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A kinetic study on the inactivation of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase by bromopyruvate

Three distinct isoenzymes of 7-phospho-2-oxo-3-deoxy-D-*arabino*-heptonate D-erythrose-4-phosphate-lyase (pyruvate phosphorylating), EC 4.1.2.15 (referred to hereafter as 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase), which catalyze the first step of the biosynthesis of the aromatic amino acids, have been detected in *Escherichia coli* K 12 (ref. 1). The formation of DAHP from erythrose 4-phosphate and phosphoenolpyruvate (PEP) by isoenzyme 1a is inhibited allosterically by phenylalanine[2]. The mechanism of action of the phenylalanine-sensitive DAHP synthase is "ping-pong" and the first substrate of the enzyme is PEP (ref. 3). MELOCHE[4] observed that bromopyruvate inactivates the 6-phospho-2-keto-3-deoxy-D-gluconate D-glyceraldehyde-3-phosphate-lyase (EC 4.1.2.14) and pyruvate protects the enzyme against the inactivating effect of bromopyruvate. We have found a similar inactivating effect of bromopyruvate on the phenylalanine-sensitive DAHP synthase and the present paper describes the kinetics of inactivation of the enzyme by bromopyruvate and the kinetic analysis of the protection of the enzyme by PEP.

The growth conditions of the wild-type *E. coli* K 12 and the purification of the enzyme were described previously[3]; bromopyruvate was prepared chemically[5].

As Fig. 1a shows, bromopyruvate inactivates the enzyme and the rate constant k_1 of the pseudo-first-order reaction may be expressed as

$$k_1 = \frac{\log E_0 - \log E_t}{t} 2.3 \qquad (1)$$

where E_0 and E_t are the amounts of active enzyme at zero time and time t respectively.

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

The values of the rate constant k_1 depend on the concentration of bromopyruvate. As shown in Fig. 1b, PEP protects the enzyme against the inactivating effect of bromopyruvate. This protective effect of PEP is specific in the sence that neither erythrose 4-phosphate, the second substrate of the enzyme, nor phenylalanine, the allosteric inhibitor, has any effect on the rate inactivation of the enzyme by bromopyruvate. The inactivation of the enzyme by bromopyruvate in the presence of PEP follows a pseudo-first-order reaction also, and the rate constant k' can be calculated according to Eqn. 1.

The reaction between enzyme E and bromopyruvate may be influenced by the presence of the substrate PEP which combines with the enzyme, forming E–PEP complex. In this case it is possible to calculate the pseudo-first-order rate constant k_2 for the reaction between bromopyruvate and the E–PEP complex and the dissociation constant K of the E–PEP complex. The dissociation constant K may be expressed as

$$K = \frac{[E]\,[\mathrm{PEP}]}{[E\text{–PEP}]} \tag{2}$$

The value of K can be determined graphically using Eqn. 3 (refs. 7, 8)

$$\frac{k'}{k_1} = \frac{K}{[\mathrm{PEP}]}\left(1 - \frac{k'}{k_1}\right) + \frac{k_2}{k_1} \tag{3}$$

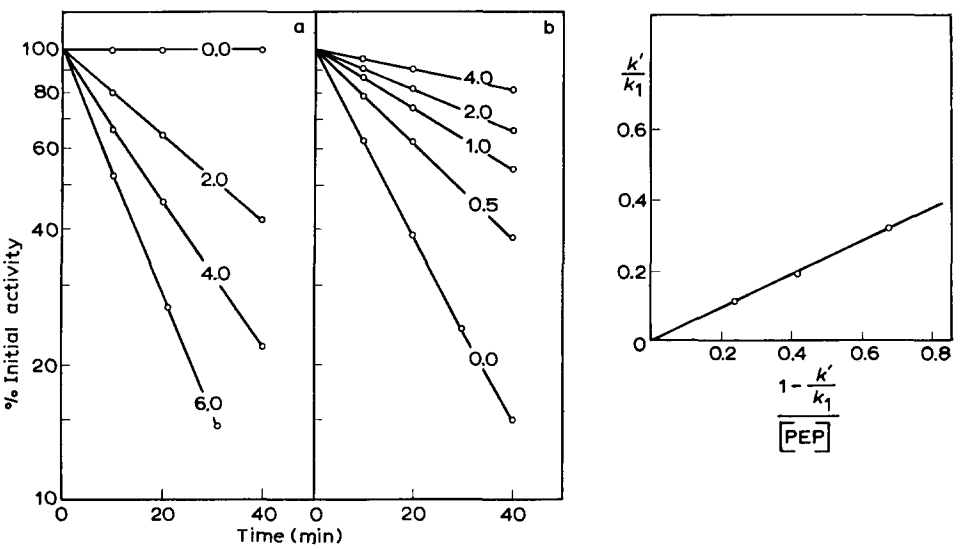

Fig. 1. The inactivation of DAHP synthase by bromopyruvate in the absence (a) and in the presence (b) of phosphoenolpyruvate. 0.1 M potassium phosphate buffer (pH 7.8) containing 1 mg of protein per ml and various concentrations of bromopyruvate as indicated (a), or 4 mM of bromopyruvate and various concentrations of PEP as indicated (b) were incubated at 22°. Samples were taken at zero time and at other times as required and diluted fifty-fold in a reaction mixture containing 100 μmoles of potassium phosphate buffer (pH 7.5), 0.5 μmoles of PEP, 0.5 μmoles of erythrose 4-phosphate in a total volume of 1 ml for the determination of enzyme activity. The mixtures were incubated at 37° and the reaction was arrested by the addition of of 0.4 ml of 10% trichloroacetic acid. The amount of DAHP formed was determined by the thiobarbituric acid method[6].

Fig. 2. Secondary plot of the pseudo-first-order rate constants k_1 and k' obtained from Fig. 1a and 1b. The slope of the straight line from the plot of k'/k_1 *vs.* $(1 - k'/k_1)/[\mathrm{PEP}]$ is equal to K.

The secondary plot of k_1 and k' obtained from Figs. 1a and 1b gives, according to Eqn. 3, a straight line as shown in Fig. 2. The dissociation constant K of the E–PEP complex is $4.5 \cdot 10^{-5}$. The intercepts on the ordinate and on the abscissa are equal to zero. This means that at infinite concentrations of PEP, k_2 becomes zero and PEP completely protects the enzyme against the inactivating effect of bromopyruvate.

On the basis of this kinetic evidence on the nature and specificity of the inactivating effect of bromopyruvate it is probable that bromopyruvate reacts with the PEP-binding site of DAHP synthase. The peptide sequence of the PEP-binding site of the enzyme labelled by bromopyruvate is under investigation.

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On the nature of the allosteric transition of *N*-acetylglutamate-5-phosphotransferase

It was found earlier that the allosteric inhibition of the activity of ATP:*N*-acetyl-L-glutamate-5-phosphotransferase* (referred to hereafter as phosphotransferase) by arginine is kinetically an apparent second-order reaction[1]. This conventionally means that two molecules of arginine are bound to one molecule of enzyme when inhibition occurs.

The present paper reports kinetic experiments and thermodynamic calculations on the allosteric transition of the phosphotransferase.

The purified enzyme was prepared as described previously[1]. The "standard" reaction mixture for the determination of enzyme activity and of inhibition contains 200 μmoles of hydroxylamine hydrochloride, 200 μmoles of Tris base, 150 μmoles of *N*-acetyl-L-glutamate, 20 μmoles of ATP, 20 μmoles of $MgCl_2$ and L-arginine as indicated in a total volume of 2.0 ml. The reaction mixtures were incubated at the temperature indicated. The reaction was arrested by addition of the hydroxamic acid reagent and the hydroxamic acid formed was determined photometrically as described previously[1].

* Proposed name.