REPRESSION AND INHIBITION OF 3-DEOXY-D-*ARABINO*-HEPTULOSONIC ACID 7-PHOSPHATE SYNTHETASE BY PARAFLUOROPHENYLALANINE IN ESCHERICHIA COLI

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Received April 29, 1964

The focal reaction in control of aromatic biosynthesis in *E. coli* is conversion of erythrose-4-phosphate and phosphoenolpyruvate to 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP). Smith et al. (1962) have demonstrated the presence of at least two DAHP synthetases in a triple aromatic mutant (83-24) of *E. coli*. These enzymes are under specific non-competitive inhibition by phenylalanine and tyrosine respectively. The end product amino acids also exert complex repressive action on the synthesis of these enzymes. The existence of multiple DAHP synthetases has been confirmed by Brown and Doy (1963) with the addition of a tryptophan repressible enzyme which is not inhibited by any of the aromatic amino acids.

In a separate publication (Previc and Binkley, 1964) we have described a slow exponential growth phase of *E. coli* in

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2. This work was supported by a research grant from the University of Illinois, and by USPHS Grants AI-3380 and CY-3231.

the presence of p-fluorophenylalanine (FPA). This phase succeeds the previously described linear growth induced in E. coli by analogs of phenylalanine (Cohen and Munier, 1959). The present report is a preliminary account of the effects of FPA on DAHP synthetase activity in the various phases of growth related to treatment with the analog.

E. coli 83-24, requiring phenylalanine, tyrosine and tryptophan, was grown in minimal salts-glucose medium plus 0.2% Difco yeast extract and 0.2% Difco vitamin-free casamino acids, then incubated 2 h in unsupplemented medium. Normal E. coli B was grown exponentially in minimal medium. Linear growth was induced by the addition of 10^{-3} M DL-FPA to normal, exponential E. coli B. The linear culture was harvested at 2.5 h post-induction at an O.D. of 0.65 (450 mμ). Inoculation for slow exponential growth was made very lightly into minimal medium plus 10^{-3} M DL-FPA from a 2.5 h linear culture and growth was continued 17 h with a mass doubling time of 127 min to an O.D. of 0.80. All the above cultures were harvested by rapid chilling to 0°C., followed by centrifugation. All subsequent handling was at 0-5°C. The cells, washed twice in 0.04 M potassium phosphate buffer, pH 7.4, were resuspended in the same buffer. Disruption was in a French pressure cell at 15,000 psig. The effluents were centrifuged at 20,000 x g for 10 min and the supernatants dialyzed 4 h against 0.04 M potassium phosphate buffer, pH 7.4. These crude extracts were stored at 0-5°C. and used within 18 h of preparation without interim freezing. Crystalline serum albumin was used as the standard for protein determinations by the method of Lowry et al. (1951). The DAHP synthetase assay was that of Srinivasan and Sprinson (1959) with the modification of Smith et al. (1962).

Extracts from de-repressed E. coli mutant 83-24 were assayed for DAHP synthetase activity and tested for inhibition of activity by phenylalanine and tyrosine. The results were essentially the same as those obtained by Smith et al. (1962). For example, at 0.05 mM, L-phenylalanine inhibited total activity by 21% and L-tyrosine by 69%. When these extracts were assayed in the presence of DL-FPA, 0.05 mM effected a 9% inhibition.

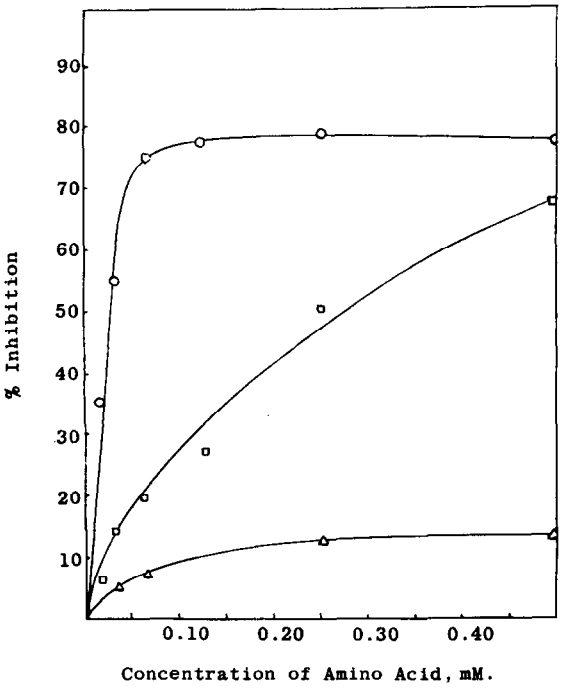

Fig. 1. Inhibition of DAHP Synthetase Activity by Aromatic Amino Acids. The reaction mixture contained: erythrose-4-phosphate, 1.0 μmole; phosphoenolpyruvate, 1.0 μmole; potassium phosphate buffer, pH 6.4, 100 μmoles; cell extract containing sufficient DAHP synthetase activity to give 30-50% conversion of substrates in the uninhibited systems; and (o) L-phenylalanine, (△) L-tyrosine, and (□) DL-parafluorophenylalanine as indicated in a total volume of 1.0 ml. The reaction mixture was incubated at 37°C. for 10 min and the amount of DAHP formed was determined by the modification of Smith et al. (1962).

Similar studies were made on E. coli B: (a) in exponential growth in minimal medium; (b) in linear growth induced by FPA; and (c) in slow exponential growth in the presence of FPA. As shown in Fig. 1, the DAHP synthetase activity of the extracts from untreated E. coli B was inhibited more extensively by phenylalanine than by tyrosine. The maximum level of inhibition by phenylalanine (79%) was attained near 0.1 mM concentration of the amino acid. Inhibition by FPA approached that by phenylalanine only at much higher levels. This same relationship was true in the extracts from the late linear phase and from the slow exponential phase. These gave inhibition patterns with the same general shapes as in Fig. 1. More significant was the overall uninhibited activity in extracts from FPA-treated cultures. Table 1 shows that the induction of linear growth by FPA resulted in a severe repression of DAHP synthetase activity, relative to untreated cells.

Table 1

DAHP Synthetase Activity in E. coli B Under Different Conditions of para-Fluorophenylalanine Treatment[a]

Supplement	Phase	DAHP Synthetase Relative Activity[b]	% Inhibition[c] PA	TYR	FPA
None	Exponential (Normal)	1.00	79	13	68
FPA	Linear	0.13	82	23	48
FPA	Slow exponential	0.05	81	29	66 (78)[d]

a. Assay procedures were same as those described in Fig. 1.
b. Relative activities are based on mg protein in the extracts.
c. Values are for 0.5 mM amino acid and are maximum only for PA.
d. This value is maximum for FPA and is at 2.5 mM.

Continued repression was evident in the slow exponential phase.

We have suggested elsewhere (Previc and Binkley, 1964) that the linear mass increase and inhibition of cell division by FPA in E. coli B are separable phenomena, the former a result of interference with phenylalanine metabolism and the latter a result of tyrosine deprivation. In the present report, FPA is shown to severely repress DAHP synthetase activities that are sensitive to both phenylalanine and tyrosine. The data give no indication of possible effects of FPA on other enzymes at later stages in the diverging pathways of aromatic biosynthesis. It does show that deficiency of DAHP, a precursor of both phenylalanine and tyrosine, probably leads to a severe imbalance in the supply of these amino acids. Resumption of cell division and exponential growth is governed by the rate at which a particular FPA level permits production of these necessary metabolites.

We are grateful to Dr. D. B. Sprinson for a sample of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate and to Dr. B. D. Davis for E. coli mutant 83-24.

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