Institutes of Health, U. S. Public Health Service. One of us (T.S.P.) was supported as an NSF Undergraduate Research Participant. The signal encouragement and advice of Dr. ROBERT M. FINK is gratefully acknowledged.

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Received October 19th, 1966

Biochim. Biophys. Acta, 132 (1967) 525–528

BBA 61125

A kinetic study of the mechanism of action of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase in *Escherichia coli* K 12

The first step in the biosynthesis of aromatic amino acids in microorganisms is the formation of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) from erythrose 4-phosphate and phosphoenolpyruvate (ref. 1). Three distinct isoenzymes of DAHP synthase (7-phospho-2-oxo-3-deoxy-D-*arabino*-heptonate D-erythrose-4-phosphate-lyase (pyruvate phosphorylating), EC 4.1.2.15) which catalyze the formation of DAHP were detected in *Escherichia coli* K 12 and W (refs. 2–4). All these DAHP synthases catalyze the formation of DAHP, but the activity of isoenzyme 1a is inhibited allosterically by phenylalanine. Isoenzyme 1b is inhibited by tyrosine and isoenzyme 1c is not inhibited by any of the aromatic end-products.

The present paper describes kinetic studies on the mechanism of action of purified phenylalanine-sensitive DAHP synthases and the results obtained are compatible with the mechanism termed "Ping-Pong"[5].

Wild-type cells of *E. coli* K 12 were grown on mineral salts medium A containing 0.2% glucose[6]. Cells harvested from logarithmic-phase culture were washed twice in 0.1 M potassium phosphate buffer (pH 7.4), disrupted by sonic treatment and the extract was centrifuged. From the supernatant the phenylalanine-sensitive DAHP synthase was purified 48-fold in comparison to the crude extract by ammonium sulfate

Abbreviations: DAHP, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate; E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate.

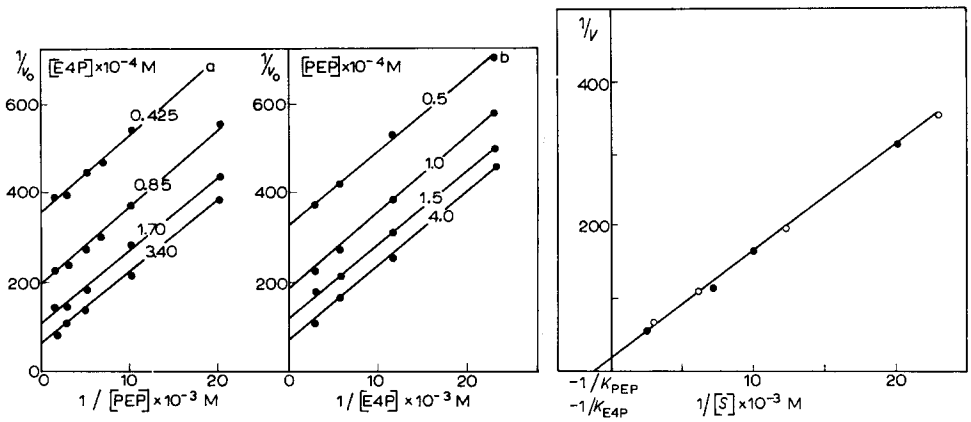


Fig. 1. Double-reciprocal plot of initial velocities (v_0) against phosphoenolpyruvate concentration at a series of fixed concentrations of erythrose 4-phosphate (a) and against erythrose 4-phosphate concentration at a series of fixed phosphoenolpyruvate concentrations (b). Initial velocity is expressed as μmoles of DAHP formed per 10 min. The reaction mixture contains 100 μmoles of potassium phosphate buffer (pH 7.5), 40 μg of protein, phosphoenolpyruvate and erythrose 4-phosphate as indicated in a total volume of 1.0 ml.

Fig. 2. Secondary plots from data of Fig. 1. Graphical analysis yields $1/V$ as intercept on the ordinate and $-1/K_{PEP}$ and $-1/K_{E4P}$ as intercepts on the abscissa. ●—●, $1/V'$ (Fig. 1a) against 1/PEP; ○—○, $1/V'$ (Fig. 1b) against 1/E4P.

fractionation and calcium phosphate gel adsorption. The purified enzyme is free from isoenzymes 1b and 1c.

The standard reaction mixture for the determination of DAHP synthase activity contains 100 μmoles of potassium phosphate buffer (pH 7.5), 0.5 μmoles of phosphoenolpyruvate and 0.5 μmoles of erythrose 4-phosphate in an total volume of 1.0 ml. The reaction mixtures were incubated at 37°. The reaction was stopped by the addition of 0.4 ml of 10% trichloroacetic acid. After sedimentation of the precipitated proteins, the amount of DAHP formed was determined by the thiobarbituric acid method[1].

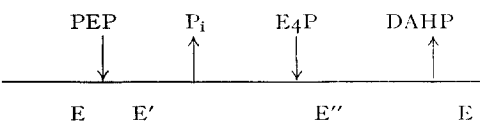
As shown in Fig. 1, the plot of the reciprocal of the initial rate of production of DAHP against the reciprocal of the phosphoenolpyruvate concentration at the four fixed concentrations of erythrocyte 4-phosphate gives a set of parallel lines. No apparent convergence of the lines at a common point is evident. The plot of the reciprocal of the erythrose 4-phosphate concentration at the four fixed concentrations of phosphoenolpyruvate also gives a set of parallel lines. The intercepts on the ordinate are the reciprocals of the apparent maximal velocities, $1/V'$, and the intercepts on the abscissae are negative reciprocals of apparent Michaelis constants, $1/K_m'$. The Michaelis constants of the enzyme for erythrose 4-phosphate (K_{E4P}) and for phosphoenolpyruvate (K_{PEP}) obtained graphically from the secondary plots of the values of $1/V'$ obtained in Figs. 1 and 2 against the reciprocal of the concentration of erythrose 4-phosphate and of phosphoenolpyruvate are 1.0 mM for both erythrose 4-phosphate and phosphoenolpyruvate (Fig. 2).

Since the apparent K_m''s for phosphoenolpyruvate and erythrose 4-phosphate calculated according to Eqns. 1 and 2 completely agree with K'_m values obtained graphically (Figs. 1 and 2):

$$K'_{PEP} = \frac{K_{PEP}}{\left(1 + \frac{K_{E4P}}{[E4P]}\right)} \quad (1)$$

$$K'_{E4P} = \frac{K_{E4P}}{\left(1 + \frac{K_{PEP}}{[PEP]}\right)} \quad (2)$$

and since the ratio of apparent Michaelis constant to apparent maximum velocity remains constant as the concentration of the fixed substrate is changed, the mechanisn of the reaction appears to be "Ping-Pong"[5,7]. Characteristics of the "Ping-Pong" mechanism are that the enzyme reacts with the first substrate and a product is released before the addition of the second substrate. According to our studies, phosphoenolpyruvate itself stabilizes the enzyme against thermal inactivation, while erythrose 4-phosphate is completely ineffective in these experiments. Furthermore, during the overall reaction P_i is released from phosphoenolpyruvate. It follows from these findings that the mechanism of reaction may be schematically represented in the following manner:



Scheme 1.

The symbols below the line represent various enzyme intermediates participating in the reaction.

A detailed analysis of the mechanism of reaction and of allosteric inhibition is under investigation.

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Received November 15th, 1966

Biochim. Biophys. Acta, 132 (1967) 528–530