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.

As shown in Fig. 1a, the inhibitory effect of arginine on the phophotransferase (E) depends on the temperature, as is the case for certain other allosteric enzymes[2-4], but the reaction order n according to Eqn. 1 (refs. 3, 5) is equal to 2 at any temperature studied:

$$\log \frac{v_0 - v_i}{v_i} = \log K_a + n \log [\mathrm{Arg}] \qquad (1)$$

where v_0 and v_i are the reaction velocities in the absence and in the presence of arginine, respectively, and K_a is the apparent association constant of the E–Arg_2 complex. As it was found that arginine (in the absence of substrates) protects the enzyme against heat inactivation[1], it was possible to determine the value of n directly for arginine. At 55° the inactivation of the free enzyme follows first-order kinetics and the rate constant k_1 is 0.086 min^{-1}. In the presence of arginine the heat inactivation of the enzyme follows pseudo-first-order kinetics and the rate constants k' in the presence of 0.03 mM, 0.05 mM, 0.07 mM, 0.1 mM and 0.15 mM of arginine are 0.06, 0.046, 0.033, 0.02 and 0.012 min^{-1}, respectively. The overall dissociation constant K of the enzyme–inhibitor complex E–I formed may be expressed as

$$K = \frac{[E]\,[I]^n}{[E\text{–}I_n]} \qquad (2)$$

From Eqn. 3 it is possible to determine the overall dissociation constant K of the E–I_n complex and the reaction order n:

$$\frac{k'}{k_1} = K\left(\frac{1 - \frac{k'}{k_1}}{I^n}\right) + \frac{k_2}{k_1} \qquad (3)$$

where k_2 is the rate constant of the inactivation of the E–I_n complex. The plot shown

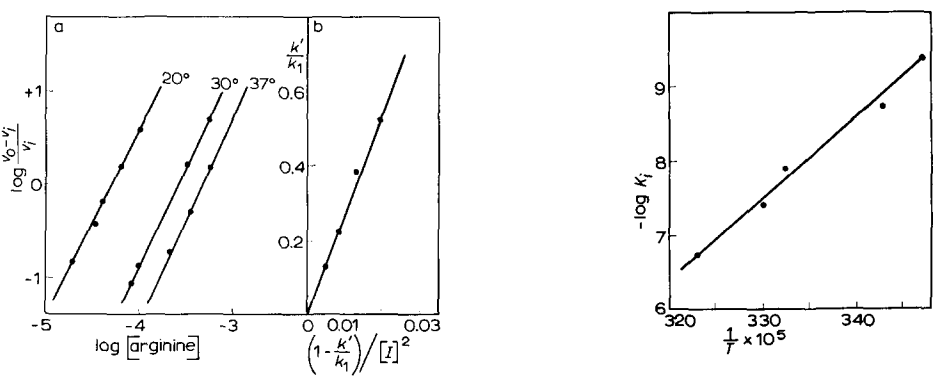

Fig. 1. Determination of the order of the reaction for the formation of the enzyme–Arg_n complex in the presence of the substrates at different temperatures (a) and in the absence of the substrates at 55° (b). For determination of n in the presence of substrates, "standard" reaction mixture was used containing arginine as indicated (a). For determination of K and n (b) 0.05 M Tris–HCl buffer containing 4 mg of protein was incubated at 55° in the absence and in the presence of different concentrations of arginine (see text). The residual activity of the enzyme was determined using the standard reaction mixture as described in the text.

Fig. 2. Effect of temperature on the apparent inhibitor constant, K_i, of arginine. Plot of $-\log K_i$ (from Table I) against the reciprocal of the absolute temperature T.

in Fig. 1b gives a straight line only if n = 2 and in this case K is equal to $2.2 \cdot 10^{-9}$ M. This means that the value of n for the formation of the E–Arg_2 complex does not depend on the presence or absence of the substrates.

The apparent inhibitor constants, K_i, for arginine, determined by the inhibition of the enzymatic reaction[1] at different temperatures, are shown in Table I. It is noteworthy that the apparent K_m for acetylglutamate is $5.0 \pm 1.0 \cdot 10^{-3}$ M and does not change in the temperature range studied. The standard enthalpy change $\Delta H°$ for the formation of the E–Arg_2 complex calculated from Fig. 2 according to the van 't Hoff equation is −50 600 cal/mole. The arginine inhibition of the phosphotransferase is an exothermic exergonic reaction as shown in Table I. The large decrease in entropy suggests that the arginine-inhibited enzyme has a more ordered

TABLE I

THERMODYNAMIC CHARACTERISTICS OF THE REACTION: $E + 2\ Arg = E\text{–}Arg_2$; $\Delta H° = -50\ 600$ cal/mole; $\Delta F° = RT \ln K_i$; $\Delta S° = \frac{\Delta H° - \Delta F°}{T}$

Temperature (°C)	K_i *(apparent, overall)*	$\Delta F°$ *(cal/mole)*	$\Delta S°$ *(cal/mole·degree)*
37	$1.6 \cdot 10^{-7}$	9 700	−132
30	$4.0 \cdot 10^{-8}$	10 300	−132
28	$8.0 \cdot 10^{-9}$	11 100	−130
20	$1.6 \cdot 10^{-9}$	11 800	−133
15	$4.0 \cdot 10^{-10}$	12 450	−132

conformation than the active enzyme, the allosteric transition involving a conformational change of the enzyme structure.

The interactions stabilizing the different conformations of the enzyme are under investigation.

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