THE EFFECTS OF ADENINE NUCLEOTIDES ON PIG HEART MALATE DEHYDROGENASE

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Observations from a number of laboratories have suggested that the relative concentrations of adenine nucleotides may play an important role in the regulation of metabolic pathways (Atkinson, 1965). For example, yeast isocitrate dehydrogenase was shown to be activated by AMP but not by ADP or ATP (Hathaway and Atkinson, 1963). Under conditions of low energy production this effect of AMP might serve as a "feedback activation" mechanism to increase the intracellular levels of NADH and subsequently ATP.

Since several other NAD^+-linked dehydrogenases such as yeast alcohol dehydrogenase (van Eys et al., 1958) and bovine liver glutamate dehydrogenase (Frieden, 1959) are also sensitive to the adenine nucleotides, it was of interest to determine whether such sensitivity is a general property of NAD^+-linked dehydrogenases. In assessing this possibility it was found that both α-glycerol phosphate dehydrogenase (Kuramitsu, unpublished results) and malate dehydrogenase are affected by adenine nucleotides. This latter enzyme is of particular interest since the results of this communication indicate the presence of separate effector sites in addition to the substrate sites, yet its molecular weight of approximately 40,000 (Wolfe and Nielands, 1956) is relatively low compared to other multisite enzymes.

MATERIALS AND METHODS

Highly purified pig heart malate dehydrogenase (720 units/mg) was obtained from C. F. Boehringer and Soehn. L-malate and oxaloacetate were purchased from Calbiochem. The nucleotides were all products of the Pabst Laboratories.

The reaction was measured in the direction of NADH oxidation using the following system: enzyme, 0.033 M phosphate, pH 7.4, 0.047 mM NADH, 0.25 mM oxaloacetate, and water in a total volume of 3.0 ml. Oxaloacetate was added to start the reaction. The rate of reduction of NAD^+ was measured in the following reaction mixture: 0.033 M Tris, pH 8.8, 1.0 mM NAD^+, 10 mM L-malate, and water in a total volume of 3.0 ml. Malate was added to initiate the reaction. The initial rates of NADH oxidation and NAD^+ reduction at room temperature were followed at 340 mμ in a Beckman model DU spectrophotometer coupled to a Gilford model 2000 multisample absorbance recorder.

RESULTS AND DISCUSSION

When malate dehydrogenase activity was measured in the direction of NADH oxidation, it was observed that AMP, ADP, and ATP inhibited the reaction (Table I). There appeared to be no marked differences in the inhibitory capacity of the three adenine nucleotides. GMP, adenosine and PP_i had little or no effect by comparison under these conditions. The inhibition produced by 1.0 mM AMP was found to be of a mixed-type with oxaloacetate and competitive with NADH, increasing the apparent K_s for NADH from 8.8 μM to 0.28 mM. The inhibition produced by ATP could also be overcome by increasing the NADH concentration.

Since NADH and NAD^+ probably bind to the same site on the dehydrogenase and there is some evidence to suggest that only one such site may exist per molecule of enzyme (Kun, 1963), it was expected that the adenine nucleotides might also act as competitive inhibitors of the reaction measured in the direction of NAD^+ reduction. Instead, all three adenine nucleotides

acted as activators for the reaction (Table II). ATP appeared to be the best activator especially at lower concentrations. The activation has been studied in detail for AMP which appeared to increase the binding of NAD^+ to the enzyme, decreasing the apparent K_s for NAD^+ from 3.7 mM to 0.79 mM without appreciably altering the V_{max} for the reaction.

TABLE I

The effects of AMP, ADP, and ATP on malate dehydrogenase (NADH oxidation)

Nucleotide Con'c (mM)	Relative activity (%) AMP	ADP	ATP
0	100	100	100
0.33	83	82	94
0.67	67	80	71
1.0	47	50	42

The activity in the absence of the nucleotides represents an absorbancy change of 0.030/min at 340 mμ.

TABLE II

The effects of AMP, ADP, and ATP on malate dehydrogenase (NAD^+ reduction)

Nucleotide Con'c (mM)	Relative activity (%) AMP	ADP	ATP
0	100	100	100
0.33	117	125	150
0.67	132	176	200
1.0	180	163	192

The activity in the absence of the nucleotides represents an absorbancy change of 0.008/min at 340 mμ.

The observation that the adenine nucleotides activated the reaction catalyzed by malate dehydrogenase in the direction of NAD^+ reduction makes it very unlikely that the effects observed are due to a simple binding of these effectors at the catalytic NAD^+-NADH site. Instead, there may be a special site or sites on the enzyme for the adenine nucleotides. If so, it might be possible to selectively inactivate the effector sites and thus desensitize the dehydrogenase to the action of the adenine nucleotides and still retain appreciable enzymatic activity. This was accomplished by carrying out the reactions in the presence of Hg^{++} (Table III). At concentrations of Hg^{++} where appreciable NADH oxidizing activity still remains, the enzyme cannot be inhibited by 1.0 mM AMP. Attempts to desensitize the enzyme by heating, treatment with 1.4 M urea, or mercaptoethanol (up to 16.5 mM), all of which cause some loss of enzymatic activity, were without success. The presence of Hg^{++} also prevents the stimulation of NAD^+ reduction by the adenine nucleotides, for example 3.4 μM Hg^{++} completely desensitized the enzyme to ATP stimulation yet the enzyme retained 70% of its original activity.

TABLE III

Desensitization of malate dehydrogenase to AMP inhibition by Hg^{++}

	ΔA_{340} mμ/min		
$Hg(NO_3)_2$ (μM)	-AMP	+AMP (1.0 mM)	Inhibition by AMP (%)
0	.0285	.0183	36
6.7	.0126	.0135	0
13.4	.0075	.0084	0

Conditions for the assays are described previously (Materials and Methods) except that $Hg(NO_3)_2$ was added prior to the addition of AMP and the substrates.

Desensitization of the dehydrogenase further supports the suggestion that these nucleotides might be bound at a site which is distinct from the NAD^+-NADH catalytic site. Separate site effects presumably result from conformational changes of the enzyme (Monod et al., 1963). Such a change of the dehydrogenase was not of sufficient magnitude to be detected on sucrose density gradient centrifugation in the presence of 1.0 mM AMP. However, preliminary experiments utilizing 5, 5'-dithiobis-(2-nitrobenzoic acid) to measure the availability of enzyme sulfhydryl groups for titration (Srere, 1965) revealed that both ATP and AMP increased the rates of titration of such groups, an indication that the nucleotides produce a conformational change of the enzyme.

These results are therefore consistent with the hypothesis that the adenine nucleotides may be acting as allosteric effectors of the pig heart malate dehydrogenase. These effects may not be limited to malate dehydrogenases from animals since AMP, ADP, and ATP also inhibit NADH oxidation of the enzyme from lemon mitochondria (Bogin and Wallace, personal communication). Furthermore, experiments carried out in this laboratory utilizing extracts of E. coli B revealed similar inhibitions.

In contrast to some other enzymes which are sensitive to adenine nucleotides (Atkinson, 1965), no immediately discernible role for adenine nucleotide regulation of malate dehydrogenase can so far be rationalized from the results presented in this communication. It may be that the nucleotides are only mimicking the action of some other naturally occurring regulator of the enzyme. These observations are being extended in order to obtain further information on the mechanisms of allosteric regulation.

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