ACTIVATION OF TYROSINE ANALOGS IN RELATION TO ENZYME REPRESSION

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Investigations in this laboratory (1) have demonstrated the existence of two enzymes in Escherichia coli 83-24 which catalyze the first step in the biosynthesis of the aromatic amino acids, the formation of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP). The activity of one of the DAHP synthetases is inhibited in vitro by phenylalanine (phe-DAHP synthetase) and the activity of the other is inhibited in vitro by tyrosine (tyr-DAHP synthetase). The synthesis of the tyr-DAHP synthetase was found to be repressed by tyrosine and by a number of structural analogs of tyrosine (2). Several of the tyrosine analogs which are as effective as tyrosine in repressing the synthesis of the tyr-DAHP synthetase in E. coli 83-24 do not inhibit the growth of the parent organism, E. coli W (2). Still another analog, 4-aminophenylalanine, which is more effective than tyrosine in repressing enzyme synthesis, inhibits the growth of E. coli W, but its growth inhibitory effects are completely overcome by shikimic acid (2).

Recent reports by several investigators (3,4,5,6) have indicated that the ability of an amino acid to regulate its own biosynthesis by feedback repression is dependent upon the ability of the amino acid to be activated and transferred to sRNA by its specific aminoacyl-RNA synthetase. Calendar and Berg (7) have shown that 3-fluorotyrosine and

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5-hydroxy-2-pyridinealanine are activated by the tyrosyl-RNA synthetases of *E. coli* B and *Bacillus subtilis*. It was, therefore, of interest to determine whether or not other analogs of tyrosine which are capable of substituting for tyrosine in repressing the tyr-DAHP synthetase but do not appear on the basis of growth data to interfere with the utilization of tyrosine for general protein synthesis, are activated by tyrosyl-RNA synthetase. The results of the present investigation show that tyrosine analogs of this type are not activated by crude or purified preparations of tyrosyl-RNA synthetase, indicating that activation of the feedback repressor is not required for the repression of the tyr-DAHP synthetase in *E. coli* 83-24.

EXPERIMENTAL

Materials. - Chromatographically pure L-phenylalanine, L-tyrosine and L-tryptophan were purchased from Calbiochem. The tyrosine analogs were the generous gifts of Dr. C. G. Skinner, Dr. L. C. Smith, and Dr. A. L. Davis. *E. coli* mutant 83-24 which is blocked after shikimic acid (8) was kindly furnished by Dr. B. D. Davis. Potassium ^{32}P-pyrophosphate was prepared by pyrolysis of potassium ^{32}P-phosphate at 400° for 1 hour and was diluted without further purification, with appropriate amounts of sodium pyrophosphate.

Preparation of Cell Extracts and Fractionation of Tyrosyl-RNA Synthetase. - Cells of *E. coli* 83-24 were grown for 17 hours at 37° in minimal medium A (9) supplemented with 0.2% Difco yeast extract and 0.2% Sheffield NZ case. The cells were harvested, washed and resuspended in 0.04 M Tris buffer, pH 7.5, containing 0.01 M β-mercaptoethanol. Cell extracts were obtained by exposing the cells to sonic oscillation, followed by centrifugation at 15,000 x g. The crude extracts were dialyzed for 2 hours at 4° against 0.04 M Tris buffer, pH 7.5, just prior to use in the ATP-PP_i exchange assays. Purified tyrosyl-RNA

synthetase was obtained from the crude extract by precipitation with ammonium sulfate between 60 and 70 per cent of saturation followed by adsorption on a DEAE-cellulose column and elution with 0.15 M potassium chloride. Preparations obtained in this manner show a 20-fold increase in the specific activity of the tyrosyl-RNA synthetase as measured by the transfer of ^{14}C-tyrosine to sRNA and contain very little, if any, phenylalanyl- and tryptophanyl-RNA synthetases.

ATP-PP_i Exchange. - The reaction mixture contained, in a total volume of 1 ml, ATP, 2 mM; magnesium chloride, 10 mM; Tris buffer, 0.1 M, pH 7.7; β-mercaptoethanol, 20 mM; sodium ^{32}P-pyrophosphate, containing approximately 3 x 10^5 c.p.m.; enzyme, either crude or purified, and L-tyrosine or tyrosine analogs, as indicated. After incubation for 10 minutes at 37°, the reaction was stopped by the addition of 0.5 ml of 7% perchloric acid, and the amount of ^{32}P incorporated into ATP was determined by a modification of the method described by Berg (10).

Enzyme Synthesis. - Cells of E. coli 83-24, grown as described above, were washed twice and resuspended at a concentration of about 1 mg dry weight of cells per ml in minimal medium A supplemented as indicated in Table II. After incubation at 37° for 2 hours, the cells were treated with toluene and assayed for tyr-DAHP synthetase (2) and ornithine transcarbamylase (11) as previously described. To measure the synthesis of β-galactosidase, the cells were incubated for 2 hours in minimal medium A containing 2 mg of lactose per ml instead of glucose, treated with toluene, and assayed for β-galactosidase as previously described (12).

RESULTS AND DISCUSSION

The ability of a number of analogs of tyrosine to be activated by crude extracts of E. coli 83-24 and by purified preparations of tyrosyl-RNA synthetase from this organism is shown in Table I. The ability of these analogs to repress the synthesis of the tyr-DAHP synthetase in E. coli 83-24 and to inhibit the growth of E. coli W has been reported

previously (2). Phenylalanine, which is one tenth as active as tyrosine in repressing the synthesis of the tyr-DAHP synthetase in E. coli 83-24, is activated by crude extracts but not by tyrosyl-RNA synthetase. Tryptophan, which has only slight activity in repressing the synthesis of the tyr-DAHP synthetase, is activated by the crude extract but not by the purified tyrosine activating enzyme. 3-Fluorotyrosine and 5-hydroxy-2-pyridinealanine repress the synthesis of the tyr-DAHP synthetase; however, these analogs inhibit the growth of E. coli W, and their growth inhibitory effects are not appreciably overcome by shikimic acid. As would be expected, these analogs, which are activated by the tyrosyl-RNA

TABLE I

Activation of Tyrosine Analogs by Crude and Purified Extracts of E. coli 83-24

Supplements	Concentration mM	Crude extract	Purified tyrosyl-RNA synthetase
		c.p.m. in ATP*	
None		123	24
L-Tyrosine	0.01	1322	1370
	0.1	2979	2710
L-Phenylalanine	0.1	1759	29
	1.0	-	32
L-Tryptophan	0.1	2846	75
	1.0	-	78
DL-3-Fluorotyrosine	0.2	1294	1550
	2.0	2287	2250
DL-5-Hydroxy-2-pyridinealanine	0.2	464	605
	2.0	1453	2040
L-4-Aminophenylalanine	1.0	162	38
DL-3-Nitrotyrosine	2.0	156	64
L-4-Nitrophenylalanine	1.0	243	21
DL-3-Thianaphthenealanine	2.0	199	33

*The reaction mixture described in the text contained ^{32}P-pyrophosphate, 320,000 c.p.m., and either crude extract, 40 μg of protein, or DEAE-cellulose fraction, 2 μg of protein. The c.p.m. reported above have been corrected for controls in which enzyme was omitted from the reaction mixture.

synthetase of E. coli B and B. subtilis (7), are also activated by the tyrosyl-RNA synthetase of E. coli 83-24.

In contrast, 4-aminophenylalanine, whose growth inhibitory effect in E. coli W is completely overcome by shikimic acid, is not activated by the tyrosyl-RNA synthetase of E. coli 83-24, nor is it activated by the tyrosyl-RNA synthetases of E. coli B or B. subtilis (13). In addition, no significant activation is observed with crude extract. This compound, however, represses the synthesis of the tyr-DAHP synthetase at a lower concentration than tyrosine. 3-Nitrotyrosine, which represses the synthesis of the tyr-DAHP synthetase at approximately the same concentrations as tyrosine, shows no evidence of activation by the tyrosyl-RNA synthetase at concentrations up to 100-fold above the effective level of tyrosine. 4-Nitrophenylalanine and 3-thianaphthenealanine, which do not inhibit the growth of E. coli W but do repress the synthesis of the tyr-DAHP synthetase in E. coli 83-24 at concentrations comparable to tyrosine, are not activated by the tyrosyl-RNA synthetase, nor is a significant activation observed with crude extract.

In separate experiments, those analogs which are not activated by the tyrosyl-RNA synthetase were tested for their ability to prevent the activation of tyrosine. At ratios of analog to tyrosine of 200 to 1, no significant reduction in the activation of tyrosine is observed. Also, of all the analogs listed in Table I, only 3-fluorotyrosine and 5-hydroxy-2-pyridinealanine were found to inhibit the transfer of ^{14}C-tyrosine to sRNA.

From these data, it appears that several analogs of tyrosine which are capable of inhibiting the synthesis of the tyr-DAHP synthetase in E. coli 83-24 are not activated by the tyrosyl-RNA synthetase or by crude extracts of this organism. The observed inhibition of synthesis of tyr-DAHP synthetase by these analogs could be the result of inhibition of general protein synthesis or the result of specific repression of enzyme

synthesis. In order to demonstrate the specificity of the inhibition, the effect of several of these analogs on the synthesis of ornithine transcarbamylase and of β-galactosidase was determined, and the results are shown in Table II. More than 20-fold increases in the amount of these enzymes are obtained under the conditions described in the Experimental section. Under these conditions, arginine represses the synthesis of ornithine transcarbamylase, and no detectable synthesis of β-galactosidase is observed when the medium is supplemented with glucose instead of lactose. In separate experiments a mixture of amino acids at a concentration of 0.05 μmoles of each amino acid per ml of medium was added during the two hour incubation period, and the synthesis of these enzymes was found to be no more than 2-fold greater than that observed in the absence of added amino

TABLE II

The Effect of Tyrosine Analogs on Enzyme Synthesis

Supplements during 2 hour incubation	Concentration mM	Enzyme Activity, Per Cent of Control		
		Tyr-DAHP synthetase	Ornithine transcarbamylase	β-Galacto-sidase
None		100 (0.6)*	100 (8.4)*	100 (3.4)*
L-Tyrosine	0.02	34	100	100
	0.05	<5	124	110
DL-3-Fluorotyrosine	0.1	<5	67	49
	0.2	<5	58	39
L-4-Aminophenylalanine	0.02	16	109	100
	0.1	<5	100	100
DL-3-Thianaphthenealanine	0.04	40	100	96
	0.2	10	116	90
L-Arginine	0.05	100	<5	110

*Micromoles of product formed per mg dry weight of cells under the assay conditions described in the text. The specific activity of the cells before the 2 hour incubation period was less than 5 per cent of these values.

acids, indicating that the endogenous supply of amino acids is not severely limiting. As seen in Table II, 3-fluorotyrosine, which is activated both by crude extracts and by purified tyrosyl-RNA synthetase, partially inhibits the synthesis of ornithine transcarbamylase and β-galactosidase, and completely inhibits the synthesis of tyr-DAHP synthetase. In contrast, 4-aminophenylalanine and 3-thianaphthenealanine, which are not significantly activated by crude extracts or by purified tyrosyl-RNA synthetase, do not inhibit the synthesis of ornithine transcarbamylase or β-galactosidase but do repress the synthesis of the tyr-DAHP synthetase. From these data, it appears that 4-aminophenylalanine and 3-thianaphthenealanine do not inhibit overall protein synthesis but do specifically repress the synthesis of the tyr-DAHP synthetase.

No evidence was obtained which would indicate that 4-aminophenylalanine and 3-thianaphthenealanine repress the synthesis of tyr-DAHP synthetase by a mechanism different than that of tyrosine, e. g. maintaining the concentration of endogenous tyrosine. Neither of these two analogs decreases the amount of tyrosine required for the growth of E. coli 83-24, and a mixture of 3-thianaphthenealanine and tyrosine are not synergistic in repressing the synthesis of the enzyme.

Therefore, it appears that, in the case of the tyr-DAHP synthetase in E. coli 83-24, repression of enzyme synthesis is probably not dependent upon the participation of the tyrosine activating enzyme involved in general protein synthesis.

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