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# INTERACTION OF RAT MUSCLE AMP DEAMINASE WITH MYOSIN

## II. MODIFICATION OF THE KINETIC AND REGULATORY PROPERTIES OF RAT MUSCLE AMP DEAMINASE BY MYOSIN

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## Summary

The problems of whether the kinetic and regulatory properties of AMP deaminase were modified by formation of a deaminase-myosin complex were investigated with an enzyme preparation from rat skeletal muscle.

Results showed that AMP deaminase was activated by binding to myosin. Myosin-bound AMP deaminase showed a sigmoidal activity curve with respect to AMP concentration in the absence of ATP and ADP, but a hyperbolic curve in their presence. Addition of ATP and ADP doubled the $V$ value, but did not affect the $K_m$ value. Myosin-bound AMP deaminase also gave a sigmoidal curve in the presence of alkali metal ions, whereas free AMP deaminase gave a hyperbolic curve. GTP abolished the activating effects of both myosin and ATP.

## Introduction

In the previous papers [1,2], we reported that in rat skeletal muscle adenylosuccinate synthetase (IMP:L-aspartate ligase (GDP-forming EC 6.3.4.4) and AMP deaminase (AMP-aminohydrolase, EC 3.5.4.6) are bound to F-actin and myosin, respectively. We suggested that this compartmentation might favor ammoniagenesis through the purine nucleotide cycle [3] glycolysis [1,4] during muscle contraction. Recently, it was reported that aldolase (D-fructose-1,6-biphosphate D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.13) in muscle is bound to actin [5] and that its interaction with actin resulted in changes in its kinetic properties [6]. We reported previously that the activity of adenylosuccinate synthetase was not changed by its interaction with actin. However,

it seemed possible that this binding to myosin might modify the regulatory properties of AMP deaminase on ammoniagenesis and glycolysis. This paper reports the studies confirming this possibility.

## Materials and Methods

Nucleotides were purchased from Sigma Chemical Co. AMP deaminase from skeletal muscle, cardiac muscle, liver and kidney, myosin, F-actin, actomyosin, heavy meromyosin (H-meromyosin) and light meromyosin (L-meromyosin) of rats were prepared as described in previous papers [1,2,7]. The preparations of myosin, F-actin, actomyosin, H-meromyosin and L-meromyosin were not contaminated with either AMP deaminase or adenylate kinase (EC 2.7.4.3).

AMP deaminase was assayed as described previously [2]. Myosin-bound AMP deaminase was assayed after homogenizing the suspension in 50 mM Tris-HCl buffer (pH 7.0) with a Potter-Elvehjem homogenizer and a Teflon pestle. Incubation was carried out with vigorous shaking. Myosin and actomyosin ATPases were assayed by the method of Sugita et al. [8]. The reaction medium for myosin ATPase assay contained enzyme solution, 1 mM ATP, 10 mM $CaCl_2$, 0.6 M KCl and 20 mM Tris-maleate buffer (pH 6.8) in a final volume of 2.0 ml. The reaction mixture for actomyosin ATPase contained enzyme solution, 1 mM ATP, 1 mM $MgSO_4$, 20 $\mu$M $CaCl_2$, 30 mM KCl and 20 mM Tris-maleate buffer (pH 6.8) in a final volume of 2 ml. These mixtures were incubated at 37°C for 30 min and the phosphate liberated from ATP was determined by the method of Fiske-SubbaRow [9]. One unit of each enzyme activity was defined as the amount catalyzing the formation of 1 $\mu$mol of product (ammonia or $P_i$) per min. Protein concentration was determined by the method of Lowry et al. [10] with bovine serum albumin as the standard.

## Results

*Effects of various muscle contractile proteins in AMP deaminase activity*

In the previous paper, we demonstrated that AMP deaminase of rat skeletal muscle, but not the other isozymes, was bound to the L-meromyosin portion of myosin [2]. Then we examined whether AMP deaminase activity was affected only by myosin, and especially its L-meromyosin portion. For this purpose, rat muscle AMP deaminase was incubated with various muscle contractile proteins in 50 mM Tris-HCl buffer (pH 7.0) at 37°C for 10 min. As described previously [2], under these conditions AMP deaminase was bound to myosin, actomyosin and L-meromyosin, but not to F-actin or the H-meromyosin portion of myosin. As shown in Table I, AMP deaminase activity could be detected in all the suspensions tested, but it was increased by about 70% on incubation with myosin, actomyosin or L-meromyosin. In contrast, the enzyme activity was not increased by incubation with H-meromyosin or F-actin. Next the myosin and actomyosin ATPase activities were determined to see if conversely they also were modified by AMP deaminase. However, the change of ATPase activity upon binding to myosin or actomyosin was not detected under our experimental conditions and they were not influenced by the addition of AMP deaminase. With F-actin, almost all the activity of AMP deaminase was

TABLE I

EFFECTS OF VARIOUS MUSCLE CONTRACTILE PROTEINS ON RAT SKELETAL MUSCLE AMP DEAMINASE ACTIVITY

Rat muscle AMP deaminase (2 $\mu$g) was incubated with various muscle contractile proteins in 50 mM Tris-HCl buffer (pH 7.0) at 37°C for 10 min and then the enzyme activity was determined in the reaction mixture and the supernatant obtained by centrifugation of the mixture at 5000 × $g$ for 10 min. n.d., not determined.

| Addition | | AMP deaminase activity | |
|---|---|---|---|
| | | Reaction mixture | Supernatant |
| None | | 100 | 100 |
| Myosin | 203 $\mu$g | 173 | 0 |
| L-Meromyosin | 145 $\mu$g | 164 | 0 |
| H-Meromyosin | 120 $\mu$g | 109 | n.d. |
| Actomyosin | 210 $\mu$g | 160 | 0 |
| F-actin | 209 $\mu$g | 104 | 86 |

recovered in the supernatant obtained by centrifugation of the reaction mixture, bearing in mind that AMP deaminase did not interact with F-actin, and thus explaining why the enzyme activity was not modified by F-actin. These findings indicate that the interaction of AMP deaminase and myosin (probably the L-meromyosin portion) results in specific change in AMP deaminase activity.

*Effect of myosin concentration on AMP deaminase activity*

A fixed amount (14 $\mu$g) of rat muscle AMP deaminase (17 units) was incubated with various amounts of myosin in 50 mM Tris-HCl buffer (pH 7.0) and then the enzyme activity was determined in the reaction mixture and in the supernatant obtained by centrifugation of the reaction mixture at 5000 × $g$ for 10 min. As shown in Fig. 1, maximum activation of AMP deaminase was observed in the presence of 106 $\mu$g of myosin. With this concentration of myosin, scarcely any AMP deaminase activity was detected in the supernatant. Assuming that the molecular weights of myosin and AMP deaminase are 470 000 and 243 000, respectively, the molar ratio of AMP deaminase to myosin for maximum activation of the deaminase was calculated to be 1 : 4. This value is similar to the binding ratio of AMP deaminase to myosin (1 : 3). This result also suggests that rat muscle AMP deaminase is activated as a result of its binding to myosin. Similar ratios were obtained on activation with actomyosin and L-meromyosin.

*Effect of myosin on AMP deaminase isozyme activities*

Table II shows that among the AMP deaminase isozyme tested, only AMP deaminase from rat skeletal muscle was activated by addition of myosin. This supports the previous conclusion that the activation of this AMP deaminase is due to its specific binding to myosin.

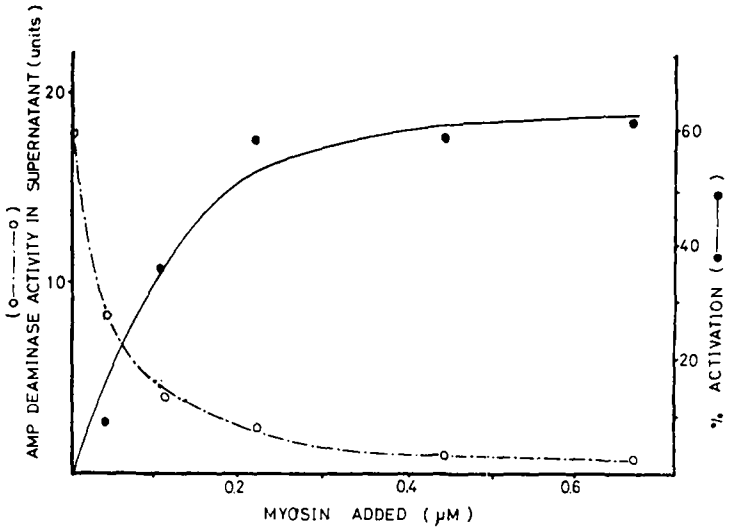


Fig. 1. Activation of rat muscle AMP deaminase by myosin. Samples of 14 μg of rat muscle AMP deaminase (specific activity, 1274 units per mg protein) were incubated with various amounts of myosin in 50 mM Tris-HCl buffer (pH 7.0) at 37°C for 10 min. AMP deaminase activity was determined in the suspension after incubation and in the supernatant of the incubation mixture obtained by centrifugation at 5000 × *g* for 10 min.

### *Effect of binding on the regulatory and kinetic properties of muscle AMP deaminase*

It is known that AMP deaminase is activated by alkali metal ions, ATP and ADP, whereas it is inhibited by GTP and GDP [11]. Table III summarizes the effects of these nucleotides and alkali metal ions on myosin-bound and free AMP deaminase activities. In these experiments, AMP deaminase was assayed in the presence of 1 mM AMP as substrate. This concentration is approximately

TABLE II

EFFECTS OF MYOSIN ON AMP DEAMINASE ISOZYMES FROM THE LIVER, KIDNEY AND CARDIAC MUSCLE

In this experiment, enzyme solutions (liver type I, 0.95 unit; liver type II, 0.66 unit; kidney type I, 0.45 unit; kidney type II, 0.38 unit; cardiac muscle, 0.74 unit; and skeletal muscle, 1.23 units) were incubated with myosin (203 μg) in 50 mM Tris-HCl buffer (pH 7.0) at 37°C for 10 min. Enzyme activities were determined in the reaction mixture and supernatant obtained by centrifugation at 5 000 × *g* for 10 min.

| Isozyme | AMP deaminase activity | |
|---|---|---|
| | Reaction mixture * | Supernatant ** |
| Skeletal muscle | 152.3 | 0.2 |
| Cardiac muscle | 104.0 | 79.9 |
| Liver type I | 101.3 | 78.3 |
| Liver type II | 102.3 | 84.5 |
| Kidney type I | 103.0 | 88.2 |
| Kidney type II | 102.8 | 90.4 |

* Percentage of the isozyme activity added.

** Percentage recovery of activity in the supernatant.

TABLE III

EFFECTS OF ALKALI METAL IONS AND NUCLEOTIDES ON MYOSIN-BOUND AND FREE AMP DEAMINASE ACTIVITY

Myosin-bound AMP deaminase was prepared as follows. AMP deaminase (95 $\mu$g) was incubated with myosin (10.1 mg) in 50 mM Tris-HCl buffer (pH 7.0) at 37°C for 10 min and then centrifuged at 5 000 × $g$ for 10 min. The precipitate was suspended in the same buffer. AMP deaminase activity was determined at 1 mM AMP concentration.

| Addition | | AMP deaminase activity (%) | |
|---|---|---|---|
| | | Free enzyme | Myosin-bound enzyme |
| None | | 100 | 143 |
| KCl | 50 mM | 205 | 154 |
| NaCl | 50 mM | 155 | 135 |
| ATP | 2 mM | 238 | 387 |
| ATP | 2 mM + GTP 2 mM | 164 | 160 |
| ADP | 2 mM | 200 | 326 |
| ADP | 2 mM + GTP 2 mM | 155 | 150 |
| GTP | 2 mM | 71 | 58 |

the physiological concentration in muscle cells [12]. The results confirm that free AMP deaminase was considerably activated by ATP or ADP. As shown in Table III, however, activation was greater with myosin plus adenine nucleotide than with adenine nucleotide alone. GTP decreased not only the activation of AMP deaminase by ATP or ADP, but also the activation by myosin. Alkali

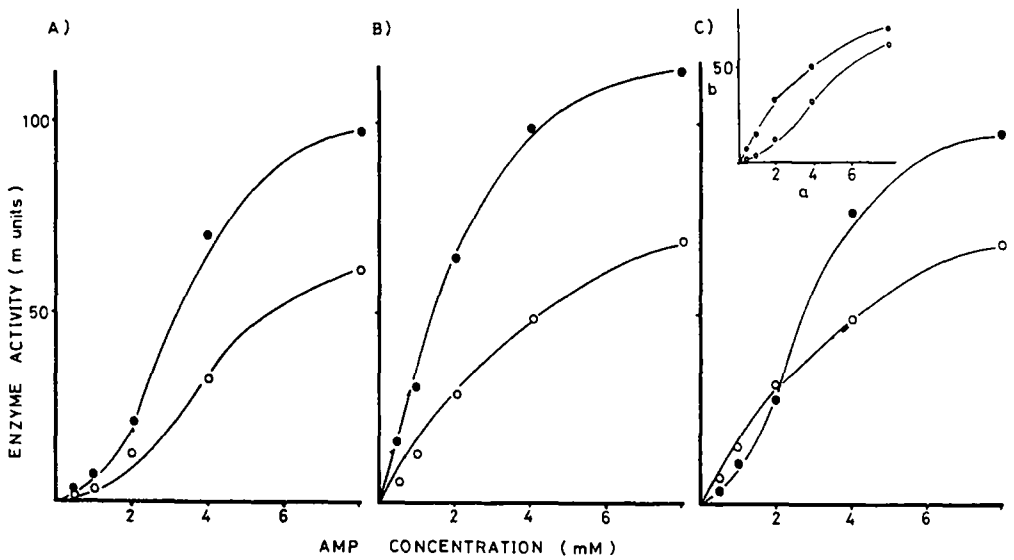


Fig. 2. Effects of ATP and KCl on the substrate-velocity curve of myosin-bound and free AMP deaminase. The reaction mixture contained enzyme solution, 50 mM Tris-maleate buffer (pH 6.5) and 4 mM ATP (B) or 50 mM KCl (C) in a final volume 0.6 ml. As controls ATP and KCl were omitted from the reaction mixture (A). ○———○, free AMP deaminase, and ●———●, Myosin-bound AMP deaminase. The inset shows the Substrate-velocity curve of free AMP deaminase in the presence or absence of 50 mM KCl ( the data were adapted from 2A and 2C). *a* and *b* indicate AMP deaminase concentration (mM) and enzyme activity (munits), respectively. ●———●, in the presence of KCl; ○———○, in the absence of KCl.

metal ions, such as potassium and sodium ions, activated free AMP deaminase. The $K_a$ values for $K^+$ and $Na^+$ were calculated to be 9.2 mM and 42 mM, respectively. However, they did not affect myosin-bound AMP deaminase. Neither myosin-bound AMP deaminase nor the free enzyme was influenced by divalent cations, glycolytic intermediates or aminoacids.

Fig. 2 shows the effects of ATP and $K^+$ on the substrate-velocity curve of myosin-bound AMP deaminase and free enzyme. In the presence of 4 mM ATP, myosin increased the $V$ about 2-fold, but did not affect the $K_m$ value for AMP (Fig. 2B). Like free AMP deaminase, myosin-bound AMP deaminase exhibited a sigmoidal curve with respect to AMP concentration in the absence of ATP (Fig. 2A), but a hyperbolic curve in the presence of ATP. However, the responses of free and myosin-bound AMP deaminases to $K^+$ were different, as shown in Fig. 2C, in the presence of 50 mM KCl, myosin-bound AMP deaminase showed a sigmoidal curve, unlike free AMP deaminase which showed a hyperbolic curve (inset of Fig. 2C). This suggests that alkali metal ions bound to an allosteric binding site of free AMP deaminase to normalize the reaction, but that myosin may block or hinder the binding of alkali metal ion to AMP deaminase and thus prevent the activating effect of KCl.

## Discussion

Previously we found by in vitro binding experiments that AMP deaminase from rat skeletal muscle is bound to myosin [2]. From these findings, we thought that the head portion of myosin was the binding site for AMP deaminase, because it interacts with actin, thus enhancing ATPase activity. However, contrary to expectation, we found that AMP deaminase was bound to the light meromyosin portion of myosin. Furthermore, we found that this interaction modified the AMP deaminase activity, although it did not change the ATPase activity of myosin. The only other modification of enzyme activity by muscle contractile proteins so far reported is that of aldolase. Arnold and Pette [6] reported that the $V$ of aldolase was almost doubled and its $K_m$ value for fructose-1,6-diphosphate was increased almost 10-fold by its interaction with actin. Similarly we found that in the presence of ATP the $V$ value of myosin-bound AMP deaminase was approximately 2-fold that of the free enzyme, whereas the $K_m$ value for AMP of myosin-bound AMP deaminase was almost the same as that of the free enzyme. With regard to the $K_m$ value of AMP deaminase, however, the effect of binding is complex in the absence of ATP.

It has been shown that AMP deaminase is regulated by three different effector groups: two for the activator group (adenine nucleotides, such as ATP and ADP, and alkali metal ions, such as $K^+$, $Na^+$ and $Li^+$) and one for the inhibitor group (guanine nucleotides, such as GTP and GDP). These groups are bound not only to different sites from each other, but also to different sites from the substrate binding site [11].

In the absence of ATP, the substrate-velocity curves of AMP deaminase were sigmoid, irrespective of whether the deaminase was bound to myosin or not, indicating a substrate cooperative effect (Fig. 2A). On addition of ATP these curves became hyperbolic (Fig. 2B). These findings suggest that myosin activates AMP deaminase by binding to a different site on the enzyme from that

for ATP. We have not yet examined the effect of GTP on the substrate-velocity curve. However, 2 mM GTP abolished the activating effect of myosin on AMP deaminase at a substrate concentration of 1 mM (Table III). KCl also normalized the substrate-velocity curve of free AMP deaminase, as shown in the inset of Fig. 2C. The *V* value of myosin-bound enzyme was also increased over that of free enzyme in the presence of KCl, but the substrate-velocity curve was sigmoidal with less than 3 mM AMP. These findings suggest that myosin is bound to a site of AMP deaminase located very close to the binding site for alkali metal ion and so abolishes the normalizing effect of $K^+$, although it increases the *V* value of the enzyme, which is independent of $K^+$. From the results, the levels of ATP and GTP seem to be more important than the level of alkali metal ion in regulation of myosin-bound AMP deaminase activity. Thus, the kinetic properties of rat muscle AMP deaminase are modified by the binding to myosin. Ashby et al. [13] reported that inorganic phosphate at a concentration of 5 mM resulted in dissociation of the complex of rabbit muscle AMP deaminase and myosin. We also observed that approximately 30% of rat muscle AMP deaminase was dissociated from the enzyme-myosin complex in the presence of phosphate at a physiological concentration range (8 mM) [12]. These findings suggest that inorganic phosphate might be involved in the regulation of AMP deaminase through the change of its distribution in muscle cells. In line with this finding, Wilson [14] found that the kinetic properties of hexokinase are also modified by change in its intracellular distribution and named this an ambiquitous enzyme. In this sense, rat muscle AMP deaminase is a typical ambiquitous enzyme. However, inorganic phosphate is an inhibitor of AMP deaminase [15] with an inhibition constant of less than 1 mM. Accordingly, the problem of whether inorganic phosphate at physiological concentration range plays a critical role in the regulation of rat muscle AMP deaminase must await further investigation.

Ogasawara et al. [16] reported that AMP deaminase could be separated into three types of isozyme by column chromatography on phosphocellulose: muscle, liver and heart types. We also demonstrated that AMP deaminase from rat skeletal muscle differed chromatographically, electrophoretically and immunochemically from the isozymes in other organs. In previous work, we showed that, of the isozymes from various organs, only the isozyme from skeletal muscle interacted with myosin from rat skeletal muscle [2]. We also showed that only the skeletal muscle enzyme was activated by interaction with myosin. These findings indicate organ specificity in activation of AMP deaminase by myosin.

As previously mentioned, the purine nucleotide cycle might work not only for ammoniagenesis, but also for the regulation of glycolysis during muscular contraction [4]. In this sense, AMP deaminase bound to myosin might play a more critical role in the regulation of ammoniagenesis and glycolysis than adenylosuccinate synthetase bound to actin, since the synthetase activity is not modified by this binding.

However, the problem of whether these in vitro findings actually reflect an in vivo regulatory function of AMP deaminase in muscle cells requires further investigation. In connection with this problem, it is interesting that it has been reported that AMP deaminase activity is markedly reduced in muscle prepara-

tion from dystrophic mice [17], humans with Duchenne type dystrophy [18], patients with hypokalemic periodic paralysis [19] and denerved rats [20]. In these muscles, no abnormality has yet been found in the function of myosin and actomyosin. Thus it is tempting to speculate that energy metabolism may be impaired in these diseased muscles because AMP deaminase is deranged and so cannot bind to myosin. Studies on these dystrophic muscles might provide a clue to the actual regulatory function of AMP deaminase.

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