Regulation of Chemoautotrophic Metabolism

III. DAHP Synthetase in *Thiobacillus neapolitanus*

D. P. KELLY

Microbiology Department, Queen Elizabeth College, London

Received August 28, 1969

*Summary.* DAHP synthetase (PODH lyase, EC4.1.2.15.) activity was demonstrated in undialysed and dialysed extracts of the wild type strain C and phenylalanine-resistant variant P4 of *T. neapolitanus.* Activity at pH 6.4 in extracts of both strains was inhibited at least 50% by 10^{-5} M phenylalanine. Strain C enzyme was inhibited at least 80% by 10^{-4} M tyrosine, but was relatively unaffected by tryptophan. Tryptophan stimulated the P4 enzyme threefold at 10^{-8}--10^{-4} M. Inhibition of the P4 enzyme by phenylalanine could be virtually completely prevented by tyrosine or tryptophan, but these acids and histidine were much less effective in preventing inhibition of the strain C enzyme. Maximum activity in extracts of both strain C and P4 was obtained at pH 8.9, at which pH DAHP synthesis was 8 times greater than at pH 6.4. Activity at pH 8.9 in dialysed extracts of P4 showed $K_M$ values of $1.43 \times 10^{-5}$ M and $4 \times 10^{-3}$ M for E-4-P and PEP respectively. $K_I$ values for competitive inhibition by L-phenylalanine were $5.4 \times 10^{-6}$ M and $1.45 \times 10^{-5}$ M respectively for ranges of concentration of E-4-P and PEP.

Inhibition of the growth of strain C by phenylalanine was concluded to be due to prevention of tyrosine and tryptophan synthesis through inhibition of DAHP synthesis. Resistance to phenylalanine was conferred on the mutant P4 by its possession of a system by which inhibition of DAHP synthesis by phenylalanine was prevented by tyrosine and tryptophan.

Inhibition of the growth of *T. neapolitanus* by phenylalanine may be due to interference by phenylalanine with the synthesis of aromatic amino acids (Kelly, 1969a, b). One enzyme likely to be under regulation by the end products of the branched pathway of aromatic amino acid biosynthesis is the initial enzyme of the pathway, DAHP synthetase, which catalyses the conversion of erythrose-4-phosphate and phosphoenolpyruvate to 3-deoxy-D-arabino-heptulosonate-7-phosphate (Doy, 1968; Doy and Brown, 1965; Gibson and Pittard, 1968; Lingens, 1968). This paper reports some of the properties of the DAHP synthetase activity in crude extracts of wild type and phenylalanine-resistant strains of *T. neapolitanus.* Inhibition of the growth of strain C by phenylalanine and resistance to inhibition in P4 may both be explained by the different responses of their DAHP synthetase enzymes to aromatic amino acids.
Materials and Methods

Large Scale Preparation of Thiobacillus neapolitanus. T. neapolitanus strain C or phenylalanine-resistant mutant P4 (Kelly, 1969a) were obtained from a continuous culture (Kelly, 1968) or from 12 litre volumes of culture grown in a New Brunswick MicroFerm Laboratory fermentor, aerated with 4 litre of air/min, stirred at 300 r.p.m and maintained at pH 6 by the automatic addition of M K₂CO₃. Organisms were harvested by centrifuging at 2°C, washed with phosphate buffer and, if not used at once, stored at −20°C as packed pellets.

Preparation of Cell-Free Extracts. Suspensions of 5–8 g fresh weight of bacteria in 10 ml 0.05 M potassium phosphate pH 6.4 were passed once through a French pressure cell at 0°–5°C under a pressure of 20,000 lb./in.². These extracts were centrifuged at 2°C for 30 min at approx. 20,000 × g. Supernatant liquids were retained as the source of enzyme, and are referred to as the crude extract.

Dialysis of the Crude Extract. Undialysed crude extracts were used in a number of the experiments described below. For dialysis, crude extract in Visking dialysis tubing was gently agitated in 200 volumes of 0.033 M potassium phosphate buffer, pH 8.0 at 2°C for 4 hr.

Measurement of Protein in Crude and Dialysed Extracts. Routinely the method of Lowry, Rosebrough, Farr and Randall (1951) was used. Check determinations by the ultraviolet absorption method of Warburg and Christian (Layne, 1957) were sometimes performed.

Assay of DAHP synthetase [or 7-phospho-2-oxo-3-deoxy-D-arabino-heptonate:D-erythrose-4-phosphate-lyase (pyruvate phosphorylating), PODH lyase, EC 4.1.2.15]. The procedure adopted was based principally on that of Srinivasan and Sprinson (1959) and Sprinson, Srinivasan and Katagiri (1962), with some modifications suggested by Smith, Ravel, Lax and Shive (1962) and Doy and Brown (1965).

Reaction mixtures for the standard assay procedure contained in a final volume of 1 ml: 100 μmoles potassium phosphate buffer (pH 6.4 or 8.9); 0.75 μmole D-erythrose-4-phosphate (sodium salt, E-4-P); 0.75 μmole trisodium phosphoenolpyruvate (PEP); 100 μl Thiobacillus extract (2–4 mg protein). Reaction was initiated by adding the extract to the other solution after 5 min preincubation at 37°C. After 6 min the reaction was stopped by adding 0.2 ml of 20% trichloracetic acid. Zero-time blanks were always prepared by adding the TCA to a reaction mixture immediately prior to the extract. Precipitates were removed by centrifuging, and DAHP in the supernatant liquids was determined. A mixture of 0.5 ml supernate with 0.5 ml 0.025 M periodic acid in 0.125 N H₂SO₄ was placed in a water bath at 37°C for 30 min; 1 ml 2% (w/v) potassium arsenite in 0.5 N HCl was added, and the iodine colour was subsequently discharged in 2–3 min at room temperature. After adding 4 ml of 0.3% (w/v) thiobarbituric acid (Sprinson, Srinivasan and Katagiri, 1962) the mixture was placed in a boiling water bath for 9 min, then cooled to about 20°C in a water bath. Routinely the pink chromophore produced by this procedure was extracted into 4 ml cyclohexanone (Warren, 1959; Doy and Brown 1965) and its absorbance read against water-saturated cyclohexanone at 549 μₘ in a Cary model 14 recording spectrophotometer or a Unicam SP. 500 spectrophotometer. Enzyme activity was frequently low, but absorbance could be determined within ± 0.0005 absorbance unit with the Cary spectrophotometer. The cyclohexanone procedure was more reliable than comparison of readings on the assay solutions at 549 and 610 or 625 μₘ as described by Doy and Brown (1965) or Minamikawa (1967).

Modifications to the Standard Procedure. To study the kinetics of the DAHP synthesis, a concentration of 1 μmole of E-4-P or PEP per ml of reaction mixture

25 Arch. Mikrobiol., Bd. 69