

Regulation of the *Escherichia coli* Tryptophan Operon by Early Reactions in the Aromatic Pathway¹

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7-Methyltryptophan (7MT) or compounds which can be metabolized to 7MT, 3-methylanthranilic acid (3MA) and 7-methylindole, cause derepression of the *trp* operon through feedback inhibition of anthranilate synthetase. Tyrosine reverses 3MA or 7-methylindole derepression, apparently by increasing the amount of chorismic acid available to the tryptophan pathway. A mutant isolated on the basis of 3MA resistance (*MAR 13*) was found to excrete small amounts of chorismic acid and to have a feedback-resistant phenylalanine 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthetase. Genetic evidence indicates that the mutation conferring 3MA resistance and feedback resistance is very closely linked to *aroG*, the structural gene for the DAHP synthetase (*phe*). Since feedback inhibition of anthranilate synthetase by L-tryptophan (or 7MT) is competitive with chorismic acid, alterations in growth conditions (added tyrosine) or in a mutant (*MAR 13*) which increase the amount of chorismic acid available to the tryptophan pathway result in resistance to 7MT derepression. Owing to this competitive nature of tryptophan feedback inhibition of anthranilate synthetase by chorismic acid, the early pathway apparently serves to exert a regulatory influence on tryptophan biosynthesis.

In *Escherichia coli*, the level of intermediates in aromatic biosynthesis is regulated by several different allosteric interactions. The end products of the pathway (principally phenylalanine, tyrosine, and tryptophan) inhibit three isoenzymatic forms of the first enzyme of the pathway, 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthetase, and further interact at the branch point with the enzymes metabolizing chorismic acid along the various specific biosynthetic routes (1, 5, 6, 9, 15, 19, 20). Thus, alterations in the levels of the allosteric effectors and the activities of the allosteric enzymes would be expected to demonstrate the complex regulatory interrelationships in the aromatic pathway.

In the preceding paper (11), evidence was presented showing that compounds capable of being metabolized to 7-methyltryptophan (7MT), i.e., 3-methylanthranilic acid (3MA) and 7-methylindole, inhibit growth and cause derepression of the *trp* operon in wild-type strains of *E. coli*. Presumably, both phenomena are manifestations of a tryptophan limitation caused by 7MT inhibition

of anthranilate synthetase. Unlike other methyltryptophan analogues, 7MT does not appear to function as an active corepressor.

In this report, we present evidence that analogue-induced derepression is antagonized by the inclusion of tyrosine in the growth medium of wild-type cells, and that mutants with a feedback-resistant DAHP synthetase (*phe*) are also refractory to this derepression. The most likely explanation for these observations is that both alterations increase the amount of chorismic acid available to the tryptophan pathway, thereby reversing the inhibitory effect of 7MT on anthranilate synthetase. Thus, the early pathway involving the production of chorismic acid apparently serves to exert a regulatory influence on tryptophan biosynthesis.

MATERIALS AND METHODS

Organisms. All mutants described were derived from W1485 *tna* (a mutant lacking tryptophanase isolated in this laboratory) except *aroG* (a mutant lacking the phenylalanine-inhibitable DAHP synthetase), obtained from K. D. Brown. The parent strain of *aroG* is W1485. The mutants were obtained by ultraviolet or nitrosoquandine treatment and plating on appropriate selective media. 3MA-resistant

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mutants were obtained by plating mutagen-treated cells on minimal agar plates containing 6.7×10^{-4} M 3MA.

Transduction procedures. Plkc transducing lysates of the mutants were prepared by the confluent lysis technique (13). Control lysates from wild-type strains and controls for sterility were done routinely.

Growth media. All strains and mutants were grown in the minimal medium of Vogel and Bonner (18) containing 0.5% glucose and other supplements as desired. The cultures were agitated on a rotary shaker at 37 C. Growth curves were obtained by growing 50-ml cultures in a 500-ml side-arm flask and estimating growth at various times by turbidity at 660 nm.

Chemicals. D-Erythrose-4-phosphate dimethylacetal dicyclohexylammonium salt was obtained from Calbiochem, Los Angeles, Calif., and converted to free erythrose-4-phosphate by the method of Ballou, Fischer, and MacDonald (2) before use. Chorismic acid was prepared as the free acid according to the procedure of Gibson (10). All other chemicals were obtained commercially.

Preparation of extracts. Cells were harvested by centrifugation, washed once in saline, and resuspended in 0.1 M K_2HPO_4 , pH 7.0, containing 10^{-3} M 2-mercaptoethanol and 10^{-3} M ethylenediaminetetraacetate (EDTA). The cells were broken by sonic oscillation, and cell debris was removed by centrifugation at $30,000 \times g$ for 30 min. For assay of DAHP synthetase, extracts were prepared in 5×10^{-2} M K_2HPO_4 , pH 7.0, containing 10^{-3} M 2-mercaptoethanol.

Enzyme assays. The α subunit of tryptophan synthetase (A protein) was assayed according to Smith and Yanofsky (16), and anthranilate synthetase was assayed as described in the preceding paper (11).

DAHP synthetase was assayed according to the procedure of Doy and Brown (7), except that the reaction mixture contained 10^{-3} M Co^{+2} (see below). After heating with thiobarbituric acid, the reaction tubes were cooled briefly and extracted with 4.0 ml of cyclohexanone to avoid the turbidity usually encountered in the assay. The concentration of DAHP in the cyclohexanone was determined spectrophotometrically at 549 nm by use of a molar extinction coefficient of 6.0×10^4 .

A unit of enzyme activity is equivalent to 1 μ mole of substrate consumed or product formed per minute at 37 C. Specific activity is given as units per milligram of protein. The protein was determined by the method of Lowry et al. (14). In some cases (relative activity), the specific activity relative to that found in W1485 *tna* grown in minimal medium was used. A relative activity of 1.0 corresponds to 0.0027 units of anthranilate synthetase per mg of protein and 0.0152 units of tryptophan synthetase A protein per mg of protein.

Co^{+2} activation of DAHP synthetase. A recent report indicated that the DAHP synthetase (*trp*) is stimulated by Co^{+2} (15). We have found that Co^{+2} appears to activate DAHP synthetase in crude extracts or partially purified preparations of W1485 *tna* which contain predominantly the DAHP synthetase (*phe*). Table 1 shows the effect of various cations on the total DAHP synthetase activity in crude extracts of W1485 *tna* which was dialyzed overnight in buffer containing

TABLE 1. Cation activation of DAHP synthetase

Cation ^a	DAHP synthetase relative activity ^b
None.....	100
Co^{+2}	182
Mg^{+2}	104
Mn^{+2}	158
Zn^{+2}	65
Fe^{+2}	69
Fe^{+3}	41
EDTA.....	16

^a Cations were added to DAHP synthetase reaction mixture at a concentration of 10^{-3} M.

^b A crude extract of W1485 *tna* dialyzed overnight in buffer containing 10^{-3} M EDTA was used as a source of enzyme.

10^{-3} M EDTA. It is apparent that Co^{+2} and Mn^{+2} significantly stimulate DAHP synthetase activity and that EDTA almost completely inhibits the reaction. Maximal activation of DAHP synthetase occurs at 10^{-3} M Co^{+2} , and this concentration was used in all subsequent experiments.

Diethylaminoethyl (DEAE)-Sephadex chromatography. DEAE-Sephadex chromatography was carried out on a column of 2.5×32 cm by use of a linear gradient elution starting with 350 ml of 10^{-2} M K_2HPO_4 , pH 7.0, plus 0.1 M KCl in the mixing bottle and 350 ml of 10^{-2} M K_2HPO_4 , pH 7.0, plus 0.6 M KCl in the inlet bottle. Eluting buffers contained 10^{-3} M 2-mercaptoethanol. Nucleic acids were not removed before crude extracts were placed on the column. At the end of the fractionation, a small amount of 10^{-2} M K_2HPO_4 , pH 7.0, containing 1.0 M KCl was washed through the column to insure that all DAHP synthetase activity had been eluted. Recovery of DAHP synthetase varied between 32 and 58%.

RESULTS

Since the analogue-induced *trp* operon derepression results from tryptophan limitation, it should be reversed by added tryptophan or precursors such as anthranilic acid and indole. Table 2 shows that these compounds did repress cultures exposed to the analogues and that an early intermediate, shikimic acid, had only a slight effect in decreasing the enzyme levels. Shikimate is a precursor of chorismic acid and, in light of the arguments presented below, we have no ready explanation for its relative ineffectiveness. Table 2 further shows that tyrosine, but not phenylalanine or a mixture of the two, effectively reversed the derepression. We felt that, as inhibition of anthranilate synthetase by tryptophan (or 7-methyltryptophan) is competitive with chorismic acid (1, 11), tyrosine reversal of 3MA and 7-methylindole derepression might be due to tyrosine causing increased chorismic acid avail-

TABLE 2. Effect of aromatic amino acids and some precursors on 3-methylantranilic acid and 7-methylindole derepression of anthranilate synthetase in W1485 *tna*

Growth conditions	Additions	Concn (μ moles/ml)	Relative activity
Minimal medium	None	—	1.0
	Phenylalanine	.30	1.4
	Tyrosine	.28	0.68
	Tyrosine + phenylalanine	.30	2.0
Minimal medium + 0.2 μ mole of 3-methylantranilic acid/ml	None	—	17.0
	L-Tryptophan	.05	1.8
	Indole	.068	2.5
	Anthranilic acid	.058	2.6
	Shikimic acid	.29	12.8
	Phenylalanine	.30	11.0
	Tyrosine	.055	8.1
	Tyrosine	.28	0.96
	Tyrosine + phenylalanine	.30	13.4
Minimal medium + 0.1 μ mole of 7-methylindole/ml	None	—	17.3
	Tyrosine	.28	4.9
	Tyrosine + phenylalanine	.30	12.9

able to the tryptophan pathway. To test this possibility, a tryptophan auxotroph, *trpD2*, was grown overnight on limiting tryptophan supplemented with tyrosine alone, or tyrosine and phenylalanine; the cells were washed and then incubated with several mixtures of the aromatic amino acids. As *trpD2* accumulates anthranilic acid under the growth conditions used in the experiment and is derepressed for anthranilate synthetase, the rate of anthranilic acid accumulation is a measure of the amount of chorismic acid available for tryptophan biosynthesis. Table 3 indicates that *trpD2* accumulated more anthranilic acid in the presence of tyrosine than in minimal media, and much more than when both phenylalanine and tyrosine were present. Under these different conditions, anthranilate synthetase was derepressed to approximately the same degree. The results support the hypothesis that tyrosine reversal of 3MA or 7-methylindole derepression is due to its influence on the amount of chorismic acid available to the tryptophan pathway.

Some mutants isolated on the basis of 3MA resistance did not derepress on medium containing 3MA or 7-methylindole (Table 4) and are unlinked by transduction to the tryptophan operon (11). Preliminary studies of 3MA resistance in one of these mutants, *MAR 13*, indi-

cated that the mutant accumulated small amounts of chorismic acid. From Table 5 it is apparent that a compound accumulated in *MAR 13* supernatant fluid (but not in W1485 *tna* or *trp E2^{FBR2-50}*) which could be converted to anthranilic acid with highly purified anthranilate synthetase. Formation of anthranilic acid from the compound which accumulated in *MAR 13* supernatant fluid required the complete anthranilate synthetase reaction mixture, including both glutamine and $MgCl_2$. This compound is probably chorismic acid. Thus, it appeared that the mutational alteration conferring 3MA resistance in *MAR 13* might affect regulation of the early pathway of aromatic biosynthesis.

DAHPSynthetase is the first enzyme specific

TABLE 3. Anthranilic acid accumulation by *trpD2* in the presence of aromatic amino acids^a

Growth conditions	Accumulation conditions	Anthranilate synthetase (units/mg)	Amt of anthranilic acid ^b /10 ⁸ cells (μ moles/ml)		
			1 hr	2 hr	3 hr
Trp	None	.103	.118	.226	.303
Trp + tyr	Tyr	.086	.135	.264	.382
Trp + tyr + phe	Tyr + phe	.088	.031	.057	.086

^a The concentrations of amino acids in the growth or accumulation media (in 10^{-2} μ moles/ml) were as follows: tryptophan (trp), 2.5; tyrosine (tyr), 28; phenylalanine (phe), 30.

^b The concentration of anthranilic acid was determined by acidifying 1.0 ml of supernatant fluid from the accumulation media with 0.1 ml of 1 N HCl and extracting with ethyl acetate. The concentration of anthranilic acid in the ethyl acetate layer was determined spectrophotometrically at an absorption maximum of 336 nm.

TABLE 4. Resistance to 3-methylantranilic acid (3MA) and 7-methylindole (7MIIn) derepression of anthranilate synthetase and A protein in *MAR 13*

Strain	Relative specific activity					
	Anthranilate synthetase			A protein		
	Minimal medium	3MA ^a	7MIIn ^a	Minimal medium	3MA	7MIIn
W1485	1.0	19.4	20.1	1.0	11.8	—
<i>MAR 13</i>	0.37	0.87	2.3	0.42	0.72	2.2

^a Strains were grown in minimal medium, or minimal medium containing 0.2 μ mole of 3MA/ml or 0.2 μ mole of 7MIIn/ml.

for aromatic amino acid biosynthesis (17). Previous reports have indicated that there are three isoenzymatic DAHP synthetases in *E. coli* (6, 15, 19, 20), one inhibited by phenylalanine, one by tyrosine, and one by tryptophan. Since DAHP synthetase plays an important role in the overall regulation of aromatic biosynthesis, a mutation giving rise to increased chorismic acid production, such as in *MAR 13*, might be due to an alteration in the amount or properties of DAHP synthetase. As shown in Table 6, the DAHP synthetase activity in *MAR 13* was only slightly inhibited by phenylalanine, whereas in W1485 *tna* more than 80% of the total DAHP synthetase was inhibited by phenylalanine. The total amount of DAHP synthetase activity in crude extracts of *MAR 13* was similar to that found in wild-type W1485 *tna*. A mutant which entirely lacks the phenylalanine-inhibitable DAHP synthetase activity, *aroG* (19), had only about 10% of the total activity found in wild-type strains. Unlike *MAR 13*, the DAHP synthetase activity not inhibited by phenylalanine in *aroG* was inhibited by tyrosine and tryptophan. Therefore, it appeared that *MAR 13* has an altered phenylalanine DAHP synthetase which renders it resistant to feedback inhibition by phenylalanine.

To determine whether the mutation to 3MA resistance was due to an altered phenylalanine

TABLE 5. Detection of a compound in *MAR 13* culture supernatant which can be converted to anthranilic acid by purified anthranilate synthetase^a

Culture supernatant fluid ^b	Amt tested	Anthranilic acid formed/ ml of supernatant fluid
	ml	μmoles
W1485 <i>tna</i>4	.0003
<i>trpE</i> ^{FBR2-50}4	.0003
<i>MAR 13</i>2	.0120
<i>MAR 13</i>4	.0108
<i>MAR 13</i> (ether extract) ^c1	.3400

^a An excess of purified anthranilate synthetase-PR transferase complex (specific activity = 2.25 units/mg) was added, and the reaction was allowed to continue until no further anthranilic acid was formed.

^b Culture supernatant fluids were tested for presence of "chorismic acid" by use of the standard anthranilate synthetase reaction mixture minus chorismic acid.

^c *MAR 13* ether extract was obtained by extracting 500 ml of *MAR 13* culture supernatant under acid conditions with ethyl ether, and evaporating (in vacuo) over 5.0 ml of 0.1 M K₂HPO₄, pH 7.0, until all ether was removed.

TABLE 6. DAHP synthetase activities and inhibition by aromatic amino acids

Strain ^a	Specific activity	Inhibition (%) ^b		
		Phenylalanine, 10 ⁻³ M	Tyrosine, 10 ⁻³ M	Tryptophan, 10 ⁻³ M
W1485 <i>tna</i>0962	83.2	21.6	19.8
<i>MAR 13</i>0978	4.6	3.9	12.6
<i>aro G</i>0067	13.5	63.5	73.0

^a Each of the strains was grown in minimal medium containing 0.5% glucose.

^b The sum of the amount of inhibition by each of the aromatic amino acids may be more than 100% owing to some cross-inhibition.

DAHP synthetase, *MAR 13* was transduced into *aroG*. More than 90% of the wild-type recombinants from this cross did not derepress on 3MA (Table 7), indicating that the 3MA-resistant locus in *MAR 13* is very closely linked to *aroG*. Table 8 shows that, in addition to not derepressing on 3MA, these recombinants also have acquired the feedback-resistant DAHP synthetase. Isolates from a control cross between wild-type W1485 *tna* and *aroG* are shown for comparison.

Crude extracts of W1485 *tna*, *MAR 13*, and *aroG* (Fig. 1) were chromatographed on DEAE-Sephadex to determine whether the relative proportions of the various isoenzymes had been altered in *MAR 13*. Although resolution of the isoenzymes was not attained, there were indications of differences in the three strains. Wild-type W1485 *tna* (Fig. 1) had one definite peak preceded by a very broad region which may be heterogeneous in composition. It is apparent that all fractions contained phenylalanine-inhibitable DAHP synthetase activity, although the major peak contained the most. *MAR 13*, however, had one main peak of DAHP synthetase activity, not inhibitable by phenylalanine. This peak was preceded by a small shoulder and may correspond to that seen in W1485 *tna*, although it appears to be considerably reduced in *MAR 13*. Apparently the tryptophan- and tyrosine-inhibitable DAHP synthetases are not present in high enough concentrations in *MAR 13* to be detected after chromatography, as those activities would likely be repressed in this strain (Table 6). Strain *aroG* (Fig. 1) showed a single narrow peak which was inhibited by tryptophan. Although 60% of the activity of *aroG* in crude extracts was inhibited by tyrosine, very little tyrosine-inhibitable DAHP synthetase activity was detected after chromatography. The reason for this is unknown.

TABLE 7. *Recombination between MAR 13 and aro G*

Donor	Recipient	No. of wild-type recombinants tested	No. derepressed on 3MA ^b
W1485 <i>tna</i>	<i>aro G</i>	23	23
MAR 13	<i>aro G</i>	37	3

^a Selection of "wild-type" recombinants was for growth in the presence of tyrosine and tryptophan, as *aro G* does not grow in the presence of these two amino acids (19).

^b The recombinants were purified by single-colony isolation and grown in the presence of 0.2 μ mole of 3MA/ml. The whole-cell assay for tryptophan synthetase (12) was used to determine whether 3MA had caused derepression.

TABLE 8. *DAHPSynthetase and A protein activities in recombinants between MAR 13 and aro G*

Recombinant	Growth conditions ^a	Specific activity		Inhibition by phenylalanine (%) ^b
		A protein	DAHPSynthetase	
W1485 <i>tna</i> \times <i>aro G</i>				
No. 1	3MA	.1057	.0780	83.7
No. 2	3MA	.1222	.0805	85.4
No. 19	Min	—	.1132	91.7
No. 21	Min	—	.1119	92.6
MAR 13 \times <i>aro G</i>				
No. 1	3MA	.0063	.0650	9.6
No. 2	3MA	.0045	.0613	10.4
No. 29	Min	—	.0902	8.2
No. 30	Min	—	.0648	5.0
No. 31	Min	—	.0877	3.6
No. 32	Min	—	.0733	5.9

^a Recombinants from crosses shown were grown overnight in minimal medium (Min) or minimal medium containing 0.2 μ moles of 3MA/ml (3MA).

^b The column indicates the per cent inhibition of DAHPSynthetase by 10^{-3} M phenylalanine.

DISCUSSION

The early pathway of aromatic biosynthesis exerts a regulatory influence on tryptophan production, because tryptophan feedback inhibition of anthranilate synthetase is competitive with chorismic acid. Thus, repression of the tryptophan operon in the presence of the derepressing analogue 7MT (or 3MA and 7-methylindole which are metabolized to 7MT) is a sensitive indicator of the amount of chorismic acid available to the tryptophan pathway.

Tyrosine at high concentrations can fully reverse 3MA derepression. Previous reports indi-

cate that tyrosine stimulates the synthesis of the phenylalanine-inhibitable DAHPSynthetase (4), the dominant DAHPSynthetase in *E. coli*. Tyrosine would be expected to repress enzymes of the tyrosine pathway as well as inhibit prephenate dehydrogenase. This would make more chorismic acid available for other pathways (i.e., the tryptophan pathway). This hypothesis is supported by the experiments which demonstrate that *trpD2* accumulates more anthranilic acid in the presence of tyrosine than in minimal medium. Also, if tyrosine increases the amount of chorismic acid

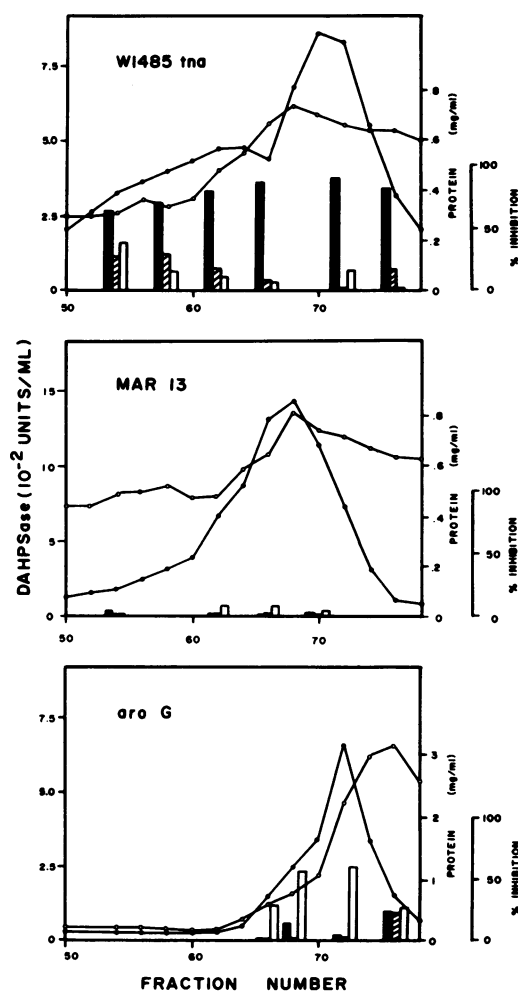


FIG. 1. DEAE-Sephadex chromatography of crude extracts of wild-type W1485 *tna*, MAR 13, and *aro G*. Solid circles indicate DAHPSynthetase activity; open circles, protein. Bar graph indicates the per cent inhibition by phenylalanine (solid bar), tyrosine (striped bar), or tryptophan (open bar). Only those fractions containing DAHPSynthetase activity are shown. A total of 120 fractions of about 6 ml each were collected.

available to the tryptophan pathway, tyrosine should indirectly reverse the normal tryptophan feedback inhibition of anthranilate synthetase, causing increased tryptophan synthesis and repression. Thus, wild-type cells grown on tyrosine alone are slightly repressed (Table 2).

Tyrosine and phenylalanine together are ineffective in reversing 3MA or 7-methylindole derepression. Also, W1485 *tna* grown in minimal medium supplemented with phenylalanine and tyrosine is slightly derepressed (Table 2). This has been noted by other investigators (3), and has been attributed to an inhibition of the phenylalanine and tyrosine isoenzymatic DAHP synthetases. Presumably, the amount of DAHP formed by the tryptophan DAHP synthetase is insufficient to allow production of tryptophan at repressing levels. This conclusion is also supported by experiments which show that *trpD2* accumulates much less anthranilic acid in the presence of phenylalanine and tyrosine than in minimal media with or without tyrosine. Thus, tyrosine and phenylalanine together do not reverse 3MA derepression because less chorismic acid is available for the tryptophan pathway.

Since tyrosine reversal of 3MA derepression appears to be related to increased amounts of chorismic acid, it should be possible to reverse 3MA derepression by supplying chorismic acid in the growth medium. However, chorismic acid cannot be utilized for growth of aromatic auxotrophs blocked prior to chorismic acid synthesis (9). Apparently, chorismic acid does not enter cells in sufficient quantities to allow growth of auxotrophs, and thus it was not possible to test the effects of chorismic acid on 3MA derepression directly. Shikimic acid, which can be utilized for growth by aromatic auxotrophs and can be converted to chorismic acid, however, does not reverse 3MA derepression. A possible explanation for this observation is that the chorismic acid formed from shikimic acid would be expected to be utilized for both tyrosine and phenylalanine biosynthesis as well as tryptophan biosynthesis. The concentration of shikimate used (or the rate at which it is metabolized) may not be high enough to raise the phenylalanine and tyrosine concentrations to levels which would cause repression of the phenylalanine and tyrosine pathways or significantly feedback-inhibit these pathways.

Although tyrosine would inhibit DAHP synthetase (*tyr*), this probably does not significantly decrease chorismic acid synthesis. But the tyrosine pathway would be repressed and prephenate dehydrogenase would be markedly inhibited, resulting in an increase in the level of chorismate for the tryptophan pathway.

MAR 13, a mutant which is resistant to 3MA

derepression, provides further evidence that alterations in the early pathway may affect tryptophan biosynthesis. This mutant appears to excrete small amounts of chorismic acid. Genetic analysis has shown that the locus which confers 3MA resistance is closely linked to *aroG*, the structural gene for the phenylalanine-inhibitable DAHP synthetase (19). The basis of 3MA resistance in this mutant appears to be an altered DAHP synthetase which is not subject to feedback inhibition by phenylalanine.

The DAHP synthetase (*phe*) is the predominant enzyme in *E. coli*, and in *MAR 13* this enzyme is feedback-resistant, resulting in a relatively large increase in the level of chorismate formed. This may result in increased levels of phenylalanine and tyrosine, which would repress and inhibit their respective pathways but would not significantly inhibit chorismate formation. The result is an excess of chorismic acid available for the tryptophan pathway which reverses 7MT induced derepression.

Ezekiel (8) has described a mutant similar to *MAR 13* in *E. coli* B Luria. This mutant, selected for resistance to thienylalanine, had a normal amount of DAHP synthetase but, unlike *MAR 13*, about half the enzyme in crude extracts was inhibited by tyrosine, and the other half was insensitive to inhibition by phenylalanine. Although crude extracts of *MAR 13* contain very low tryptophan- and tyrosine-inhibitable DAHP synthetase activity, there appeared to be a possibility that the mutation in *MAR 13* resulted in a complete loss of DAHP synthetase (*phe*) and a concomitant increase in the amount of the other isoenzymes. Therefore, crude extracts of *MAR 13* were chromatographed on DEAE-Sephadex. Previous reports (19, 20) have indicated that the DAHP synthetase isoenzymes could be separated by DEAE cellulose chromatography. Preliminary experiments in this laboratory with DEAE cellulose chromatography resulted in poor recoveries, so DEAE-Sephadex, which gave better recoveries and might be expected to give better separation, was used. However, separation of the DAHP isoenzymes by DEAE-Sephadex chromatography into definite peaks was not obtained. This may be due to the relatively low levels of the tyrosine and tryptophan isoenzymes present in crude extracts, or possibly to other factors. However, it is evident that the major peak of enzyme activity in W1485 *tna* is inhibited by phenylalanine, whereas in *MAR 13* this peak is not inhibited by phenylalanine or by tyrosine and tryptophan. Thus, it is unlikely that the mutation in *MAR 13* results in increased amounts of the tyrosine or tryptophan DAHP synthetase; rather, it probably is due to an altered phenylalanine DAHP syn-

thetase which is not feedback-inhibited by phenylalanine. This conclusion is also supported by genetic evidence which indicates that the mutation conferring 3MA resistance and feedback resistance to the DAHP synthetase (*phe*) is closely linked to *aroG*.

Growth of wild-type cells in the presence of tyrosine or mutations to a feedback-resistant DAHP synthetase (*phe*) appear to increase the amount of chorismic acid available for tryptophan biosynthesis which alters the regulatory properties of the tryptophan operon. Thus, the competitive nature of tryptophan feedback inhibition of anthranilate synthetase by chorismic acid appears to be an important regulatory function which allows for increased tryptophan biosynthesis under conditions where more of the early intermediates are being produced.

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LITERATURE CITED

1. Baker, T. I., and I. P. Crawford. 1966. Anthranilate synthetase. Partial purification and some kinetic studies on the enzyme from *Escherichia coli*. *J. Biol. Chem.* **241**:5577-5584.
2. Balou, C. E., H. O. L. Fischer, and D. L. MacDonald. 1955. The synthesis and properties of D-erythrose 4-phosphate. *J. Amer. Chem. Soc.* **77**:5967-5970.
3. Brown, K. D. 1968. Regulation of aromatic amino acid biosynthesis in *Escherichia coli* K12. *Genetics* **60**:31-48.
4. Brown, K. D., and C. H. Doy. 1966. Control of three isoenzymic 7-phospho-2-oxo-3-deoxy-D-arabino-heptonate-D-erythrose-4-phosphate lyases of *Escherichia coli* W and derived mutants by repressive and inductive effects of the aromatic amino acids. *Biochim. Biophys. Acta* **118**:157-172.
5. Cotton, R. G. H., and F. Gibson. 1965. The biosynthesis of phenylalanine and tyrosine; enzymes converting chorismic acid into prephenic acid and their relationships to prephenate dehydratase and prephenate dehydrogenase. *Biochim. Biophys. Acta* **100**:76-88.
6. Doy, C. H. 1967. Tryptophan as an inhibitor of 3-deoxy-arabino-heptulosonate 7-phosphate synthetase. *Biochem. Biophys. Res. Commun.* **26**:187-192.
7. Doy, C. H., and K. D. Brown. 1965. Control of aromatic biosynthesis: the multiplicity of 7-phospho-2-oxo-3-deoxy-D-arabinoheptonate-D-erythrose-4-phosphate-lyase (pyruvate phosphorylating) in *Escherichia coli*. *Biochim. Biophys. Acta* **104**:377-389.
8. Ezekiel, D. H. 1965. False feedback inhibition of aromatic amino acid biosynthesis by β -2 thienylalanine. *Biochim. Biophys. Acta* **95**:54-62.
9. Gibson, F., and J. Pittard. 1968. Pathways of biosynthesis or aromatic amino acids and vitamins and their control in microorganisms. *Bacteriol. Rev.* **32**:465-492.
10. Gibson, M. K., and F. Gibson. 1965. Preliminary studies on the isolation and metabolism of an intermediate in aromatic biosynthesis: chorismic acid. *Biochem. J.* **90**:248-256.
11. Held, W. A., and O. H. Smith. 1970. Mechanism of 3-methyl-anthranilic acid derepression of the tryptophan operon in *Escherichia coli*. *J. Bacteriol.* **100**:209-217.
12. Ito, J., and I. P. Crawford. 1965. Regulation of the enzymes of the tryptophan pathway in *Escherichia coli*. *Genetics* **52**:1303-1316.
13. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**:190-206.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
15. Pittard, J., J. Camakaris, and B. J. Wallace. 1969. Inhibition of 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthetase (*trp*) in *Escherichia coli*. *J. Bacteriol.* **97**:1242-1247.
16. Smith, O. H., and C. Yanofsky. 1962. Enzymes involved in the biosynthesis of tryptophan, 794-806. In S. P. Colowick and N. O. Kaplan (ed.), *Methods in enzymology*, vol. 5. Academic Press Inc., New York.
17. Srinivasan, P. R., and D. B. Sprinson. 1959. 2-Keto-3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase. *J. Biol. Chem.* **234**:716-722.
18. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. *J. Biol. Chem.* **218**:97-106.
19. Wallace, B. J., and J. Pittard. 1967. Genetic and biochemical analysis of the isoenzymes concerned in the first reaction of aromatic biosynthesis in *Escherichia coli*. *J. Bacteriol.* **93**:237-244.
20. Wallace, B. J., and J. Pittard. 1967. Chromatography of 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthetase (*trp*) on diethylaminoethyl cellulose: a correction. *J. Bacteriol.* **94**:1279-1280.