buffer, washed twice with buffer, and resuspended in minimal medium containing all required growth factors except the aromatic amino acids. Enough medium was added to the final suspension to give an optical density reading of 65 in the EEL colorimeter (Evans Electroelenium, Ltd., Halstead, England) using the blue filter (OBl0) and 1.27-cm tubes (approximately 10^6 cells per ml). Such cell suspensions were distributed in 15-ml quantities into two 100-ml flasks. At zero-time, L-tryptophan solution (10^{-4} \text{M}) was added to one flask to give a final concentration of 5 \times 10^{-4} \text{M} L-tryptophan. Both flasks were incubated at 37°C in a shaker water bath. Samples (0.5 ml) were removed from each flask at 30-min intervals and pipetted into
0.5 ml of cold 10% trichloracetic acid. The precipitate was removed by centrifugation and 0.4 ml of the supernatant fluid was used in the assay for DAHP and DAH (the dephosphorylated form of DAHP).

**Estimation of DAHP and DAH.** The method described by Brown and Doy was used (4). The effect of tryptophan on the development of the chromogen was tested as follows. Immediately prior to the addition of periodate, suitable concentrations of tryptophan were added to samples in which tryptophan had not been present either during the accumulation or during the period of enzyme reaction. The optical density readings obtained with these tubes were compared with results from similar tubes to which no tryptophan had been added. Final readings were corrected for any effects of tryptophan on the actual colorimetric assay.

**Assay of DAHP synthetase.** The method described by Doy and Brown (7) was used except that CO\(^{3+}\) was added to the reaction mixture at a final concentration of 10\(^{-4}\) M.

**Isolation of mutants.** The conditions under which cells were treated with the mutagen N-methyl-N’-nitro-N-nitrosoguanidine were those described by Adelberg, Mandel, and Chen (2).

**Preparation of cell-free extracts.** The conditions under which cell-free extracts were prepared by use of the French Press to break the cells has been described previously (9).

**Sonic treatment of cell suspensions.** Cells were suspended in 0.1 M phosphate buffer (pH 7.0) to a final concentration of approximately 10\(^{8}\) cells per ml and were sonically treated for 40 sec using an MSE ultrasonicator.

**RESULTS**

Strain AB2891 possesses only a single isoenzyme, DAHP synthetase (trp), and will not grow in minimal medium supplemented with tryptophan unless phenylalanine and tyrosine are also added (12). If DAHP synthetase (trp) is not inhibited by tryptophan, this growth inhibition must be caused entirely by the repressive effects of tryptophan on the formation of DAHP synthetase (trp). Mutations in the trpR gene, previously described by Cohen and Jacob (5), cause constitutive synthesis of the enzymes of the tryptophan operon and also of DAHP synthetase (trp) (3; J. Pittard and J. Camakaris, unpublished data). If DAHP synthetase (trp) is not inhibited by tryptophan, therefore, tryptophan should have no effect on the growth rate of a derivative of strain AB2891 which has integrated the trpR\(^{-}\) allele.

**Formation of a trpR\(^{-}\) derivative of AB2891.** A strain possessing only the single isoenzyme DAHP synthetase (trp), and also possessing a mutation in the trpR gene, was constructed as follows. Strain W3110 trpR\(^{-}\) is a female strain which was obtained from C. Yanofsky. It has a mutation in the trpR gene which will be referred to in this paper as trpR363. W3110 trpR\(^{-}\) was converted into a male donor (JP61) by infection with the F-merogenote, F-lac, from strain JP57. Strain JP61 was then mated with AB2891, and selection was made for arg\(^{+}\) recombinants. Since JP61 transfers genes in the order lac\(^{+}\) trpR363 arg\(^{+}\), a high percentage of the arg\(^{+}\) recombinants were expected to have inherited the trpR363 allele. Ten arg\(^{+}\) recombinants were purified, and
cell-free extracts were prepared from cultures grown in minimal medium supplemented with phenylalanine, tyrosine, and tryptophan and in minimal medium alone. The extracts were assayed for DAHP synthetase (trp) and for anthranilate synthetase. Four of the 10 recombinants produced both of these enzymes constitutively and were shown still to possess only a single isoenzyme, DAHP synthetase (trp). One of these strains, JP62, was chosen for further study.

Inhibition of growth of a trpR- derivative of AB2891 by tryptophan. Like the parent strain AB2891, JP62 grows slowly in minimal medium with a mean generative time of 3 hr. This relatively slow growth rate reflects the organism’s decreased ability to carry out the first reaction of aromatic biosynthesis as a result of the mutations affecting DAHP synthetase (tyr) and DAHP synthetase (phe). Growth rates of AB2891 and JP62 can be returned to normal by the addition of all the aromatic amino acids and vitamins to the growth medium. Although the formation of DAHP synthetase (trp) is no longer repressed by tryptophan in strain JP62, the addition of tryptophan to minimal medium considerably decreases its growth rate. The addition of $5 \times 10^{-4}$ M L-tryptophan causes a 75% reduction in growth rate, to give a new mean generation time of 12 hr.

Effect of tryptophan on the accumulation of DAHP. To confirm that the inhibitory effect of tryptophan on the growth of JP62 was a result of the inhibition of DAHP synthetase activity, an $aroB^-$ mutation affecting the enzyme converting DAHP to dehydroquininate was introduced into JP62. The recombinant strain which possesses the mutant alleles $aroF363$, $aroG365$, $aroB351$, and $trpR363$ is JP65. When cell suspensions of JP65 are washed and incubated at 37 C in minimal medium that does not contain the aromatic amino acids, DAHP and its dephosphorylated derivative DAH (DAH(P)) are accumulated and excreted by the cells (12). The formation of DAH(P) by these cells depends entirely on the activity of DAHP synthetase (trp) which is being produced constitutively. Therefore, the effect of added tryptophan on the accumulation of DAH(P) by these cells was studied to determine whether DAHP synthetase (trp) was feedback-inhibited in vivo. The results indicated that the addition of L-tryptophan ($5 \times 10^{-4}$ M) at time zero causes an 80% decrease in the rate at which DAH(P) accumulates (Fig. 1). Cell suspensions were sonically treated (at zero-time and at 120 min) and assayed for DAHP synthetase activity. The amount of DAHP synthetase activity did not increase during the 120 min, and the level of activity found in cells incubated in the presence of tryptophan was the same as that found in cells incubated in the absence of tryptophan, confirming that no significant repression or derepression of DAHP synthetase occurred during the course of the experiment. Both growth tests and accumulation studies, therefore, clearly indicate that tryptophan can inhibit the activity of DAHP synthetase (trp).

Isolation of feedback-resistant mutants. Further evidence to support the conclusion that DAHP synthetase (trp) is inhibitable by tryptophan comes from a study of mutant strains derived from AB2891 and able to grow in the presence of tryptophan. Strain AB2891 was treated with nitrosoguanidine, and the survivors were plated to minimal medium containing $5 \times 10^{-4}$ M L-tryptophan. Only those colonies which grew on this medium and which were still unable to make either functional DAHP synthetase (tyr) or DAHP synthetase (phe) were examined further. Some of these mutants, although able to grow in the presence of added tryptophan, did show a reduction in growth rate when tryptophan was added to the medium. Strain JP6 was an example of such a mutant strain. Other strains showed
no reduction in growth rate (J. Pittard and J. Camakaris, in preparation). Strain JP66 grows more quickly than AB2891 in minimal medium (mean generation time of 87 min compared to 180 min; Table 2). When tryptophan is added to the minimal medium, however, strain AB2891 will not grow at all, whereas strain JP66 grows with a mean generation time that is only approximately 1.5 times longer than that observed in minimal medium. The growth rate of a trpR363 derivative of JP66, JP68, is also included in Table 2. It is interesting to note that with this strain the addition of tryptophan to minimal medium does not cause any decrease in growth rate, whereas in the case of the trpR363 derivative of AB2891, JP62, the addition of tryptophan changes the mean generation time from 180 to 720 min. The addition of all the aromatic amino acids plus shikimate to the medium causes these four strains to grow at a normal rate. Enzymic studies of JP66 failed to show any alteration to the repressibility of DAHP synthetase (trp) in this organism, indicating that its ability to grow in the presence of tryptophan did not result from any change in repressibility of DAHP synthetase (trp). The DAHP synthetase activities in cell-free extracts prepared from both JP66 and AB2891 grown in the presence and in the absence of aromatic amino acids are shown in Table 3. The failure to demonstrate any change in repression of DAHP synthetase (trp) in JP66 suggests that its ability to grow in the presence of tryptophan may result from a change in the feedback sensitivity of this enzyme.

**Table 2. Growth rates of mutant strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean generation times when grown in minimal medium supplemented with</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No supplement</td>
<td>Tryptophan</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>min</td>
</tr>
<tr>
<td>AB2891</td>
<td>180</td>
<td>—</td>
</tr>
<tr>
<td>JP62</td>
<td>180</td>
<td>720</td>
</tr>
<tr>
<td>JP66</td>
<td>87</td>
<td>144</td>
</tr>
<tr>
<td>JP68</td>
<td>84</td>
<td>78</td>
</tr>
</tbody>
</table>

* In minimal medium supplemented with tryptophan, strain AB2891 shows no increase in optical density during incubation for 8 hr at 37 C.

**Table 3. Repressibility of DAHP synthetase (trp)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specific activity of DAHP synthetase (trp) in extracts prepared from cells grown in</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimal medium</td>
<td>Minimal medium plus tyrosine, phenylalanine, tryptophan, and shikimic acid</td>
</tr>
<tr>
<td>AB2891</td>
<td>1.95</td>
<td>0.5</td>
</tr>
<tr>
<td>JP66</td>
<td>2.66</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* L-Phenylalanine and L-tyrosine were 10⁻⁴ M; L-tryptophan, 10⁻⁵ M; and shikimic acid, 10⁻⁴ M.

cotransducible with aroH were carried out as follows. Strain AB2891 has the genotype aroF363, aroG365, aroH* for the three structural genes for the DAHP synthetase isoenzymes. Strain JP66 is isogenic with AB2891, except that it may contain a mutation in the aroH gene which renders DAHP synthetase (trp) feedback-resistant. Strain AB3248 has the genotype aroF363, aroG365, aroH367 and requires the aromatic amino acids for growth since it cannot make functional DAHP synthetase. In a phage P1-mediated transduction experiment in which AB3248 is the recipient (either AB2891 or JP66 are the donors) and in which transductants are selected on minimal medium not containing aromatic amino acids, selection is being made for the transfer of the aroH gene. If the aroH gene of strain JP66 carries a mutation which causes feedback-resistant DAHP synthetase (trp) to be formed, nearly all the transductants from the cross in which JP66 is the donor should be able to grow in the presence of added tryptophan. On the other hand, none of the transductants from the cross in which AB2891 is the donor would be expected to grow on this medium. When these experiments were carried out, at least 90% of the transductants obtained with JP66 as donor were able to grow in the presence of tryptophan. No transductants with this property were obtained, however, from the transduction in which AB2891 was the donor. Although these results do not prove that the mutation causing tryptophan resistance is located within the gene aroH, the close linkage between the two supports this conclusion.

**Effect of tryptophan on the accumulation of DAHP(P) by feedback-resistant strains.** Both the aroB351 and the trpR363 mutations were introduced into strain JP66 to make a recombinant strain JP69, which is identical with JP65 except for the mutation in the aroH gene conferring feedback resistance. Accumulations were carried out, as they were for JP65, in the presence and in the absence of added tryptophan (Fig. 2). In this case, the addition of tryptophan had no
effect on the accumulation of DAH(P), confirming the conclusion that DAH synthetase (trp) made by strain JP66 is feedback-resistant.

One other strain isolated from AB2891 is JP70. This strain produces DAH synthetase (trp) constitutively. Since its growth rate is not affected by tryptophan, its DAH synthetase (trp) activity is presumably also feedback-resistant. Accumulation studies were carried out with an aroB351 derivative of JP70. The results confirmed the conclusion that the DAH synthetase (trp) made by this strain is also feedback-resistant (Fig. 3).

In vitro inhibition of DAH synthetase (trp). By working with strain AB2891, it is possible to study the characteristics of DAH synthetase (trp) in crude cell-free extracts. We have found that upon incubation with ethylenediaminetetra-acetate (EDTA), cell-free extracts of this strain lose all DAH synthetase activity. The addition of Co²⁺ (10⁻⁴ M, as sulphate) restores this activity to a value twice the initial value obtained in the absence of EDTA. The addition of Co²⁺ (10⁻⁴ M) to crude cell-free extract to which no EDTA has been added also causes an approximate twofold increase in activity. Furthermore, when these extracts are titrated for sensitivity to tryptophan in the presence of Co²⁺, it is possible to demonstrate feedback inhibition (Fig. 4). Included in this figure are two titrations carried out on the two feedback-resistant strains, JP65 and JP70.

![Graph](http://jb.asm.org/)

**Fig. 2.** Effect of tryptophan on the accumulation of DAH(P) by washed cell suspensions of strain JP69. Symbols are as expressed in Fig. 1.

The DAH synthetase produced by these strains is much less sensitive to tryptophan, particularly at lower levels of inhibitor concentration. The largest inhibition of wild-type DAH synthetase (trp) that was observed was 60% (in the presence of Co²⁺ and using L-tryptophan at a concentration of 10⁻⁴ M). In the absence of added Co²⁺, an inhibition of 20% was observed with this concentration of L-tryptophan.

**DISCUSSION**

These results clearly indicate that DAH synthetase (trp) of E. coli K-12 is inhibited by tryptophan in vivo. When Co²⁺ is added to the reaction mixture, it is also possible to demonstrate inhibition of this enzyme activity in vitro. The addition of Co²⁺ stimulates DAH synthetase (trp) activity in cell-free extracts and reveals its sensitivity to inhibition by tryptophan. Because, however, Co²⁺ does not have to be added in order to show inhibition in vivo, its effect in the in vitro experiments is presumably to correct some alteration to enzyme conformation or state of aggregation which has occurred during the
preparation of cell-free extracts. Neither in vivo nor in vitro tests have revealed more than an 80% inhibition of enzyme activity.

Mutant strains with a feedback-resistant DAHP synthetase (trp) have been isolated. Some of these are also derepressed for the enzyme DAHP synthetase (trp), whereas others show normal repressibility. In the case of the strains that are both derepressed and feedback-resistant, it has not yet been possible to determine whether these changes are the result of one or two mutations occurring in the aroH gene.

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LITERATURE CITED