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EFFECTS OF VARIOUS LIGANDS ON INTERACTION OF AMP DEAMINASE WITH MYOSIN

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Summary

Purified rat muscle AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) binds tightly to rat myosin. The binding is abolished in the presence of low concentrations of various ligands. Pyrophosphate and GTP at concentrations as low as 0.1 μM were effective in abolishing the interaction between two proteins. Other nucleoside triphosphates were less effective than GTP and the concentrations required for 50% inhibition were approximately 0.3 to 0.7 μM. ADP and AMP are also effective in inhibiting the interaction between two proteins, but they are less effective than the nucleoside triphosphates; 50% inhibition occurred at 34 μM with ADP and at 1 mM with AMP. Creatine phosphate and inorganic phosphate showed 50% inhibition at 5 to 6 mM. All of the compounds, which affected AMP deaminase activity, were effective in abolishing the interaction of the enzyme with myosin; however, the interaction-abolishing effects of the compounds are not parallel with their inhibitory effects on the deaminase activity. Although there exist three parental isozymes of AMP deaminase in the rat, all three enzymes interacted with myosin.

Introduction

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) catalyzed the deamination of AMP to form IMP and ammonia. The enzyme, widely distributed in animal tissues [1], is found in high concentrations in muscle, where it is closely associated with myosin [2,3]. Currie and Webster showed that AMP deaminase activity associated with rat muscle actomyosin has been dissociated by precipitation of actomyosin at low ionic strength in the presence of inorganic phosphate [3]. Recently, Ashby and Frieden [4] clearly showed that purified rabbit muscle AMP deaminase binds tightly to myosin, heavy meromyosin, and Subfragment 2, but does not bind to light meromyosin nor to Subfragment 1, and

that the binding is abolished in the presence of relatively low concentrations of phosphate, an inhibitor of AMP deaminase.

It is our interest to determine the effects of other ligands on their binding, since many substances including phosphate are known as effectors for this enzyme. In this paper, we show that the interaction of rat muscle AMP deaminase with myosin may be affected by the presence of various ligands. Nucleoside triphosphates and pyrophosphate at concentrations as low as 1 μM were effective on abolishing the interaction between two proteins. Although there exist three parental AMP deaminase isozymes in the rat [5], all three isozymes were found to interact with myosin.

Materials and Methods

Nucleotides were obtained from Boehringer, Mannheim, or Sigma Chemical Co. Other reagents were commercial preparations of the highest purity available.

AMP deaminase was purified from rat leg muscle by the method described previously [5] with some minor modifications. The preparation exhibits a single polypeptide band on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. One unit of enzyme activity is defined as the amount of enzyme required to catalyze the deamination of 1 μmol of AMP per min under the conditions described previously [5].

Myosin was prepared from rat muscle by the method of Perry [6], purified further by chromatography on DEAE-Sephadex [7] and dialyzed exhaustively against 5 mM imidazol/HCl (pH 6.5) containing 0.5 M KCl. The protein concentration was determined from the absorbance at 280 nm using $A_{280}^{1\%} = 5.43$ [8].

Binding experiments were carried out by a modification of the procedure of Ashby and Frieden [4]. 10 μg of myosin in 5 mM imidazole/HCl (pH 6.5) containing 0.5 M KCl was mixed with 0.5 unit (approximately 0.3 μg) of AMP deaminase in the same solution in a final volume of 50 μl. The mixture was then diluted with 9 volumes of 5 mM imidazole/HCl (pH 6.5) containing various concentrations of ligands and 0.02% of bovine serum albumin, to a KCl concentration of 50 mM. After centrifugation at 20 000 $\times g$ for 10 min at 4°C, the supernatant was assayed for enzyme activity as described previously [5]. The addition of bovine serum albumin was required under our binding conditions, since the enzyme lost its activity quickly at low protein concentration. Bovine serum albumin seems unlikely to affect the interaction of the enzyme with myosin, because the comparable results of the binding are obtained in the absence of bovinse serum albumin, but with a reduced enzyme activity.

Effects of various ligands on enzyme activity were examined at 25°C by following the change in absorbance at 265 nm. The reaction mixture of 0.6 ml contained 50 mM imidazole/HCl (pH 6.5), 0.1 mM AMP, 100 mM KCl and various concentrations of ligands.

Results and Discussion

The binding of AMP deaminase to myosin in the absence and presence of potassium phosphate is shown in Fig. 1. In this experiment, myosin amount

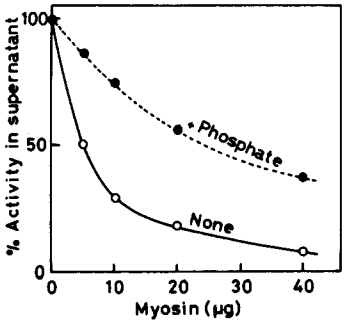

Fig. 1. Binding of rat muscle AMP deaminase to myosin in the absence and presence of phosphate. AMP deaminase (0.5 unit) in 5 mM imidazole/HCl (pH 6.5) containing 0.5 M KCl was mixed with various amounts of myosin in the same buffer to a final volume of 50 μl. The solution was then diluted with 9 volumes of 5 mM imidazole/HCl (pH 6.5) containing 0.02% bovine serum albumin or with 9 volumes of the same solution containing 11.1 mM potassium phosphate. After centrifugation, the supernatant was assayed for AMP deaminase activity.

was varied from 0 to 40 μg and AMP deaminase amount kept constant (0.5 unit). In the presence of 40 μg of myosin, most of the AMP deaminase was bound to myosin and removed from the supernatant by centrifugation, however the inhibitory effect of phosphate on the binding of two proteins was less with 40 μg of myosin than that obtained with 10 μg of myosin. When myosin amount was decreased to less than 10 μg, the amount of enzyme bound to myosin was reduced and the effect of phosphate on the binding of two proteins was lowered. The most appropriate amount of myosin seemed to be 10 μg, thus the experiments were all carried out using 10 μg of myosin and 0.5 unit of AMP deaminase as described under Materials and Methods. Under these conditions 29% of AMP deaminase did not bind to myosin and remained in the supernatant after centrifugation, but the effect of various ligands on interaction of the enzyme with myosin is most predominant.

The concentrations of myosin and the AMP deamiase used in this study are much lower than those used by Ashby and Frieden [4]. These authors carried out the binding experiments by measuring the optical density at 280 nm or by analyzing shlieren patterns after analytical centrifugation, whereas our experiments were carried out by measuring the enzyme activity to reduce the amounts of two proteins.

The effects of various concentration of nucleoside triphosphates and other compounds on binding of the deaminase to myosin are summarized in Fig. 2. Pyrophosphate is most effective amongst a variety of compounds tested and is effective at concentrations as low as 0.1 μM. GTP is effective as pyrophosphate. Other triphosphates were comparatively less effective; the concentrations required for 50% inhibition were approximately 0.3 μM with ATP and 0.7 μM with dATP, and the other triphosphates such as CTP, dCTP, dGTP, UTP and TTP showed 50% inhibition at the concentrations similar to those obtained by ATP and dATP. ADP and AMP are also effective in inhibition of binding of the deaminase to myosin, whereas they are less effective than nucleoside triphosphates; 50% inhibition occurred at 34 μM by ADP and at 1 mM by AMP. Creatine phosphate and inorganic phosphate appeared to be equipotent in

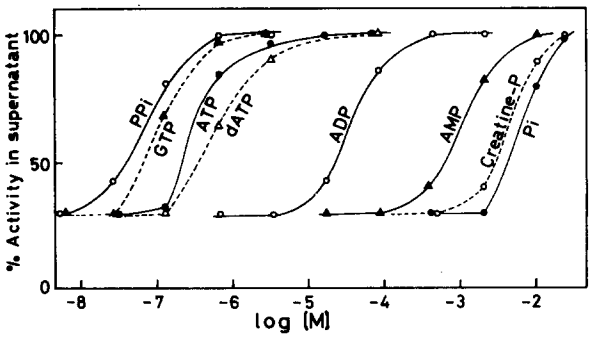

Fig. 2. Effect of various ligands on binding of muscle AMP deaminase to myosin. 10 μg of myosin in 5 mM imidazole/HCl (pH 6.5) containing 0.5 M KCl was mixed with 0.5 unit of rat muscle AMP deaminase in the same buffer. The solution was then diluted with 9 volumes of 5 mM imidazole/HCl (pH 6.5) containing various concentrations of ligands and 0.02% bovine serum albumin to a KCl concentration of 50 mM. After centrifugation, the supernatant was assayed for the enzyme activity. Various ligands used are indicated by figures identifying the curve.

inhibiting the interaction of the enzyme with myosin, 50% inhibition occurring at 5 to 7 mM.

Nucleoside triphosphate, ADP, creatine phosphate and inorganic phosphate, all at concentrations considered to be physiological, have been reported to exert a profound effect on rat muscle AMP deaminase activity [9—13]. From the binding studies, it was shown that pyrophosphate and deoxyribonucleoside triphosphates affected the binding of the enzyme to myosin at concentration less than 1 μM. Therefore, the inhibitory effects of these compounds and the known effectors on the deaminase activity were examined. Inorganic phosphate showed 50% inhibition at concentration of 550 μM. Pyrophosphate was more effective in inhibiting the enzyme activity than inorganic phosphate; 50% inhibition occurred at 30 μM. The nucleoside triphosphates were potent inhibitor; at concentrations as low as 20 μM, the enzyme showed 32, 7, 25 and 26% of the original activity by ATP, GTP, CTP and UTP, respectively. The deoxyribonucleoside triphosphates were as effective as ribonucleosides; 25, 10, 30 and 10% of the original activity remained by dATP, dGTP, dCTP and TTP, respectively, at concentration of 20 μM. The concentrations required for 50% inhibition were less than 10 μM by all nucleoside triphosphates. Among the compounds examined, GTP was found to be the most potent inhibitor; 50% inhibition occurred at 1.6 μM. Since ADP is known as an activator [10—12] and AMP as the substrate, all of the compounds affecting AMP deaminase activity were effective in abolishing the interaction of the enzyme with myosin. The potent inhibitors for enzyme activity are generally potent in abolishing the interactions between two proteins, however pyrophosphate is most potent in abolishing the interactions in spite of its low inhibitory effect on the enzyme activity.

Previously, we demonstrated that there are three parental isozymes (A, B and C) in the rat [5]. Isozyme A is the form found in muscle; B, the major form in liver; C, the form found in heart. They differed from one another with respect to their chromatographic behavior, their kinetic properties, and their reactions with specific antibodies. It seems clear that AMP deaminase A from

rat muscle interacts with myosin to form a complex. Therefore, it is of interest to know whether there is the isozymic specificity in binding of the deaminase to myosin. Homogeneous liver enzyme and partially purified heart enzyme were prepared by the method described previously [5,14], and they were tested for their binding to myosin using the same amount of enzyme (0.5 unit). In these experiments, 74% of liver enzyme and 70% of heart enzyme were bound to 10 μg of myosin. Under the same binding condition, 71% of muscle enzyme was bound. These results indicate that there is no isozymic specificity in binding of the enzyme to myosin.

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