ALLOSTERIC MODIFICATION OF ADENYLATE DEAMINASE ACTIVITY

INITIATION OF ADENOSINE DEAMINASE ACTIVITY BY POTASSIUM IONS

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Activation of purified adenylate deaminase from the duck myocardium by K^+ ions is accompanied by a change in the substrate specificity of the enzyme and appearance of ability to deaminate adenosine and adenine. Adenosine deaminase activity appears with K^+ in a concentration exhibiting maximal stimulating effect (0.15 M) and it increases with an increase in the K^+ concentration, parallel with a decrease in Hill's coefficient. It can be concluded from the pH dependence, the character of inhibition by phosphate, and the effect of cations of the alkali metals that deamination of adenosine takes place at natural combining sites of adenylate deaminase, the conformation of which is modified by the activator.
KEY WORDS: *adenylate deaminase; allosteric modification; activation by K^+; adenosine deaminase activity.*

Adenylate deaminases from various organs and tissues are allosteric enzymes whose activity is controlled by cations of alkali metals and by nucleotides [6, 8, 10]. In most cases K^+ is the most effective activator of the enzyme; in the presence of K^+, moreover, the kinetic curves of V as a function of [S] for adenylate deaminase become hyperbolic in shape [8].

In the study of the properties of purified adenylate deaminase from duck myocardium described below it was found that besides an increase in affinity for AMP, the action of K^+ also causes qualitative changes in the substrate specificity (transformation) of the enzyme, expressed as the appearance of ability to deaminate adenosine and adenine. From the phenomenologic point of view allosteric transformation is one form of allosteric effect of ligands.

EXPERIMENTAL METHOD

Purification of adenylate deaminase consisted of homogenization of duck myocardial tissue, chromatography on phosphocellulose [7], precipitation with $(NH_4)_2SO_4$ (23 g to 100 ml material), removal of the salt on a 1.6 × 30 cm column with Sephadex G-25, equilibrated with 0.03 M Na-phosphate buffer, pH 7.2 (elution with 1 M KCl), adsorption of the enzyme on DEAE-Sephadex A-25 (1.6 × 5 cm column), and desorption with 0.03 M Na-phosphate buffer containing 0.2 M NaCl. Adenylate deaminase activity was determined at 37°C as the quantity of ammonia formed during the reaction. The reaction was carried out in 0.05 M imidazole-HCl buffer, pH 6.5, for 3 min in the case of AMP and 15 min in the case of the other substrates; the enzyme concentration was 10 μg/ml. Ammonia was measured by means of Nessler's reagent after isothermic distillation [1].

EXPERIMENTAL RESULTS

As Table 1 shows, besides stimulating adenylate deaminase, KCl also caused a change in substrate specificity, so that the enzyme began to deaminate adenine derivatives not containing phosphate residues. This effect was exhibited most clearly by KCl in concentrations of $\geq$ 0.15 M (Fig. 1). In this concentration, KCl lowered Hill's coefficient (n) for AMP from 1.9 to 1.4 and gave the maximal stimulating effect. A further increase in the KCl concentration led to a fall in the value of n to 1 (Table 1) and to an increase in affinity for adenosine. This indicates that adenosine deaminase activity arises as a result of con-

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TABLE 1. Changes in Kinetic Parameters of Adenylate Deaminase under Influences of KCl

Substrate	Without KCl			— 0,5 M KCl		
	s.a.	$S_{0,5}$	n	s.a.	$S_{0,5}$	n
10 mM ATP	0	—	—	0	—	—
10 mM ADP	0	—	—	0	—	—
10 mM AMP	126	2,9	1,9	129	2,1	1,1
0,4 mM AMP	2,2			13,4		
10 mM adenosine	0	—	—	2,7	—	—
10 mM adenine	0	—	—	1,4	—	—

Legend. s.a.) Specific activity in micromoles NH_3/mg protein/min; $S_{0.5}$) substrate concentration (in mM) at which reaction velocity is 50% of maximal.

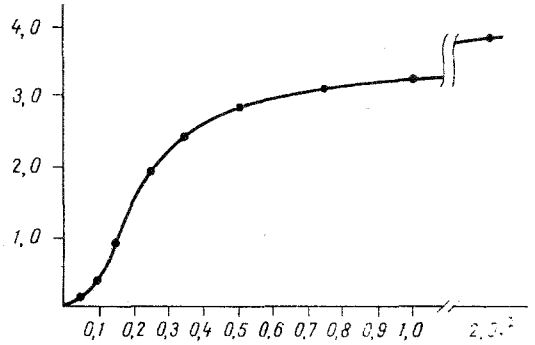

Fig. 1. Velocity of deamination of adenosine by adenylate deaminase as a function of KCl concentration. Abscissa, KCl concentration (in M); ordinate, velocity of deamination (in μmoles NH_3/mg protein/min). Adenosine concentration 10 mM.

formational changes in the combining sites. Within the limits of solubility of adenosine, its deamination by adenylate deaminase obeyed the equation for a reaction of zero order (Fig. 2). Heating adenylate deaminase to 80°C for 3 min led to complete loss of its activity, evidence that the reaction was enzymic in character. The dependence of the rate of deamination of adenosine on pH was similar to the pH dependence for AMP and the curve had two maxima, at pH 6.2 and 6.8. Besides K^+ ions, other ions stimulating enzyme activity and, in particular, Na^+ and to some extent Li^+ (but not Mg^{++} or Ca^{++}), possessed the ability to modify adenylate deaminase activity in a concentration of 0.5 M, but Li^+ was much more effective than Na^+ as an activator of adenylate deaminase. Phosphate did not facilitate the appearance of ability to deaminate adenosine, but inhibited K^+-dependent activity (Fig. 3). Inhibition of both adenosine deaminase and adenylate deaminase activity was allosteric in character (n = 1.9). The results suggest that the deamination of adenosine and AMP takes place at the same combining sites of adenylate deaminase. The effect of K^+ on the enzyme is probably to modify the conformation of that part of the combining site which is responsible for binding the phosphate of AMP. The change taking place is part of a change induced essentially by the process of binding of the phosphate residue, i.e., it is part of the preparation for the catalytic act. It is the basis for the increase in the affinity for AMP^+ and the appearance of the ability to deaminate adenosine and adenine produced by the action of K^+. In other words, K^+ reduces the dependence of the beginning of catalysis on occupation of the phosphate-binding region of the combining site. Stimulation of adenylate deaminase activity by ATP is evidently connected with its influence on another part of the combining site. During stimulation by ATP ability to deaminate d-AMP and 2'-AMP appears, but deamination of adenosine and deoxyadenosine is not observed [3]. In this case strict

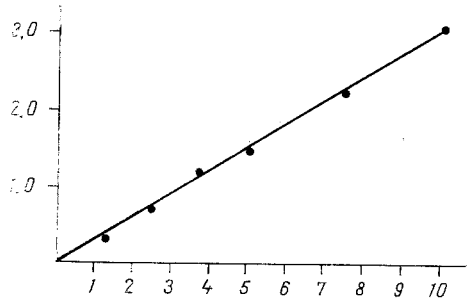

Fig. 2

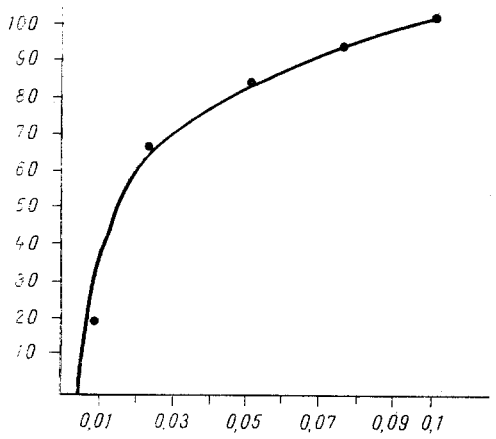

Fig. 3

Fig. 2. Velocity of deamination of adenosine as a function of substrate concentration. Abscissa, adenosine concentration (in mM); ordinate, velocity of deamination (in μmoles NH_3/mg protein/min). KCl concentration 0.5 M.

Fig. 3. Inhibition of adenosine deaminase activity of adenylate deaminase by phosphate. KCl concentration 0.5 M. Value of n, determined by method of Taketa and Pogel [9], is 1.9. Abscissa, concentration of Na-phosphate buffer, pH 6.5 (in M); ordinate, degree of inhibition (in %).

dependence of the catalytic act on the binding of phosphate residues is preserved and an increase in the affinity of the enzyme for the riboside and (or) the adenine residue of AMP probably takes place.

Since the transforming activity of K^+ is observed within a physiological range of concentrations, deamination of adenosine by adenylate deaminase can take place *in vivo*. This indicates the possibility of using adenylate deaminase directly to control the activity of adenosine, which is a vasodilator [2, 5], and hence, the coronary blood flow. This type of modification can also be used as a compensatory mechanism in diseases accompanied by absence of adenosine deaminase in the body [4].

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