BBA 61148

Thermodynamic properties of the allosteric transition of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase

The activity of one of the three 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, catalyzing the first step of biosynthesis of aromatic amino acids in *Escherichia coli* K12 is inhibited by phenylalanine[1]. It was previously found that the temperature strongly influences the effect of allosteric inhibitors or activators on the allosteric enzymes[2–5]. During our studies on the thermodynamics of allosteric transition, we have observed that the inhibitor constant K_i of DAHP synthase for phenylalanine does not depend on the temperature, in contrast to the substrate binding constant K_s of enzyme for phosphoenolpyruvate (PEP) which is strongly temperature dependent.

This paper reports kinetic experiments and thermodynamic calculations on the allosteric transition of the phenylalanine sensitive DAHP synthase.

Growth conditions of the wild-type *E. coli* K12 and the purification of enzyme were as described previously[6]. Since both PEP, the "first substrate", and phenylalanine stabilize the enzyme against the inactivation by heat, it was possible to determine the inhibitor constant (K_i) of enzyme for phenylalanine and the substrate binding constant (K_s) for PEP directly. As Fig. 1a shows, the heat inactivation of enzyme at 50° follows first-order kinetics and the rate constant k_1 is 0.64 min^{-1}. PEP stabilizes the enzyme and the heat inactivation of enzyme follows pseudo-first-order kinetics. The rate constant k' in the presence of 0.14 mM, 0.28 mM and 0.56 mM of PEP are 0.27, 0.144 and 0.088 min^{-1}, respectively. The dissociation constant K_s of enzyme–PEP complex was determined graphically according to O'SULLIVAN AND COHN[7]

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

The secondary plot of k_1 and k' obtained from Fig. 1a gives, according to Eqn. 1, a straight line as shown in Fig. 1b. The dissociation constant K_s at 50° is $1.1 \cdot 10^{-4}$. The

TABLE I

THERMODYNAMIC CHARACTERISTICS OF THE REACTION: $E + \mathrm{PEP} = E - \mathrm{PEP}$

$\Delta H^\circ = -45500$ cal/mole; $\Delta F^\circ = RT \ln K_s$; $S^\circ = \frac{\Delta H^\circ - \Delta F^\circ}{T}$

Temperature (°C)	K_s	*ΔF° (cal/mole)*	*ΔS° (cal/mole · degree)*
40	$3.0 \cdot 10^{-5}$	−6400	−124
50	$1.1 \cdot 10^{-4}$	−6800	−120
55	$4.0 \cdot 10^{-4}$	−5100	−123
60	$1.1 \cdot 10^{-3}$	−4500	−123

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

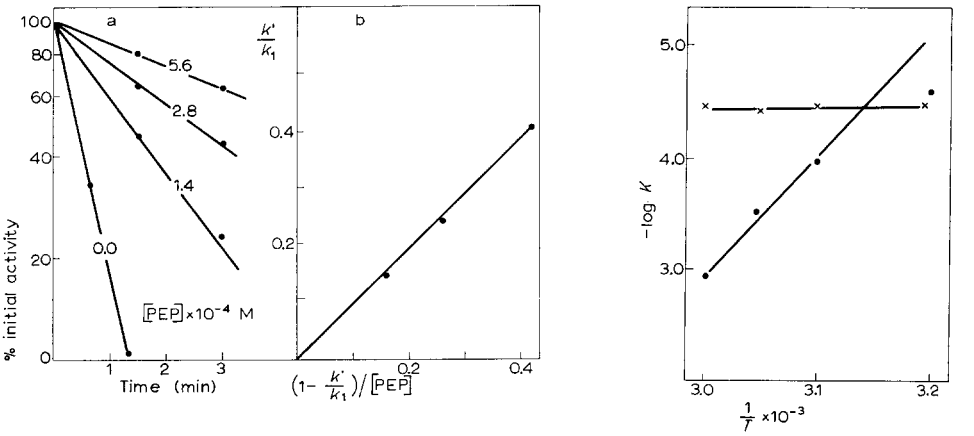

Fig. 1. Heat inactivation of DAHP synthase in the absence and in the presence of PEP (a). 0.1 M Tris–HCl buffer (pH 7.5) containing 0.5 mg of protein per ml and various concentrations of PEP as indicated was incubated at 50°. Samples were taken at zero time and/or other times as required and diluted 50-fold in a reaction mixture containing 100 μmoles of potassium phosphate buffer (pH 7.5), 0.5 μmole of PEP, 0.5 μmole of erythrose 4-phosphate in a total volume of 1 ml for determination of enzyme activity. The mixtures were incubated at 37° and the reaction was arrested by the addition of 0.4 ml of 10% trichloroacetic acid. The amount of DAHP formed was determined by the thiobarbituric acid method (b). Secondary plot of the first-order rate constants k_1 and k' obtained from Fig. 1a. The slope of the straight line from the plot of k'/k_1 *vs.* $(1 - k'/k_1)/[\text{PEP}]$ is equal to K_s.

Fig. 2. Effect of temperature on the substrate binding constant, K_s for PEP and on the inhibitor constant K_i for phenylalanine. The plot of $-\log K_s$ (●—●) and $-\log K_i$ (×—×) *vs.* the reciprocal of the absolute temperature T.

K_s for PEP was determined using the described method, at other temperatures also and as shown in Fig. 2 and Table I, it decreases with the decreasing temperature. Phenylalanine also stabilizes the enzyme against the inactivating effect of heat and we determined the dissociation constant K_i for phenylalanine at different temperatures as described above. As Fig. 2 shows, K_i does not depend on the temperature in contrast to the previously studied cases[2–5]. The standard enthalpy change, $\Delta H°$, for the formation of enzyme–PEP complex calculated from Fig. 2 according to the van 't Hoff equation is −45500 cal/mole. The large decrease in entropy (as shown in Table I) suggests that the enzyme–PEP complex has a more ordered conformation than the free enzyme.

It was proposed earlier that because of the fluctuation of the polypeptide chain(s) the molecules of an enzyme may have many different conformations, each being in equilibrium with the others[8]. If temperature influences this equilibrium, then a temperature shift increases or decreases the probability of formation of certain conformation(s) of enzyme molecules suitable for binding the substrate(s) or the allosteric effector, or both of them. If we compare the effect of temperature on the allosteric enzyme of the arginine pathway reported previously[5] with the results presented in this paper on DAHP synthase, it seems probable that these two enzymes represent two different conformational possibilities. In the previously reported case[5], the decreasing temperature favors the transition of enzyme to its inhibitor binding conformation, while the same temperature shift favors the transition of DAHP synthase to its substrate binding conformation.

The molecular mechanism of the allosteric transition of DAHP synthase is under investigation.

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Received August 9th, 1967