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Effect of Tryptophan Analogs on Derepression of the *Escherichia coli* Tryptophan Operon by Indole-3-Propionic Acid

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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 modification 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* aporepressor 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 noncoordinate manner, with the later enzymes of the operon being relatively more repressed 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-aporepressor complex on *trp* operon expression.

The efficiency of regulation of tryptophan biosynthesis in *Escherichia coli* resides in the ability 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 *trpR* gene product, the aporepressor (10, 15, 20). Tryptophan or a metabolic derivative of tryptophan modulates the frequency of elongation of mRNA through the attenuator region (3, 12). In addition, 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. Studies 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 interaction of the aporepressor and L-tryptophan or certain analogs, such as 6-methyltryptophan (5, 10, 14, 15, 18). These studies precluded the involvement 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-methyltryptophan (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 synthase and the β subunit of tryptophan synthase, respectively. Therefore these experiments required a system in which repression of *trp* operon expression by tryptophan analogs could be reliably quantitated.

Repression of *trp* operon expression in *E. coli* cells growing in minimal medium is slight, because 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

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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.

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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-*arabino*heptonate D-erythrose-4-phosphate-lyase (pyruvate-phosphorylating) (DAHP synthase) (EC 4.1.2.15) activity which is resistant to allosteric inhibition by phenylalanine (8).

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 10^8 cells per ml. Cultures were incubated until the density reached 4×10^8 to 5×10^8 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 KH_2PO_4-K_2HPO_4, 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 phosphoribosytransferase [*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 that 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:

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

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-DL-, 6-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 chromato-

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 β subunit (Fig. 1). The activities of anthranilate synthase and the tryptophan synthase β 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 β 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).

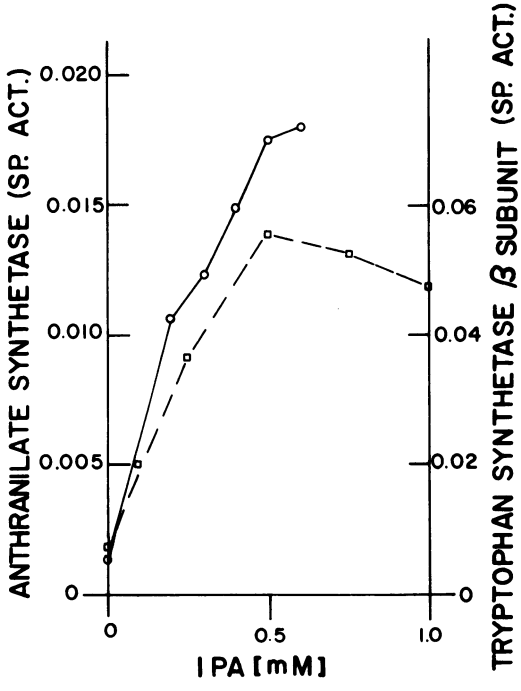

FIG. 1. *Concentration dependence of IPA derepression of the trp operon. The specific activities of anthranilate synthase* (○) *and the tryptophan synthase β subunit* (□) *were determined in extracts from cultures incubated with the designated concentrations of IPA for 45 min.*

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 μM 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 β 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 μM 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-, 5-fluoro-, 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 extents 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-

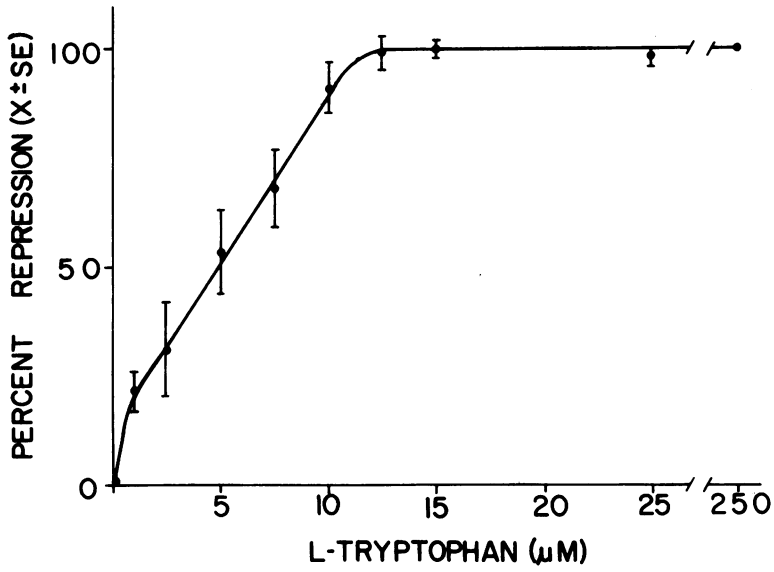

FIG. 2. *Effect of L-tryptophan concentration on repression of the trp operon. W3110 tna was incubated with 0.25 mM IPA and various concentrations of L-tryptophan. The specific activities of anthranilate synthase and the tryptophan synthase β subunit were determined. The percent repression, mean ± standard error ($\bar{x} \pm SE$) from two independent experiments, was calculated as described in the text.*

TABLE 1. *DL-Tryptophan prevention of IPA derepression*[a]

Concn (mM)			Anthranilate synthase		Tryptophan synthase β subunit	
IPA	L-Tryptophan	DL-Tryptophan	Sp act (EU[b]/mg)	Relative repression (%)	Sp act (EU/mg)	Relative repression (%)
			0.00142		0.0087	
0.25			0.00986		0.0446	
0.25	0.025		0.00098		0.0066	
0.25		0.025	0.00116	98	0.0073	98
0.25	0.25		0.00111		0.0066	
0.25		0.25	0.00130	98	0.0069	99

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

[b] EU, Enzyme units.

sion by 5-hydroxytryptophan was a function of the analog concentration. However, the degree of repression by D-tryptophan and 7-azatryptophan was not a function of the analog concentration (Table 2).

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 indoleglycerol-phosphate 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*

Analog	Relative repression (%)[a]			
	0.025 mM analog		0.25 mM analog	
	Anthranilate synthase	Tryptophan synthase β subunit	Anthranilate synthase	Tryptophan synthase β subunit
4-Methyl-DL-tryptophan	95	95	96	96
5-Methyl-DL-tryptophan	93	97	94	96
5-Fluorotryptophan	89	95	97	100
6-Methyl-DL-tryptophan	97	93	93	92
6-Fluorotryptophan	99	99	99	97
D-Tryptophan[b]	25 ± 5	21 ± 5	24 ± 8	28 ± 14
7-Aza-DL-tryptophan	43	41	47	41
5-Hydroxy-DL-tryptophan	19	29	54	63
7-Methyl-DL-tryptophan[c]	43 ± 3	85 ± 3	65 ± 8	88 ± 2
N-Acetyl-L-tryptophan	13	8	1	4
Tryptamine	10	0	7	0
L-Tryptophan methyl ester[d]	25	8	100	100
L-Tryptophan ethyl ester[d]	24	11	100	100
DL-Tryptophan butyl ester[d]	0	0	100	100

[a] Relative repression of anthranilate synthase and the tryptophan synthase β subunit was calculated by the equation described in the text.
[b] Mean ± standard error of three independent experiments.
[c] Mean ± standard error of five independent experiments.
[d] Repression observed was probably due to hydrolysis and liberation of tryptophan during the incubation period (see text).

TABLE 3. *Effect of 7-MT on IPA depression of trp operon enzymes*[a]

Concn (mM)			Anthranilate synthase		Anthranilate phosphoribosyl transferase		Indole-3-glycerol-phosphate synthase		Tryptophan synthase			
									β subunit		α subunit	
IPA	L-tryptophan	7-MT	Sp act (EU/mg)	Rel. rep. (%)	Sp act (EU/mg)	Rel. rep. (%)	Sp act (EU/mg)	Rel. rep. (%)	Sp act (EU/mg)	Rel. rep. (%)	Sp act (EU/mg)	Rel. rep. (%)
—[c]	—	—	0.00141		0.00112		0.00132		0.0095		0.0131	
—	—	0.25	0.00184		ND[d]		ND		0.0064		0.0131	
0.25	—	—	0.00738		0.00394		0.00638		0.0345		0.0459	
0.25	0.025	—	0.00107		0.00102		0.00155		0.0075		0.0114	
0.25	—	0.025	0.00501	38	0.00223	58	0.00206	82	0.0126	82	0.0202	75
0.25	—	0.25	0.00414	52	0.00206	64	0.00181	87	0.0109	88	0.0173	83

[a] 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.
[b] EU, Enzyme units.
[c] —, None.
[d] 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.

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

Analog	Analog concn (mM)	L-Tryptophan concn (mM)	Inhibition (%)
		0.01	24
		0.02	37
		0.10	87
		1.0	100
7-MT	0.01		14
	0.033		42
	0.10		84
IPA	0.10		0
	1.0		0
	0.10	0.01	25
	0.20	0.01	30
	0.01	0.02	40
	0.02	0.02	41
	0.10	0.02	35

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

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.

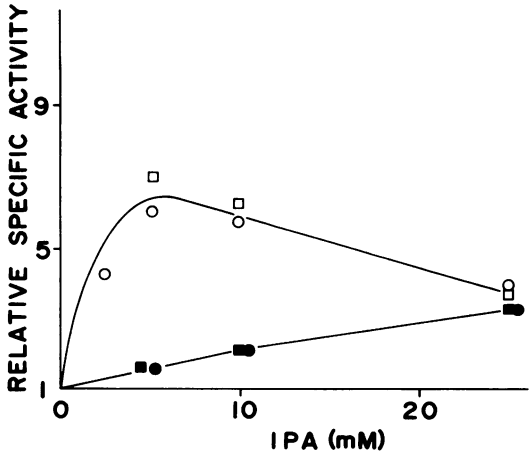

FIG. 3. *IPA derepression in wild-type and aroG strains. The specific activities of the tryptophan synthetase α (circles) and β (squares) subunits were measured in extracts of cultures incubated in the designated concentrations of IPA for 45 min. The responses of E. coli W3110 strains tna (open symbols) and aroG13 tna (closed symbols) were compared.*

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

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-fluorotryptophan, 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 noncoordinately 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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