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Robert J. Pauley  
*Marquette University*

Walter W. Fredricks  
*Marquette University*

Oliver H. Smith  
*Marquette University*

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ROBERT J. PAULEY,† WALTER W. FREDRICKS, AND OLIVER H. SMITH*
Department of Biology, Marquette University, Milwaukee, Wisconsin 53233

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The abilities of 14 tryptophan analogs to repress the tryptophan (trp) operon
have been studied in Escherichia coli cells derepressed by incubation with 0.25
mM indole-3-propionic acid (IPA). trp operon expression was monitored by
measuring the specific activities of anthranilate synthase (EC 4.1.3.27) and the
tryptophan synthase (EC 4.2.1.20) β subunit. Analogs characterized by modifi-
cation or removal of the α-amino group or the α-carboxyl group did not repress
the trp operon. The only analogs among this group that appeared to interact with
the trp aaporepressor were IPA, which derepressed the trp operon, and D-tryptophan.
Analogs with modifications of the indole ring repressed the trp operon to
various degrees. 7-Methyl-tryptophan inhibited anthranilate synthase activity
and consequently derepressed the trp operon. Additionally, 7-methyltryptophan
prevented IPA-mediated derepression but, unlike tryptophan, did so in a non-
coordinate manner, with the later enzymes of the operon being relatively more
derepressed than the early enzymes. The effect of 7-methyltryptophan on IPA-
mediated derepression was likely not due to the interaction of IPA with the
allosteric site of anthranilate synthase, even though feedback-resistant mutants
of anthranilate synthase were partially resistant to derepression by IPA. The
effect of 7-methyltryptophan on derepression by IPA was probably due to the
effect of the analog-aaporepressor complex on trp operon expression.

The efficiency of regulation of tryptophan biosynthesis in Escherichia coli resides in the abil-
ity of tryptophan to interact at three different controlling sites. Negative control of tryptophan
(trp) operon mRNA synthesis is mediated by the interaction of tryptophan and the trpP gene
product, the aaporepressor (10, 15, 20). Tryptophan or a metabolic derivative of tryptophan
modulates the frequency of elongation of mRNA through the attenuator region (3, 12). In addi-
tion, tryptophan functions as an allosteric inhibitor of anthranilate synthase, the first enzyme in
the tryptophan biosynthetic pathway (2).

Structural analogs of L-tryptophan have had an important role in the development of our
current knowledge of the mechanisms by which tryptophan regulates its own biosynthesis. Studi-
es with tryptophan analogs have demonstrated that repression control of trp operon mRNA
synthesis, both in vivo and in vitro, requires the formation of a functional repressor by the inter-
action of the aaporepressor and L-tryptophan or certain analogs, such as 6-methyltryptophan (5,
10, 14, 15, 18). These studies precluded the in-
volvement of tryptophanyl-tRNA in repression control, although similar studies have indicated
that tryptophanyl-tRNA may be involved in the control of trp mRNA elongation through the
attenuator site (12). Experiments with 7-methyl-
tryptophan (7-MT) have demonstrated that this
analog affects trp operon expression as a result
of its interaction with the first enzyme in the
tryptophan biosynthetic pathway (8).

The aim of the studies described in this report
was to evaluate tryptophan analogs for their
ability to repress the in vivo synthesis of the
trpE and trpB gene products, anthranilate syn-
thase and the β subunit of tryptophan synthase,
respectively. Therefore these experiments re-
quired a system in which repression of trp op-
eron expression by tryptophan analogs could be
reliably quantitated.

Repression of trp operon expression in E. coli
cells growing in minimal medium is slight, be-
cause the trp operon is partially repressed under
these conditions. However, treatment of cells
with indole-3-propionic acid (IPA) causes rapid
and coordinate derepression of the trp operon
that is prevented by L-tryptophan (1, 11, 13). In

† Present Address: Department of Cell Biology, Baylor
College of Medicine, Houston, TX 77030.
vitro studies have shown that derepression of trp operon expression by IPA was due to a direct effect of IPA on the trp aporepressor (17). Therefore, IPA apparently decreases the level of functional trp repressor in cells, causing derepression of the trp operon. Consequently, the abilities of tryptophan analogs to repress the trp operon can be evaluated by determining, relative to L-tryptophan, each analog's ability to prevent IPA-mediated derepression. The effects of various modifications in tryptophan structure upon the ability of L-tryptophan to repress trp operon expression are described. In addition, the relationship of repression control of trp operon expression and allosteric control of anthranilate synthase by tryptophan analogs is discussed.

(11) This paper was taken in part from a thesis submitted to Marquette University, Milwaukee, Wis., by R. J. P. in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Bacterial strains. The strains of E. coli K-12 used in this study were derived from either W3110 tna or W1485 tna, which were isolated in this laboratory and lack detectable tryptophanase activity. Mutants described in this study were isolated after UV mutagenesis. The anthranilate synthase activities of the trpE44 tna and trpE45 tna mutants were resistant to inhibition by L-tryptophan. aroG13 tna was characterized by its resistance to growth inhibition by 3-methylanthranilic acid and has 7-phospho-2-keto-3-deoxy-D-arabinohexonate 5-erythrose-4-phosphate-lyase (pyruvate-phosphorylating) (DAHP synthase) (EC 4.1.2.15) activity which is resistant to allosteric inhibition by phenylalanine (9).

Culture conditions. Cultures of E. coli strains were routinely grown at 37°C with vigorous aeration on a rotary shaker in the minimal medium of Vogel and Bonner (19) supplemented with 0.2% (wt/vol) glucose as a carbon source. Cells from an overnight late log- or early stationary-phase culture were recovered by centrifugation at 5,000 × g for 15 min (4°C), suspended in the same volume of cold saline (0.9% NaCl), and centrifuged again. The cells were resuspended in 0.1 volume of fresh medium and used to inoculate fresh medium (37°C) to an initial density of 106 cells per ml. Cultures were incubated until the density reached 4 × 108 to 5 × 109 cells per ml. Supplements, as specified in Results, were then added to each culture, and incubation was continued for 45 min. Culture flasks were chilled in an ice bath for 5 min, and then the contents were transferred to chilled centrifuge tubes. Cells were recovered by centrifugation and washed with an equal volume, followed by 0.1 volume, of cold saline.

Preparation of cell extracts. Cell extracts were prepared by suspending the washed cells in 2 ml of cold homogenization buffer per g (wet weight) of cells. Homogenization buffer, pH 7.0, contained 0.1 M KH2PO4-K2HPO4, 1 mM 2-mercaptoethanol, and 1 mM ethylenediaminetetraacetic acid. Cells were disrupted by intermittent sonic oscillation for 2 min. Cell debris was removed by centrifugation at 20,000 × g for 45 min at 4°C. The cell extracts were stored at -15°C until assayed.

Enzyme assays. Anthranilate synthase (chorismate pyruvate-lyase [amino-accepting], EC 4.1.3.27) was assayed by the method of Held and Smith (8). Assays for anthranilate phosphoribosyltransferase [N-(5'-phosphoribosyl)-anthranilate:pyrophosphate phosphoribosyltransferase, EC 2.4.2.18] and indole-3-glycerol-phosphate synthase [1-(2'-carboxyphenylamino)-1-deoxyribulose-5-phosphate carboxy-lyase (cyclizing), EC 4.1.1.48] activities were performed according to the methods of Creighton and Yanofsky (4). Tryptophan synthase, α and β subunits, (L-serine hydro-lyase (adding indole-glycerol-phosphate), EC 4.2.1.20) was assayed by the procedures of Smith and Yanofsky (16).

One enzyme unit was defined as the amount of enzyme which catalyzed the production of one µmole of product or the consumption of one µmole of substrate in 1 min at 37°C. Specific activity is given in enzyme units per milligram of protein. Protein was determined by the method of Lowry et al. (9).

Relative repression was calculated by comparing the level of a particular trp operon gene product synthesized in the presence of an analog and IPA to the level of that gene product synthesized in the presence of L-tryptophan and IPA by the formula:

\[
\frac{([SA [IPA] - SA [IPA + analog])/[SA [IPA] - SA [IPA + L-tryptophan]]) \times 100}{\text{relative repression (percent),}}
\]

where SA [IPA] was the enzyme specific activity from cells treated with 0.25 mM IPA, SA [IPA + analog] was the enzyme specific activity from cells treated with 0.25 mM IPA and the designated concentration of analog, and SA [IPA + L-tryptophan] was the enzyme specific activity from cells treated with 0.25 mM IPA plus L-tryptophan at the same concentration as indicated for the analog.

Chemicals. Chorismic acid was prepared by the method of Gibson (6). N-Acetyl-L-, 4-methyl-L-5-methyl-DL-, and 7-aza-DL-tryptophan were purchased from Cyclo Chemical Co., Los Angeles, Calif. Tryptamine hydrochloride, 5-fluorotryptophan, and 6-fluorotryptophan were acquired from Aldrich Chemical Co., Milwaukee, Wis. Sigma Chemical Co., St. Louis, Mo., supplied 5-hydroxy-DL-tryptophan, 7-methyl-DL-tryptophan, and DL-tryptophan butyl ester hydrochloride. IPA was purchased from Eastman Organic Chemical Div., Eastman Kodak Co., Rochester, N. Y. L-Tryptophan, d-tryptophan, DL-tryptophan, L-tryptophan methyl ester hydrochloride, L-tryptophan ethyl ester hydrochloride, and 5-methyl-DL-tryptophan were acquired from Schwarz/Mann, Orangeburg, N. Y. All analogs were freshly prepared in neutralized minimal medium except for IPA, which was prepared in ethyl alcohol. The final alcohol concentration in cell cultures did not exceed 3% (vol/vol). All other chemicals were commercially available.

All tryptophan analogs were examined by paper chromatography in an n-butyl alcohol–acetic acid–water (90:10:20) solvent system. The chromatography
grams were sprayed with solutions of ninhydrin or p-dimethylaminobenzaldehyde. The only analog, except for D-tryptophan, that contained detectable amounts of a substance that had the same $R_f$ as tryptophan was DL-tryptophan butyl ester.

RESULTS

The tryptophan analog IPA derepresses trp operon expression at the level of transcription and translation in vivo (1, 11, 13). IPA derepresses the trp operon because it apparently competes with L-tryptophan for binding to the aporepressor (11, 15). Based upon these observations, the abilities of tryptophan analogs to repress trp operon expression were determined by comparing the level of trp operon expression observed in the presence of an analog and IPA to the level of trp operon expression observed in the presence of L-tryptophan and IPA.

Concentrations of IPA in excess of 0.1 mM caused significant increases in the specific activities of anthranilate synthase and the tryptophan synthase $\beta$ subunit (Fig. 1). The activities of anthranilate synthase and the tryptophan synthase $\beta$ subunit were significantly greater ($P \leq 0.05$) in cells treated with 0.25 mM IPA than in untreated cells assayed in 22 independent experiments. From these latter measurements, the specific activities (mean ± standard error) of anthranilate synthase and the tryptophan synthase $\beta$ subunit were 0.00783 ± 0.00073 and 0.035 ± 0.0025, respectively, in IPA-treated cultures, as compared with 0.00132 ± 0.00051 and 0.0066 ± 0.0022, respectively, in control cultures without IPA. IPA also inhibited cell growth under the same conditions (data not shown).

L-Tryptophan repression of trp operon expression was analyzed by incubating cells with 0.25 mM IPA and various amounts of L-tryptophan. L-Tryptophan repression was concentration dependent (Fig. 2). Maximal and one-half-maximal repression were observed at approximately 12 and 5 mM L-tryptophan, respectively.

Based upon the results presented in Fig. 1 and 2, the ability of tryptophan analogs to repress trp operon expression was evaluated by determining the effect of an analog, at concentrations of 0.025 and 0.25 mM, upon derepression by 0.25 mM IPA. Analog concentrations of 0.025 and 0.25 mM were used, since L-tryptophan caused maximal repression over this range.

Table 1 illustrates the method used to analyze the abilities of tryptophan analogs to repress trp operon expression, and the results are controls for analogs that were racemic mixtures. DL-Tryptophan repressed synthesis of anthranilate synthase and the tryptophan synthase $\beta$ subunit to the same extent as L-tryptophan at either concentration; i.e., the relative repression was approximately 100%. Therefore, the D isomer of tryptophan did not significantly reduce the ability of 12.5 mM L-tryptophan to repress trp operon expression.

The same method was used to evaluate the abilities of 14 tryptophan analogs to repress trp operon expression. The results are presented in summary form in Table 2. Control cultures containing only 0.25 mM analog were also routinely assayed. Only 7-MT derepressed the trp operon in control cultures containing 0.25 mM analog alone.

The tryptophan analogs 4-methyl-, 5-methyl-, 5fluoro-, 6-methyl-, and 6-fluorotryptophan repressed enzyme synthesis to the same extent as L-tryptophan, and therefore these analogs may form a functional complex with the aporepressor. In addition, at concentrations of 0.01 and 0.005 mM, 6-methyltryptophan repressed enzyme synthesis to the same extent as equivalent concentrations of L-tryptophan (data not shown).

D-Tryptophan, 7-azatryptophan, and 5-hydroxytryptophan only partially repressed trp operon expression (Table 2). The degree of repres-
Fig. 2. Effect of L-tryptophan concentration on repression of the trp operon. W3110 tna was incubated with 0.25 mM IPA and various concentrations of L-tryptophan. The specific activities of anthranilate synthase and the tryptophan synthase β subunit were determined. The percent repression, mean ± standard error (± SE) from two independent experiments, was calculated as described in the text.

TABLE 1. DL-Tryptophan prevention of IPA derepression

<table>
<thead>
<tr>
<th>Conc (mM)</th>
<th>Anthranilate synthase</th>
<th>Tryptophan synthase β subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sp act (EU/ mg)</td>
<td>Relative repression (%)</td>
</tr>
<tr>
<td>IPA</td>
<td>L-Tryptophan</td>
<td>DL-Tryptophan</td>
</tr>
<tr>
<td>0.25</td>
<td>0.00142</td>
<td>0.00116</td>
</tr>
<tr>
<td>0.25</td>
<td>0.00986</td>
<td>0.00111</td>
</tr>
<tr>
<td>0.25</td>
<td>0.00988</td>
<td>0.00130</td>
</tr>
<tr>
<td>0.25</td>
<td>0.025</td>
<td>0.00116</td>
</tr>
<tr>
<td>0.25</td>
<td>0.025</td>
<td>0.00111</td>
</tr>
<tr>
<td>0.25</td>
<td>0.025</td>
<td>0.00130</td>
</tr>
</tbody>
</table>

* Conditions for cell culture and enzyme assays are described in the text. Relative repression was calculated as described in the text.

The analog 7-methyltryptophan (7-MT) is not readily classified with regard to its ability to repress trp operon expression, because repression of anthranilate synthase and the tryptophan synthase β subunit was not coordinate with the latter enzyme being repressed to a greater extent (Table 2). Noncoordinate repression was also observed with 0.05 mM 7-MT (data not shown).

N-Acetyltryptophan and tryptamine did not significantly repress the trp operon, even at 0.25 mM (Table 2). The methyl, ethyl, and butyl esters of tryptophan also did not repress the trp operon at 0.025 mM, but maximal repression was observed at 0.25 mM (Table 2). The possibility that repression at the higher concentration of the esters was due to production of tryptophan, as a result of removal of the ester group, was analyzed by determining the effect of the esters on the growth of a starved tryptophan auxotroph, W3110 tna trpA. The ester-substituted analogs supported growth of the tryptophan auxotroph under conditions similar to the repression studies, indicating that the esters were hydrolyzed (data not shown).

The possibility that noncoordinate repression
by 7-MT was due to an effect of 7-MT upon either the synthesis or the activity of anthranilate synthase or the tryptophan synthase β subunit was evaluated by determining the effect of 7-MT on IPA-mediated derepression of all trp operon enzymes. The results (Table 3) indicated that 7-MT repressed synthesis of indoleglycerolphosphate synthase and both the β and the α subunits of tryptophan synthase to the same degree. Synthesis of anthranilate phosphoribosyltransferase was repressed to a lesser degree than the tryptophan synthase β subunit but more than anthranilate synthase. Also, these results show that 7-MT alone slightly derepressed anthranilate synthase, whereas synthesis of the tryptophan synthase β and α subunits was not derepressed. Noncoordinate derepression of trp operator proximal and distal gene products by 7-MT has been observed previously (8). Therefore, the low relative repression of anthranilate synthase compared with the tryptophan synthase β and α subunits may be due to derepression of anthranilate synthase by 7-MT.

### Table 2. Summary of analog repression studies

<table>
<thead>
<tr>
<th>Analog</th>
<th>0.025 mM analog</th>
<th>0.25 mM analog</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anthranilate</td>
<td>Tryptophan</td>
</tr>
<tr>
<td></td>
<td>synthase</td>
<td>synthase β sub-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unit</td>
</tr>
<tr>
<td>4-Methyl-DL-tryptophan</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>5-Methyl-DL-tryptophan</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>5-Fluorotryptophan</td>
<td>97</td>
<td>95</td>
</tr>
<tr>
<td>6-Methyl-DL-tryptophan</td>
<td>97</td>
<td>93</td>
</tr>
<tr>
<td>6-Fluorotryptophan</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>D-Tryptophan</td>
<td>25 ± 5</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>7-Aza-DL-tryptophan</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td>5-Hydroxy-DL-tryptophan</td>
<td>19</td>
<td>29</td>
</tr>
<tr>
<td>7-Methyl-DL-tryptophan</td>
<td>43 ± 3</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>N-Acetyl-L-tryptophan</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>L-Tryptophan methyl ester</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>L-Tryptophan ethyl ester</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>DL-Tryptophan butyl ester</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Relative repression of anthranilate synthase and the tryptophan synthase β subunit was calculated by the equation described in the text.

### Table 3. Effect of 7-MT on IPA repression of trp operon enzymes

<table>
<thead>
<tr>
<th>Conc (mM)</th>
<th>Anthranilate synthase</th>
<th>Anthranilate phosphoribosyl transferase</th>
<th>Indole-3-glycerolphosphate synthase</th>
<th>Tryptophan synthase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sp act (EU/mg)</td>
<td>Rel. rep. (%)</td>
<td>Sp act (EU/mg)</td>
<td>Rel. rep. (%)</td>
</tr>
<tr>
<td>IPA L-tryptophan</td>
<td>7-MT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.00141</td>
<td>0.00112</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.00184</td>
<td>ND</td>
</tr>
<tr>
<td>0.25</td>
<td>—</td>
<td>—</td>
<td>0.00738</td>
<td>0.00034</td>
</tr>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>—</td>
<td>0.00107</td>
<td>0.00102</td>
</tr>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>0.025</td>
<td>0.00025</td>
<td>0.00023</td>
</tr>
<tr>
<td>0.25</td>
<td>0.25</td>
<td>0.025</td>
<td>0.00044</td>
<td>0.00056</td>
</tr>
</tbody>
</table>

* The conditions for cell culture and determination of the specific activity, as well as the relative repression (Rel. rep.), of indicated enzymes are described in the text.

EU, Enzyme units.

* — None.

ND, Not determined.
Mutants with a feedback-resistant DAHP synthase or with a feedback-resistant anthranilate synthase are not derepressed by 7-MT (8). The ability of IPA to derepress the trp operon enzymes was tested with both types of feedback-resistant mutants. An aroG strain, with a feedback-resistant DAHP synthase, required approximately 10 times the concentration of IPA to achieve the same relative level of derepression seen with 0.25 mM IPA in W3110 tna (Fig. 3). Although not shown in Fig. 3, enzyme activities for two anthranilate synthase feedback-resistant strains, trpE44 tna and trpE45 tna, closely approximated the values on the lower curve, for the aroG strain. The increase in IPA concentration required for derepression may be due to an increase in endogenous tryptophan in feedback-resistant mutants, since from Table 4 it is apparent that IPA did not inhibit anthranilate synthase activity under conditions in which L-tryptophan and 7-MT were strong inhibitors. In addition, IPA did not affect L-tryptophan inhibition of enzyme activity.

**DISCUSSION**

Direct measurement of the interaction between the trpR gene product, the aporepressor, and L-tryptophan has been difficult, because efforts to purify the aporepressor have met with only partial success (15, 18, 20). Consequently, analysis of the interaction of tryptophan and tryptophan analogs with the aporepressor has been inferred from measurements of trp operon expression (5, 10, 14, 17). However, the effect of a tryptophan analog on trp operon expression is dependent not only upon the interaction of the analog with the trp aporepressor but also upon the interaction of the analog-aporepressor complex with the trp operator locus. Therefore, the inability of certain analogs to repress trp operon expression to the same degree as L-tryptophan may be a result of the properties of the analog-aporepressor complex.

In the studies described in this report, the abilities of tryptophan analogs to interact with the trp aporepressor and to form functional complexes have been analyzed by comparing each analog to L-tryptophan for its ability to prevent IPA-mediated derepression of the trp operon. Derepression by IPA was concentration dependent (Fig. 1 and 3) and was prevented by L-tryptophan in a concentration-dependent manner (Fig. 2). Therefore, the α-amino group of L-tryptophan is probably required for the analog-aporepressor complex to interact with the trp operator locus. In addition, the aporepressor apparently may have less affinity for IPA than for L-tryptophan, because 15 μM L-tryptophan completely prevented derepression by 250 μM IPA. The reduction in the level of trp operon enzymes by high concentrations of IPA (Fig. 3) was due to a reduction in the rate of translation of trp mRNA and not to repression of trp mRNA synthesis (unpublished data).

Among the analogs that apparently did not interact with the aporepressor, because they had no significant effect on the IPA-mediated derepression, were tryptamine, N-acetyltryptophan, and the methyl, ethyl, and butyl esters of

**Table 4. Effect of 7-MT and IPA on anthranilate synthase activity**

<table>
<thead>
<tr>
<th>Analog</th>
<th>Analog concn (mM)</th>
<th>L-Tryptophan concn (mM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-MT</td>
<td>0.01</td>
<td>0.01</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>IPA</td>
<td>0.10</td>
<td>0.01</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.01</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.02</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.02</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.02</td>
<td>30</td>
</tr>
<tr>
<td></td>
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<td>0.02</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.02</td>
<td>41</td>
</tr>
</tbody>
</table>

* Dialyzed crude extract of W3110 tna was used as a source of the enzyme. Assay reaction mixtures contained 0.05 mM chorismic acid.

**Fig. 3. IPA derepression in wild-type and aroG strains.** The specific activities of the tryptophan synthetase α (circles) and β (squares) subunits were measured in extracts of cultures incubated in the designated concentrations of IPA for 45 min. The responses of E. coli W3110 strains tna (open symbols) and aroG13 tna (closed symbols) were compared.
tryptophan (Table 2). Therefore, removal or substitution of the α-carboxyl group and substitution of the α-amino group significantly reduced the ability of L-tryptophan to form a functional complex with the aporepressor.

Other analogs, such as 4-methyl-, 5-methyl-, 5-fluoro-, 6-methyl-, and 6-fluoro-tryptophan, prevented IPA-mediated derepression (Table 2), suggesting that they increased the concentration of functional repressor. Similar observations with certain of these analogs have been previously reported (5, 10, 14, 17, 20). Partial repression of trp operon expression was observed with D-tryptophan, 7-aza- and 5-hydroxytryptophan, and 7-MT. Repression by 7-aza- or D-tryptophan was not concentration dependent. Thus, these analogs may form only a partially active complex with the aporepressor. Concentration-independent repression by D-tryptophan may be due to the enzymatic conversion of D-tryptophan to L-tryptophan in cells (7). The possibility that repression by other analogs may be due to metabolism cannot be ruled out. The observation that methyl- or fluoro-group substitutions at C-5 had little or no effect on repression, whereas either a methyl-group substitution at C-7 or a hydroxyl-group substitution at C-5 reduced the level of repression, could reflect the interaction of these sites on L-tryptophan with the aporepressor or their role in determining a particular conformation of the repressor.

These studies predict that some purification of the trp aporepressor could be achieved by affinity chromatography, using tryptophan coupled to the support matrix through the indole ring. Efforts to purify the trp aporepressor by affinity chromatography in this laboratory have not been successful. However, the low affinity of the repressor for the trp operator locus apparently precluded detection of the aporepressor by the DNA binding methods that were used for assay.

The effect of 7-MT on IPA-mediated derepression was unique, because repression was noncoordinate (Tables 2 and 3). Noncoordinate repression may be due, at least in part, to the fact that 7-MT caused noncoordinate derepression of the trp operon (Table 3) (8). Under our assay conditions, the level of repression was dependent upon the concentrations of IPA, of the analog, and of endogenous tryptophan. Consequently, the level of trp operon expression may be affected by the decrease in endogenous tryptophan due to feedback inhibition of anthranilate synthase activity by 7-MT. The conclusion that 7-MT partially repressed trp operon expression by interacting with the aporepressor while it noncoordinateley derepressed the trp operon by limitation of endogenous tryptophan is not contradictory, because 7-MT was a weaker corepressor than L-tryptophan.

It was of interest that feedback-resistant mutants of anthranilate synthase and DAHP synthase, which were resistant to derepression by 7-MT (8), required higher concentrations of IPA to derepress the trp operon than did wild-type strains (Fig. 3). This observation, in itself, might imply that the feedback site of anthranilate synthase has a role in IPA-mediated derepression of the trp operon, as has been observed with 7-MT (8). However, the evidence presented in Table 4 indicates that IPA neither inhibited anthranilate synthase activity nor interacted with the feedback site. Since IPA apparently does not interact with the allosteric site of anthranilate synthase, the concentration dependence of IPA derepression in feedback-resistant mutants may be due to an increase in the concentration of intracellular tryptophan in these mutants. Tryptophan operon enzymes are repressed to a greater extent in feedback-resistant mutants grown in minimal medium than are their parent strains (data not shown) (8), and higher levels of tryptophan have been observed in the culture medium from feedback-resistant mutants than in that from their parent strain (8). Therefore, these observations indicate that the intracellular level of tryptophan is probably higher in feedback-resistant mutants than in wild-type strains and again point out the importance of the general aromatic pathway and the regulatory status of anthranilate synthase on the control of trp operon expression (8).

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LITERATURE CITED


