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PURIFICATION AND PROPERTIES OF THE 3-DEOXY-D-*ARABINO*-HEPTULOSONATE-7-PHOSPHATE SYNTHASE (PHENYLALANINE SENSITIVE) OF *ESCHERICHIA COLI* K12

I. PURIFICATION OF ENZYME AND SOME OF ITS CATALYTIC PROPERTIES

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SUMMARY

1. The phenylalanine-sensitive allosteric first enzyme (isoenzyme 1a) of the aromatic amino acid biosynthetic pathway, 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (7-phospho-2-oxo-3-deoxy-D-*arabino*-heptonate D-erythrose-4-phosphate-lyase (pyruvate-phosphorylating), EC 4.1.2.15) has been purified 160-fold from *Escherichia coli* K12. The molecular weight of the enzyme is 160 000.

2. The enzyme has a broad pH optimum between pH 6 and 8. The initial velocity data obtained follow regular Michaelis–Menten kinetics without any detectable kinetic evidence for subunit interaction. On the basis of kinetic experiments the mechanism of enzyme action is ping-pong, and the first substrate is phosphoenolpyruvate. The absolute Michaelis constant of the enzyme for both phosphoenolpyruvate and erythrose 4-phosphate is 1.0 mM. Co^{2+}, at a concentration of 1 mM, increases the enzyme activity about 2-fold. Among other ions tested, Mn^{2+} slightly activates the enzyme, while other heavy metal ions, such as Cu^{2+} and Zn^{2+}, are inhibitory.

3. Both elevated and low temperatures reversibly inactivate the enzyme. Phosphoenolpyruvate protects the enzyme against the inactivating effect of heat. The K_s of the enzyme for phosphoenolpyruvate depends on the temperature and decreases with decreasing temperature.

INTRODUCTION

The first step in the biosynthesis of aromatic acids and some other aromatic compounds in microorganisms and plants is the formation of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) from erythrose 4-phosphate (erythrose-4-*P*) and phosphoenolpyruvate (PEP) (ref. 1). Three distinct isoenzymes of DAHP synthase

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

(7-phospho-2-oxo-3-deoxy-D-*arabino*-heptonate D-erythrose-4-phosphate-lyase (pyruvate-phosphorylating), EC 4.1.2.15) were detected in *Escherichia coli* K12 (refs. 2, 3). All these DAHP synthases catalyze the formation of DAHP but the activity of isoenzyme 1a and 1b are inhibited allosterically by phenylalanine and tyrosine, respectively. The activity of isoenzyme 1c is not inhibited by any of the aromatic end products.

The present paper describes the purification of the phenylalanine-sensitive DAHP synthase (isoenzyme 1a) and some properties of the enzyme.

MATERIALS AND METHODS

Chemicals

The inorganic chemicals used were of A.R. grade. L-Phenylalanine, L-tyrosine, PEP barium salt, and D-glucose 6-phosphate barium salt were obtained from Reanal, Budapest. Thiobarbituric acid was obtained from Fluka, Buchs. Erythrose-4-*P* was prepared from D-glucose 6-phosphate according to BALLOU[4] and purified by ion-exchange chromatography, as described previously[5]. β-Niphegal (*o*-nitrophenyl-β-D-galactoside) was prepared chemically[6].

β-Galactosidase (EC 3.2.1.23) was purified from *E. coli* ML 308 (ref. 7), crystalline muscle lactate dehydrogenase (EC 1.1.1.27) was kindly supplied by Dr. J. SÜDI.

Gel filtration with Sephadex

Sephadex G-200 was allowed to swell for 3 days in 50 mM Tris–HCl buffer (pH 7.5) at room temperature, and the buffer was occasionally decanted. The hydrated gel in buffer was deaerated under vacuum prior to use. The gel was poured into a 2.4 cm × 50 cm column and equilibrated overnight at 2° by passing 50 mM Tris–HCl buffer (pH 7.5) through the column. The sample solution, 2.0 ml, was carefully layered under the buffer solution above the gel, and 3.0-ml fractions were collected. Each of the fractions was assayed for enzyme activity. The flow rate was maintained at 16–20 ml/h.

Organism and growth conditions

Wild-type strain of *E. coli* K12 and minimal medium A containing 0.2% of glucose were used[8]. The cultures were grown at 37° on a gyrotory shaker and harvested in the early stationary phase by centrifugation at 2°. The sedimented cells were washed twice with 0.05 M KCl solution by centrifugation and either stored for 2–6 days in a frozen state at −20° before use, or suspended in 0.025 M potassium phosphate buffer (pH 7.4) for the preparation of a crude extract.

DAHP-synthase assay

The crude extract of *E. coli* K12 grown on minimal medium and harvested in the early stationary phase contains mostly the phenylalanine-sensitive enzyme and only a small amount of isoenzyme 1b and 1c. For determination of the amount of the individual isoenzymes in a mixture, the following method was used. After determination of the overall activity of DAHP synthase in the crude extract, the decrease of overall activity measured in the presence of 1.0 mM phenylalanine was considered to represent isoenzyme 1a, and the decrease in the presence of 1.0 mM tyrosine to be

equivalent to 90% of isoenzyme 1b. The residual activity of DAHP synthase of the crude extract measured in the presence of 1.0 mM phenylalanine and 1.0 mM tyrosine was considered to represent isoenzyme 1c.

For the determination of activity of the enzyme standard reaction mixture was used containing 50 μmoles of Tris–HCl buffer (pH 7.5), 0.5 μmole of PEP, 0.5 μmole of erythrose-4-*P*, water and enzyme in a total volume of 1.0 ml and the mixtures were incubated at 37°. The reaction was arrested by the addition of 0.4 ml of 10% trichloroacetic acid and the precipitated proteins were removed by centrifugation. The amount of DAHP formed was determined by the thiobarbituric acid method[1].

One unit of enzyme is defined as the amount that catalyzes the formation of 1.0 mμmole of DAHP in 1 min. Specific activity is expressed as units per mg of protein. Protein was determined by the phenol method of LOWRY *et al.*[9].

β-Galactosidase assay

The assay system contained 20 μmoles of *o*-nitrophenyl-β-D-galactoside, 200 μmoles of sodium phosphate buffer (pH 7.6), water and suitably diluted enzyme in a final volume of 2.0 ml. The reaction mixtures were incubated at 30° for 10 min and the reaction was arrested by the addition of 1.0 ml of 1 M sodium carbonate. The amount of *o*-nitrophenol liberated was determined spectrophotometrically at 420 mμ. A unit of enzyme is defined as the amount which catalyzes the formation of 12 mμmoles of *o*-nitrophenol per min.

Lactate-dehydrogenase assay

For the determination of enzymatic activity standard reaction mixture was used containing 100 μmoles of Tris–HCl buffer (pH 7.6), 1.0 μmole of potassium pyruvate, 0.5 μmole of NADH in a total volume of 3.0 ml. The reaction was followed by measuring the decrease in absorbance at 340 mμ. One unit of enzyme is defined as the amount that catalyzes the formation of 0.1 μmole of NAD^+ per min.

RESULTS AND DISCUSSION

Purification of enzyme

Step 1. Crude extract. Washed cells, obtained from 8 l of culture were suspended in 120 ml of 0.025 M potassium phosphate buffer (pH 7.4) and desintegrated by sonic treatment with cooling in an ice bath (M.S.E. 20 kcycles, 100 W). Whole cells and cell debris were removed by centrifugation at 40 000 × *g* for 30 min. The contributions of the individual isoenzymes in the crude extract are: 1a, 90%; 1b, 8%; 1c, not detectable.

Step 2. Fractionation with $(NH_4)_2SO_4$. The crude extract obtained in Step 1 is treated with solid $(NH_4)_2SO_4$ to a saturation of 40% (28 g/100 ml). The inactive precipitate is removed by centrifugation, and the addition of solid $(NH_4)_2SO_4$ is continued to a saturation of 55% (11 g/100 ml). The precipitate is sedimented at 40 000 × *g* for 20 min and dissolved in 20 ml of potassium phosphate buffer (pH 7.4). The enzyme in phosphate buffer is dialyzed against 100 vol. of the same buffer overnight. After the $(NH_4)_2SO_4$ fractionation the activity of isoenzyme 1b and 1c are not detectable.

Step 3. Calcium phosphate gel adsorption and elution. 54 ml of calcium phosphate

gel (20.1 mg dry wt. per ml) were sedimented by centrifugation and suspended in the same volume of 0.025 M potassium phosphate buffer (pH 6.5). After 10 min the gel was again centrifuged, and the supernatant was discarded. The pH of the enzyme solution obtained in Step 2 was adjusted to 6.4 by careful addition of 0.025 M KH_2PO_4, and this solution was added to the sedimented calcium phosphate gel prepared previously. The gel was suspended in the enzyme solution as uniformly as possible and allowed to stand for 30 min. After centrifugation the gel was washed in the centrifuge, first with 20 ml of 0.025 M potassium phosphate buffer (pH 6.5), then three times with 7.0 ml of 0.20 M phosphate buffer (pH 6.4), and finally with 7.0 ml of 0.1 M potassium phosphate buffer (pH 7.0). The enzyme was eluted with three successive 7.0-ml portions of 0.20 M potassium phosphate buffer (pH 7.8), and the active portions pooled.

Step 4. Fractionation with acetone. The enzyme solution obtained in Step 3 (22 ml) was placed in a bath maintained between $-3°$ and $-5°$, and 18 ml of acetone ($-15°$) were added slowly with stirring. The inactive precipitate was removed by centrifugation at $-10°$ and discarded. To the supernatant 8.8 ml of acetone ($-15°$) were added as before. The precipitated enzyme was centrifuged at $-10°$ and dissolved in 0.10 M Tris–HCl buffer (pH 7.5). The enzyme is free of isoenzymes 1b and 1c (less than 5%).

The purified enzyme is stable, and it could be stored in the frozen state at $-20°$ for weeks without any detectable loss in activity.

The purification procedure is summarized in Table I.

TABLE I

ENZYME PURIFICATION

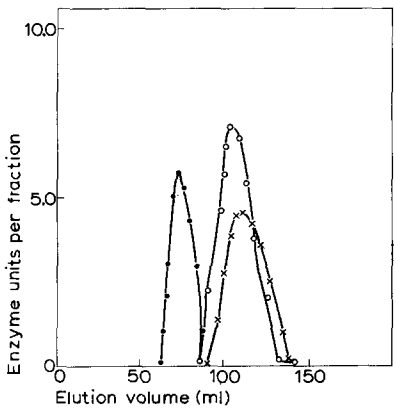
Step	*Fraction*	*Vol. (ml)*	*Total units*	*Total protein (mg)*	*Specific activity*	*Yield (%)*
1	Crude extract	120	9600	2400	4	100
2	$(NH_4)_2SO_4$ fractionation	20	7680	540	14	80
3	Calcium phosphate gel treatment	22	4560	39	115	47
4	Acetone fractionation	10	3840	6	640	40

Size of enzyme molecule

The molecular weight of the enzyme was determined by a molecular sieving procedure using Sephadex G-200, as described by ANDREWS[10]. Column calibration was carried out with enzymes of known molecular weight, β-galactosidase and lactate dehydrogenase (M_4). As Fig. 1 shows, the DAHP synthase is eluted in a single peak, and the estimated molecular weight of the enzyme calculated from its elution volume is 160 000. We could not detect any shift in the elution volume of enzyme in the presence of either PEP, the first substrate of enzyme, or the allosteric inhibitor, phenylalanine.

The effect of pH on the reaction velocity and on the phenylalanine inhibition

As shown in Fig. 2 the pH optimum of the catalytic reaction is broad, ranging



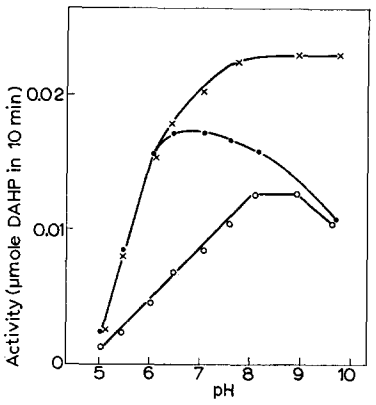


Fig. 1. Gel filtration of DAHP synthase on Sephadex G-200. A mixture of 1.8 mg of DAHP synthase (○—○), 0.12 mg of β-galactosidase (●—●), and 0.2 mg of lactate dehydrogenase (×—×) dissolved in a total volume of 2.0 ml of 0.05 M Tris–HCl buffer (pH 7.6). The enzymes were eluted from the column under the conditions described in MATERIALS AND METHODS.

Fig. 2. pH dependence of the initial rate of DAHP formation and of allosteric inhibition. The standard reaction mixture was used, except for the buffer which contained 30 μg of protein either in the absence (●—●) or in the presence (×—×) of 1.0 mM $CoCl_2$. In the inhibition experiments the standard reaction mixture was also used, containing 30 μg of protein and 0.02 mM of phenylalanine (○—○). 0.05 M Tris buffer was used, and the mixtures were incubated at 37° for 10 min.

from 6.0 to 8.0. Enzyme activity falls rapidly below pH 6.0 and gradually between 8.0 and 9.5. pH 7.5 was chosen for the standard assay of the enzyme. The rate of reaction does not change if Tris–maleate, Tris–succinate or phosphate buffer was used instead of Tris–HCl buffer. Co^{2+} activates the enzyme, and in the presence of 1.0 mM $CoCl_2$, the enzyme activity shows a broad maximum above pH 7.6 and does not change between 7.6–9.7. The inhibitory effect of phenylalanine strongly depends on pH and increases with decreasing pH (Fig. 2).

The effect of bivalent ions

It was previously found that after EDTA treatment Co^{2+} activates the tyrosine-

TABLE II

EFFECT OF BIVALENT IONS ON THE ACTIVITY OF ENZYME

The standard reaction mixture was used containing 30 μg of protein and the ions as indicated. The compound to be tested and the substrates were added simultaneously to the assay mixture and incubated at 37° for 10 min. The amount of DAHP formed was determined as described under MATERIALS AND METHODS.

Addition	*Concn. (mM)*	*Activity (%)*
None	—	100
Co^{2+}	0.4	190
Mn^{2+}	0.4	140
Cu^{2+}	0.4	1
Zn^{2+}	0.4	40
MoO_4^{2-}	0.4	100

sensitive DAHP synthase (isoenzyme 1b), and the enzyme contains firmly bound Co^{2+} (ref. 11). There was reason to suppose that the phenylalanine-sensitive enzyme is similar to the tyrosine-sensitive enzyme in this respect. Although the phenylalanine-sensitive enzyme is active without addition of any activating agent, its activity increases in the presence of Co^{2+}, as already shown in Fig. 2, and its activating effect depends on the pH. Among other ions tested, Mn^{2+} activates the enzyme slightly (Table II), while the other ions are either ineffective or inhibitory.

Initial velocity pattern

The initial velocity of DAHP formation in the presence of a saturating concentration of erythrose-4-*P* and various concentrations of PEP follows the regular Michaelis–Menten kinetics. The dependence of the initial rate of DAHP synthesis on erythrose-4-*P* concentration in the presence of a saturating concentration of PEP follows the regular Michaelis–Menten kinetics without any kinetic evidence of subunit interaction, as shown in Figs. 3 and 4. We have previously observed that kinetic

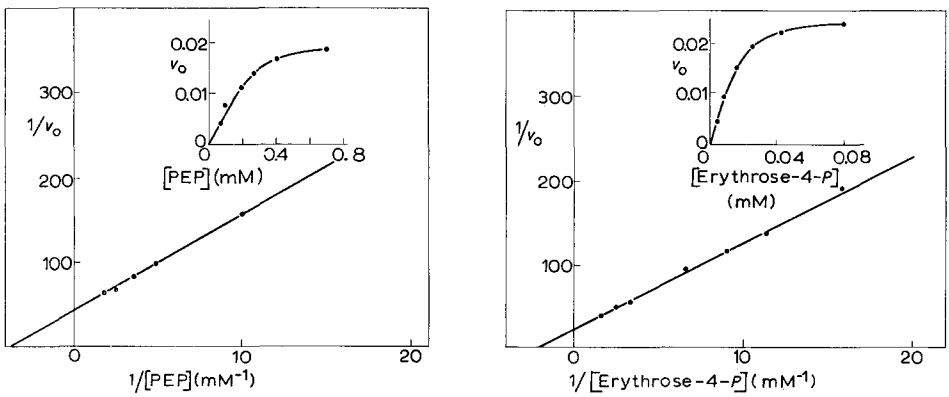
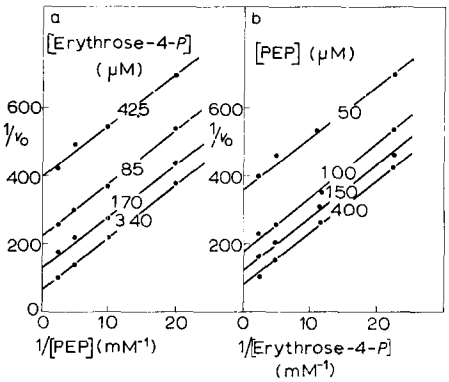


Fig. 3. Effect of PEP concentration on the initial rate of DAHP formation. The reaction mixture contained 50 μmoles of Tris–HCl buffer (pH 7.6), 0.36 μmole of erythrose-4-*P*, 20 μg of protein and PEP as indicated in a total volume of 1.0 ml. The initial velocity, v_0, is expressed as μmoles of DAHP formed per 10 min per ml of reaction mixture.

Fig. 4. Effect of erythrose-4-*P* concentration on the initial velocity of DAHP synthesis. The reaction mixture contained 50 μmoles of Tris–HCl buffer (pH 7.6), 0.5 μmole of PEP, 20 μg of protein and erythrose-4-*P* as indicated in a total volume of 1.0 ml. The initial rate, v_0, is expressed as in Fig. 3.

evidence indicates that a ping-pong mechanism of action is involved for both the tyrosine-sensitive DAHP synthase (isoenzyme 1b) and the phenylalanine-sensitive enzyme (isoenzyme 1a)[11,12]. As shown in Fig. 5 the plot of the reciprocal of the initial rate of DAHP synthesis against the reciprocal of PEP concentration at four fixed concentrations of erythrose-4-*P* gives a set of parallel lines. The plot of the reciprocal of the erythrose-4-*P* concentration against the reciprocal of the initial rate of DAHP formation at four fixed concentrations of PEP also gives a set of parallel lines, as shown in Fig. 5. In both cases there is no apparent tendency of the curves to intersect at a common point to the left of the ordinate. The intercepts on the ordinate are the reciprocals of the apparent maximal velocities, $1/v'_{max}$, and the intercepts on the



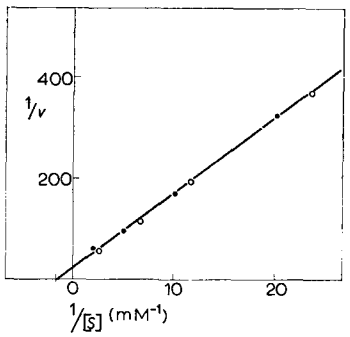


Fig. 5. Double reciprocal plot of the initial rate of DAHP formation (a) *vs.* PEP concentration in the presence of a series of fixed concentrations of erythrose-4-*P*, and (b) *vs.* erythrose-4-*P* concentration in the presence of a series of fixed concentrations of PEP. The reaction mixture contained 50 μmoles of potassium phosphate buffer (pH 7.5), 40 μg of protein, PEP and erythrose-4-*P* as indicated in a total volume of 1.0 ml. The initial rate, v_0, is expressed as in Fig. 3.

Fig. 6. Secondary plot of the data from Fig. 5 for determination of the absolute Michaelis constants of enzyme for both substrates. The graphical analysis yields the absolute Michaelis constants $-1/K_{PEP}$ and $-1/K_{Erythrose\text{-}4\text{-}P}$ as intercepts on the abscissa. ○, intercepts ($1/v_{max}$) taken from Fig. 5a; ●, intercepts taken from Fig. 5b.

abscissa are negative reciprocals of the apparent Michaelis constant, $-1/K'_m$. The absolute Michaelis constants of enzyme for PEP (K_{PEP}) and for erythrose-4-*P* ($K_{Erythrose\text{-}4\text{-}P}$) were determined graphically from secondary plots of the data obtained in Fig. 5. As shown in Fig. 6, the absolute Michaelis constants of the enzyme for both PEP and erythrose-4-*P* are 1.0 mM. The reaction appears to proceed by a ping-pong mechanism[13], indicating that the enzyme reacts with the first substrate and the first product is released before the addition of the second substrate. Since during the overall reaction P_i is released from PEP, and according to our studies PEP itself stabilizes the enzyme against thermal inactivation (Fig. 7), while erythrose-4-*P* is completely ineffective in these experiments, the first substrate is PEP. On the basis of the kinetic evidence presented the reaction pathway of enzyme and substrate interaction may be represented as follows:

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E' + C \qquad (1)$$

$$E' + B \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E'B \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E + D \qquad (2)$$

where A and B are substrates, in this case PEP and erythrose-4-*P*; C and D are products, inorganic phosphate and DAHP, respectively; and *E* and *E'* are different forms of the enzyme. It was found by DeLeo and Sprinson[14] that the P_i released in the first part of the catalytic pathway (Eqn. 1) contained the ^{18}O from the C–O–P oxygen of PEP and not from $H_2{}^{18}O$. These results, in agreement with the kinetic experiments described, indicate that the first part of the catalytic pathway (Eqn. 1) is the formation of a pyruvyl–enzyme intermediate, *E'*, and the elimination of inorganic phosphate from the enzyme–phosphoenolpyruvate complex formed initially.

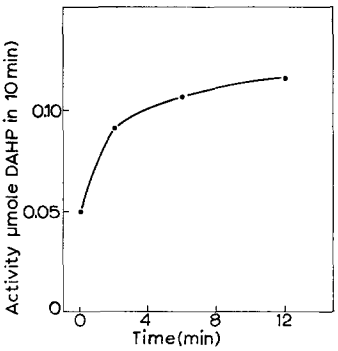


Fig. 7. Reactivation of the cold inactivated DAHP synthase. The enzyme was stored in the frozen state at −20°, and after thawing at 0°, it was diluted in 0.05 M Tris–HCl buffer (pH 7.5) to a concentration of 1.0 mg/ml. A sample of this solution was incubated at 20°, and at the times indicated, aliquots were diluted 20-fold to prevent further reactivation. The activity of enzyme in the diluted aliquots was determined using the standard reaction mixture, as described under MATERIALS AND METHODS.

Effect of temperature on the activity of enzyme

It was found that the enzyme is very stable in the frozen state at −20°, but after thawing its full activity appeared only after some 15 min of incubation at 20°, as shown in Fig. 7. Elevated temperatures reversibly inactivate the enzyme, too. Under the conditions described in Fig. 8, after 3 min of incubation at 50° the residual activity is only 20% of the original activity. The rate of reactivation of the enzyme depends on the temperature and on the concentration of the enzyme (Fig. 8). If the enzyme was diluted after the inactivation period we never observed any re-

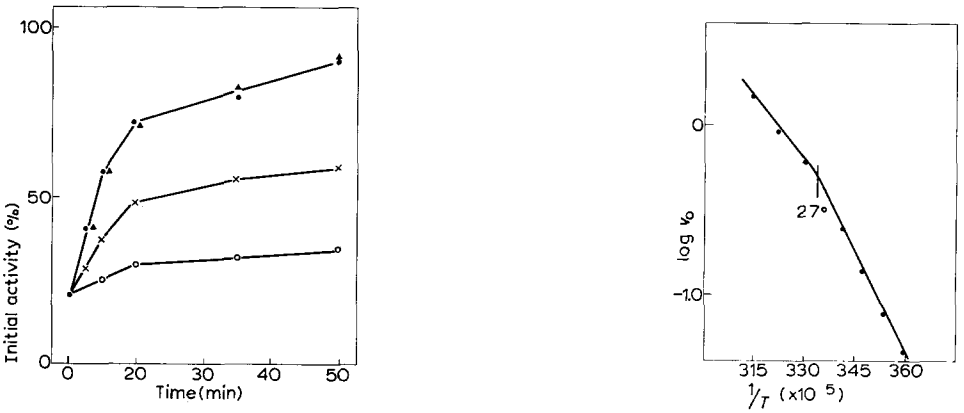


Fig. 8. Reactivation of the heat-inactivated DAHP synthase under various conditions. 4 ml of enzyme solution containing 0.6 mg of protein per ml in 0.1 M Tris–HCl buffer (pH 7.5) were incubated at 50° for 3 min. 1-ml samples of the heat-inactivated enzyme were transferred to tubes (zero time) and were incubated at 0° (×—×), at 20° (●—●), at 20° in the presence of 0.4 mM PEP (△—△) and at 20° after 10-fold dilution (○—○). At time zero and at other times aliquots were taken and diluted 20-fold in the same buffer to prevent futher reactivation. The activity of enzyme in the diluted aliquots were determined using standard reaction mixture, as described under MATERIALS AND METHODS.

Fig. 9. Arrhenius plot of the initial rate ($\log_{10}$) of DAHP synthesis against the reciprocal of absolute temperature. The initial velocity, v_0, is expressed as μmoles of DAHP formed per min per mg of protein.

activation either in the presence or in the absence of PEP or phenylalanine. All these observations can be explained by the widely accepted theory that because of the fluctuation of the polypeptide chain(s), the molecules of an enzyme may have many different conformations, which are in equilibrium with one another[15,16]. If temperature influences this equilibrium, then a temperature shift may increase or decrease the probability of formation of certain conformation(s) of enzyme molecules with or without catalytic activity. Another indication of the conformational flexibility of DAHP synthase is that the plot of the logarithm of the initial velocity against the reciprocal of the absolute temperature, the Arrhenius plot, does not give a straight line, as shown in Fig. 9 (ref. 17). At 27° a marked break can be seen and the activation energy, E, above this temperature is 11 400 cal/mole, while below this temperature it is 18 200 cal/mole. All these observations indicate indirectly but strongly that the conformation of the phenylalanine-sensitive DAHP synthase molecule is rather flexible.

Effect of temperature on the K_s of enzyme for PEP

According to the reaction pathway deduced from kinetic experiments in the presence of PEP, an intermediate of the enzyme, E', is formed through the E–PEP complex. It was previously observed that PEP stabilizes the enzyme against the inactivating effect of heat and it was proposed that the properties of the E–PEP complex and E' might be different from E (ref. 18). As presented in Fig. 10 the thermal inactivation of DAHP synthase follows first-order kinetics and the first-order rate constant of the process is given by

$$k_1 = \frac{1}{t} \ln \frac{E_0}{E} \tag{3}$$

where E_0 and E are the concentrations of the active enzyme at zero time and time t, respectively. The reaction between the enzyme, E, and PEP may be expressed as a bimolecular reaction

$$E + \text{PEP} \rightleftharpoons E\text{–PEP}$$

If the system contains a large excess of PEP, the heat inactivation follows apparent first-order kinetics, as shown in Fig. 10. The apparent first-order rate constant, k', of the process can also be calculated according to Eqn. 3. From the rate constants obtained at different PEP concentrations it is possible to determine graphically the dissociation constant, K_s, of the E–PEP complex, according to Eqn. 4 (refs. 19, 20)

$$\frac{k'}{k_1} = \frac{K_s}{[\text{PEP}]} \left(1 - \frac{k'}{k_1}\right) + \frac{k_2}{k_1} \tag{4}$$

where k_2 is the first-order rate constant of heat inactivation of E'. The secondary plot of k' and k_1 obtained in Fig. 10 as k'/k_1 *vs.* $(1 - k'/k_1)/[\text{PEP}]$, according to Eqn. 4, gives a straight line, as shown in Fig. 11, and the slope of this line is equal to K_s. The dissociation constant of the E–PEP complex, K_s, at 50° is $1.1 \cdot 10^{-4}$ M. The K_s of enzyme for phosphoenolpyruvate was determined using the method described at other temperatures as well. As shown in Fig. 12 and Table III, it decreases with decreasing temperature. The standard enthalpy change, $\Delta H°$, for the formation of

TABLE III

THERMODYNAMIC CHARACTERISTICS OF THE REACTION: E + PEP = E–PEP
$\Delta H^\circ = -45\,500$ cal/mole; $\Delta F^\circ = RT \ln K_s$; $\Delta S^\circ = (\Delta H^\circ - \Delta F^\circ)/T$.

Temp.	K_s	ΔF° *(cal/mole)*	ΔS° *(cal/mole per 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

E–PEP complex, calculated from Fig. 12 according to the Van 't Hoff equation, is −45 500 cal/mole. The large decrease of entropy, as indicated in Table III, suggests that the E–PEP complex has a more ordered conformation than the free enzyme.

It was observed previously that the temperature strongly influences the effect of allosteric activators and inhibitors on some allosteric enzymes[21–24]. Comparing the behavior of the group of allosteric enzymes previously studied with the results

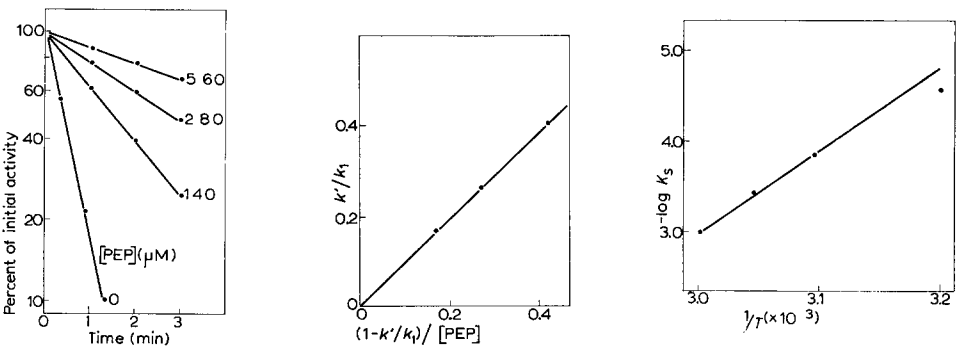


Fig. 10. Heat inactivation of DAHP synthase in the presence and absence of PEP. Samples of 0.1 M Tris–HCl buffer (pH 7.5) containing 0.5 mg of protein and various concentrations of PEP, as indicated, were incubated at 50°. Aliquots were taken at zero time and at other times as required and diluted 50-fold in the standard reaction mixture for determination of the residual activity, as described under MATERIALS AND METHODS.

Fig. 11. Secondary plot of the first-order rate constants k' and k_1 obtained from Fig. 10. The slope of the straight line from the plot of k'/k_1, *vs.* $(1 - k'/k_1)/[\text{PEP}]$ according to Eqn. 4 is equal to K_{PEP}.

Fig. 12. Effect of temperature on the K_s of enzyme for PEP. The plot of $-\log K_s$ against the reciprocal of the absolute temperature.

presented in this paper on DAHP synthase it seems that the effect of temperature is just the opposite. In the cases previously reported[21–24] a decreasing temperature favors the transition of enzymes to their allosteric effector-binding conformation, while the same temperature shift favors the transition of DAHP synthase to its substrate-binding conformation.

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