THE CONTROL OF SHIKIMIC ACID SYNTHESIS BY TYROSINE AND PHENYLALANINE

Remedios G. Fradejas, Joanne M. Ravel and William Shive

Clayton Foundation Biochemical Institute
and the Department of Chemistry
The University of Texas, Austin

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The ability of tyrosine to inhibit phenylalanine biosynthesis in Escherichia coli (Texas strain), as well as the enhancement of this effect in cells which had been cultured in the presence of high concentrations of phenylalanine, led to the suggestion that tyrosine served in a mechanism of control of the biosynthesis of phenylalanine and related metabolites synthesized through a common pathway (Beerstecher and Shive, 1947a). The close relationship of tyrosine and phenylalanine biosynthesis originally demonstrated with metabolic antagonists (Beerstecher and Shive, 1947b) is now known to involve common intermediates (Davis, 1950) rather than a direct conversion. Subsequently, it was demonstrated that the triple auxotroph E. coli mutant 83-24, requiring aromatic amino acids, does not effectively feed the quintuple aromaticless auxotroph 83-1 unless the concentration of either phenylalanine or tyrosine in the medium is reduced, suggesting that phenylalanine and tyrosine prevent the accumulation of shikimic acid (Davis, 1951).

In the present investigation, the effects of tyrosine and phenylalanine in the control of their biosynthesis to the stage of shikimic acid formation have been studied in vivo and in vitro. Each amino acid alone partially inhibits the formation of the cyclic intermediates related to shikimic acid, and a synergistic control of the biosynthesis, reducing the formation of the intermediates to a low level, is observed on addition of both amino acids.

E. coli mutant 83-24[1] was grown for 17 hours in minimal medium A (Davis and Mingioli, 1950) supplemented with 0.2 per cent Difco yeast extract and 0.2 per cent Sheffield NZ-case. The cells were harvested, washed twice with 0.04 M potassium phosphate buffer, pH 7.4, and used for whole cell experiments. To prepare cell-free extracts, these cells were resuspended at a concentration of 1 mg. per ml. dry weight of cells (determined turbidimetrically) in unsupplemented minimal medium A and allowed to incubate 2 hours at 37°. The cells were then harvested again, washed, resuspended in 0.04 M potassium phosphate buffer, pH 7.4, and exposed to sonic oscillation in a Raytheon 10 kc oscillator for 15 minutes. Whole cells and cellular debris were removed by centrifugation at 18,000 x g for 30 minutes. The incubation mixtures (details given in footnotes to Table I) were assayed for cyclic intermediates by a previously described microbiological procedure (Kalan, Davis, Srinivasan, and Sprinson, 1956) using Aerobacter aerogenes mutant A170-143SI which responds equally well to dehydroquinic, dehydroshikimic, and shikimic acids. Assaying the incubation mixtures using E. coli mutant 83-1, which does not respond to dehydroquinic acid, gave comparable results, indicating that the end product actually formed is either dehydroshikimic acid or shikimic acid.

As demonstrated in Table I, tyrosine reduces the accumulation of shikimic acid or its dehydro derivative by intact cells of E. coli 83-24 to a level of 40 per cent of the control. Further increases in the concentration of tyrosine have little additional effect. Phenylalanine, while exerting little effect alone, greatly augments the inhibition by tryosine resulting in suppression of the accumulation of the intermediates below 1 per cent of the amount obtained in the absence of the two amino acids. A similar relationship can be demonstrated in cell-free preparations as indicated in Table I. Either tyrosine or phenylalanine reduces the formation of the cyclic intermediates from glucose-6-phosphate to about 35 per cent of the

[1] The mutant strains used in this work were kindly furnished by Dr. B. D. Davis.

Table I

The Effects of Tyrosine and Phenylalanine on the Accumulation of Cyclic Intermediates in Aromatic Biosynthesis

Supplements, μmoles per ml.		Accumulation, % of control	
L-Tyrosine	L-Phenylalanine	Whole cells*	Cell-free extracts**
0	0	100	100
0.03	0	46	65
0.1	0	42	34
0.3	0	38	37
3.0	0	--	35
0	0.03	74	64
0	0.1	79	60
0	0.3	78	38
0	3.0	--	35
0.01	0.01	29	58
0.03	0.03	16	52
0.1	0.1	<1	19
0.3	0.3	--	10

*Washed cells of E. coli 83-24 were resuspended at a concentration of 1 mg. per ml. dry weight of cells in minimal medium A containing glucose as the substrate and supplemented as indicated. After 2 hours incubation at 37°, the cells were removed by centrifugation; the supernatant solution heated for 15 minutes at 100°; and aliquots were assayed for cyclic intermediates. The accumulation of the cyclic intermediates by the unsupplemented control was equivalent to 0.79 μmoles of shikimic acid per ml.

**The cell-free extract of E. coli mutant 83-24 (0.1 ml. containing approximately 3 mg. of protein) was incubated with 20 μmoles of sodium glucose-6-phosphate, 5 μmoles of magnesium chloride, 25 μmoles of potassium phosphate buffer, pH 7.4, and supplements as indicated, in a total volume of 1 ml. After 2 hours incubation at 37°, the reaction was stopped by the addition of 0.03 ml. of 6 N hydrochloric acid. The reaction mixture was brought to pH 7 by the addition of 6 N potassium hydroxide; heated for 10 minutes at 100°; the precipitate removed by centrifugation; and aliquots assayed for cyclic intermediates. The amount of product synthesized by the unsupplemented control was equivalent to 0.3 μmoles of shikimic acid.

amount obtained in the absence of the two amino acids. Further increases in the concentrations of either amino acid do not reduce the conversion appreciably more. However, the addition of both tyrosine and phenylalanine produces a synergistic effect and further decreases the amount of the cyclic intermediates formed from glucose-6-phosphate. The addition of erythrose-

4-phosphate[2] and phosphoenolpyruvate (1 μmole of each) to the inhibited system does not increase the production of the cyclic intermediates even though in these experiments these substances effectively replace glucose-6-phosphate in producing the active intermediates as has been previously reported (Srinivasan, Katagiri and Sprinson, 1959). Thus, the conversion of erythrose-4-phosphate and phosphoenolpyruvate to dehydroquinic acid appears to be under the control of phenylalanine and tyrosine, each of which partially limits the conversion. This multiple metabolite control of the biosynthesis of an intermediate common to a group of end-products apparently is a general type of control mechanism since certain end-products of aspartic acid metabolism appear to control their own biosynthesis in such a manner (Wahba and Shive, 1954). The interaction of the enzyme with the controlling factors to form complexes with reduced enzymatic activity could account for this type of control. However, further investigation of these inhibitory effects are necessary to elucidate more exactly the mechanisms involved.

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[2]The cyclohexylammonium erythrose-4-phosphate dimethylacetal was a generous gift of Dr. C. E. Ballou and was converted to the sodium salt of erythrose-4-phosphate as previously described (Ballou et al., 1955).