ACTIVATION OF RABBIT CARDIAC AMP AMINOHYDROLASE BY ADP: A COMPONENT OF A MECHANISM GUARDING AGAINST ATP DEPLETION

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1. Introduction

While it has been recognized for many years that AMP aminohydrolase (EC 3.5.4.6) is subject to activation by a variety of monovalent cations and by nucleoside triphosphates (see [1]), the role of nucleotide activation has been difficult to reconcile with the ambiguous metabolic function of this enzyme. For example, Setlow et al. [2] have proposed that AMP aminohydrolase plays a role in maintaining a balance between pools of adenine and guanine nucleotides, while Chapman and Atkinson [3] have interpreted the apparent allosteric properties of rat liver AMP aminohydrolase in terms of a mechanism to protect directly against wide excursions in energy charge. In assessing the metabolic significance of the purine nucleotide cycle in muscle, of which the AMP aminohydrolase reaction is a component, Tornheim and Lowenstein [4] have suggested that AMP deamination might protect indirectly against ATP depletion, by virtue of the fact that removal of AMP would cause net conversion of ADP to ATP via readjustment of the adenylate kinase system:

$$2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP} \qquad (1)$$

In the course of a study of the enzymes of the purine nucleotide cycle in cardiac muscle, we have found that heart AMP aminohydrolase is exquisitely sensitive to activation by ADP. This property is most easily reconciled with a role for this enzyme which, in conjunction with adenylate kinase, provides a buffer against ADP accumulation in the contracting muscle.

2. Materials and methods

AMP aminohydrolase activity was assayed throughout the purification scheme and in preliminary experiments, using a procedure based upon detection of ammonia with Nessler's reagent. In this assay, a suitable quantity of enzyme was added to 1 ml of 10 mM AMP dissolved in 0.2 M Na-citrate, pH 6.0. After incubation for 10 min at 25°C, 0.5 ml of ethanol was added, followed by 0.1 g of Permutit. The latter, to which the ammonia is absorbed was washed twice with 5 ml of water, and absorbance at 425 nm was measured following the addition of 0.25 ml 0.1 M NaOH, 9 ml of water and 0.5 ml of Nessler's reagent. An alternate assay procedure based on that of Chapman and Atkinson [3] was used for kinetic experiments. This assay involves the use of glutamate dehydrogenase to couple the production of ammonia to the oxidation of NADH. The published procedure was used, except that all experiments were repeated with double the amount of glutamate dehydrogenase in order to ensure that AMP aminohydrolase activity was rate-limiting. The rate of decrease in absorbance at 340 nm was measured with the aid of a Cary model 15 spectrophotometer.

Rabbit cardiac AMP aminohydrolase was purified approx. 200-fold from frozen rabbit hearts (Type 3, mature) supplied by Pel-Freez Biologicals, Inc., Rogers, Arkansas. The procedure used was based roughly on that developed [5] for isolation of the enzyme from skeletal muscle and involved salt fractionation with Li_2SO_4, ion exchange chromatography on cellulose phosphate and gel filtration on Sepharose 4B.

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In order to remove contaminating ATP from commercial preparations of ADP, solutions of ADP were incubated with excess glucose, hexokinase and $MgCl_2$ immediately before use and the ADP was isolated chromatographically [6].

3. Results

The pronounced allosteric activation of AMP aminohydrolase by ADP is shown in fig.1. In the absence of ADP the plot is distinctly sigmoid; when the same data are interpreted by means of a Hill plot, an excellent straight line is obtained with slope $n = 3.1$ and $[S]_{0.5} = 0.94$ mM. In the presence of ADP the sigmoidicity disappears, giving rise to a Hill coefficient of 0.90 and at least a 20-fold reduction in the value for $[S]_{0.5}$. In practical terms, it may be seen that the presence of 1–2 mM ADP results in approx. a 100-fold increase in the rate of hydrolysis at physiological substrate concentrations (below 0.5 mM – see [7]).

Activation of the enzyme is also observed in the presence of ATP (fig.2), but much higher concentrations of ATP than ADP are required to produce a demonstrable effect. In separate experiments, we have estimated that the concentrations of nucleotide required for half-maximal activation at 0.1 mM AMP are 0.65 mM for ADP and approx. 90 mM for ATP. These results clearly suggest that ADP is an important

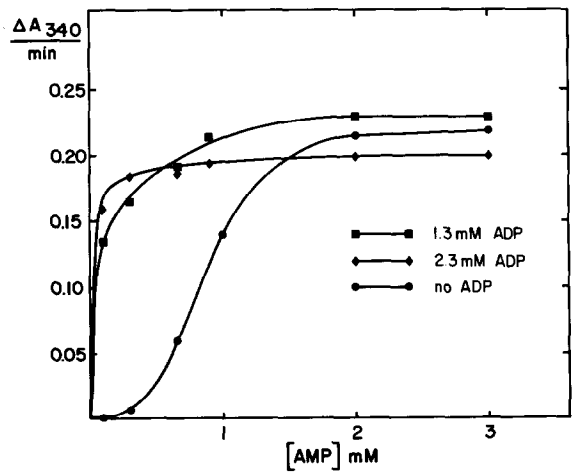

Fig.1. Activation of aminohydrolase by ADP. Details of experimental procedure are given in text.

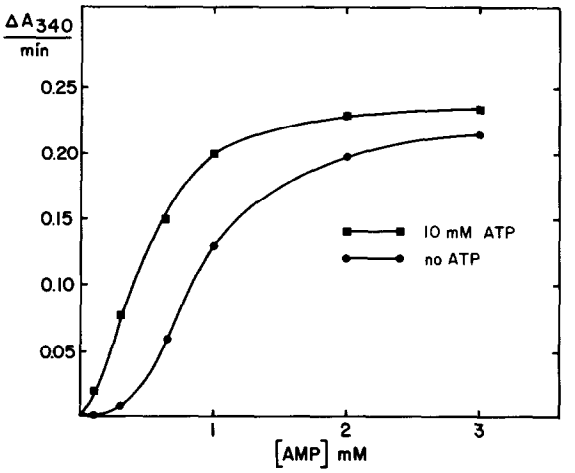

Fig.2. Activation of AMP aminohydrolase by ATP. Details of experimental procedure are given in text.

physiological regulator of the activity of AMP aminohydrolase, and that the activation observed with ATP may be representative of less favorable binding at an allosteric site designed for ADP.

4. Discussion

Specific allosteric activation of the activity of AMP aminohydrolase by ADP may be easily reconciled with the physiological role suggested for this enzyme by Tornheim and Lowenstein [4]. Thus, the allosteric properties of AMP aminohydrolase provide for negligible activity at physiological concentrations of AMP until ADP has accumulated. High rates of conversion of ATP to ADP resulting from prolonged strong contraction of the muscle will result in activation of AMP aminohydrolase, reduction in [AMP], and readjustment of the adenylate kinase equilibrium (Eqn. 1) with net conversion of ADP to ATP at the expense of the overall pool of adenine nucleotides. Such activation might be regarded as an emergency measure, providing short-term but immediate rescue from conditions favoring ADP accumulation. Clearly, uncontrolled activity would not be beneficial because of the eventual net consumption of energy required for the regeneration of AMP from IMP via the purine nucleotide cycle.

Acknowledgement

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