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Effect of temperature on the K_s for homoserine and the K_i for threonine of homoserine dehydrogenase from *Chlamidomonas reinhardti*

It was previously found that the formation of enzyme–substrate complex or of enzyme–allosteric effector complex of some allosteric enzymes is an exergonic process[1–3]. During our studies on the thermodynamics of allosteric transition, we have observed that both the substrate-binding constant (K_s) for homoserine and the inhibitor constant (K_i) for threonine of L-homoserine:NADP$^+$ oxidoreductase (EC 1.1.1.3), referred to hereafter as homoserine dehydrogenase, strongly depend on the temperature.

This paper reports kinetic experiments and thermodynamic calculations on the allosteric transition of homoserine dehydrogenase.

Growth conditions of the *minus* mating-type strain of *Chlamydomonas reinhardti*

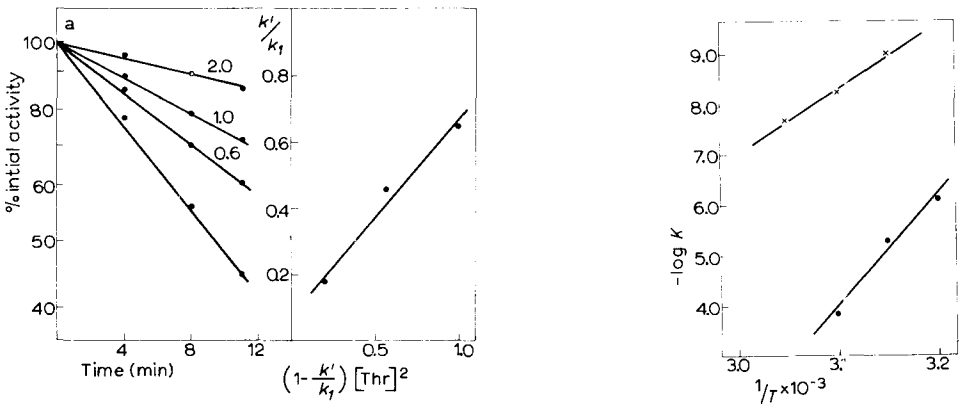

Fig. 1. Heat inactivation of homoserine dehydrogenase at 50°. a. 0.033 M Tris–HCl buffer (pH 7.4) containing 0.4 mg of protein per ml and various concentrations of threonine as indicated was incubated at 50°. Samples were taken at different times and diluted in a reaction mixture containing 300 μmoles of Tris–HCl buffer (pH 9.1), 3 μmoles of EDTA, 150 μmoles of L-homoserine and 10 μmoles of NAD$^+$ in a total volume of 3.0 ml for the determination of residual enzyme activity. The reduction of NAD$^+$ was measured by following the absorbance change at 340 mμ in cuvettes with a 1-cm light path, at 25°. b. Secondary plot of the apparent first-order rate constants k_1 and k' obtained from Fig. 1a.

Fig. 2. Effect of temperature on K_s for homoserine and on K_i for threonine. The plot of $-\log K_s$ (●—●) and $-\log K_i$ (×—×) *vs.* the reciprocal of the absolute temperature T.

No. 90 and the preparation of the crude extract were as described previously[4]. From the crude extract the homoserine dehydrogenase was purified 72-fold by ammonium sulfate fractionation, negative alumina Cγ gel adsorption and hydroxylapatite adsorption.

Since both homoserine and threonine stabilize the enzyme against inactivation by heat, it was possible to determine the K_i of enzyme for threonine and the K_s for homoserine directly. The inactivation of the free enzyme follows first-order kinetics and the rate constant, k_1, at 55° is 0.031 min^{-1}. Threonine stabilizes the enzyme and

heat inactivation of the enzyme follows apparent first-order kinetics. The rate constant, k', in the presence of 0.06, 0.1 and 0.2 mM threonine is 0.019, 0.0135 and 0.006 min^{-1}, respectively. The over-all K_i of the enzyme–threonine complex, E–I_n, was determined graphically according to the method of O'SULLIVAN AND COHN[5]

$$\frac{k'}{k_1} = \frac{K_i}{[I]^n}\left(1 - \frac{k'}{k_1}\right) + \frac{k_2}{k_1}$$

where n, the number of moles of inhibitor per enzyme molecule, is equal to 2 and k_2 is the apparent first-order rate constant of the inactivation of E–I_n complex. The plot of k'/k_1 *vs.* $(1 - k'/k_1)/[\text{threonine}]^2$ gives a straight line as shown in Fig. 1a and the slope of this line is equal to K_i. The K_i at 50° is $6.0 \cdot 10^{-9}$ M. The K_i for threonine was determined using the same method and, as shown in Fig. 2 and Table I, it decreases with decreasing temperature.

The inactivation of the enzyme in the presence of homoserine follows apparent first-order kinetics also and from the plot of k'/k_1 *vs.* $(1 - k'/k_1)/[\text{homoserine}]^2$ we

TABLE I

THERMODYNAMIC CHARACTERISTICS OF THE REACTION: E + 2 Thr = E–Thr_2

Temp.	K_i	ΔF^0 *(cal/mole)*	ΔS^0 *(cal/mole per degree)*
55°	$1.8 \cdot 10^{-8}$	−10 800	−147
50°	$6.0 \cdot 10^{-9}$	−12 100	−146
45°	$9.3 \cdot 10^{-10}$	−13 100	−145

could calculate the K_s of enzyme for homoserine at different temperatures. As Fig. 2 and Table II show, it decreases with decreasing temperature.

The standard enthalpy change, $\Delta H°$, from Fig. 2 according to the van 'tHoff equation for the formation of enzyme–$(\text{threonine})_2$ complex is −59 700 cal/mole and for the enzyme–$(\text{homoserine})_2$ complex, it is −109 000 cal/mole. As shown in Tables I and II, the formation of these complexes is an exothermic exergonic process. The large decrease of entropy suggests that the complexes have a more ordered conformation than the free enzyme.

TABLE II

THERMODYNAMIC CHARACTERISTICS OF THE REACTION: E + 2 HOMOSERINE = E–HOMOSERINE

$\Delta H° = -109\,000$ cal/mole. The equation for ΔF^0 and $\Delta S°$ as in Table I.

Temp.	K_s	ΔF^0 *(cal/mole)*	ΔS^0 *(cal/mole per degree)*
50°	$1.6 \cdot 10^{-4}$	−5600	−320
45°	$3.1 \cdot 10^{-6}$	−8000	−319
40°	$8.1 \cdot 10^{-7}$	−8700	−321

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