Adenosinetriphosphatase Activity of Squid Myosin*¹Takahide TSUCHIYA*², Noboru YAMADA*^{2,3}, Hisayo MORI*²,
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Myosin of the obliquely striated mantle muscle of squid, *Ommastrephes sloani pacificus*, was isolated by the authors' method and its ATPase profiles were studied under various reaction conditions. With Ca^{2+} (5 mM) or Mg^{2+} (1 mM) present and in 600 mM KCl, the activity—pH curve showed 2 peaks, at pH 7.0 and near pH 8.5, where the activity with Ca^{2+} was higher than that with Mg^{2+} . The activity—ionic strength curve gave a peak at 0.2 M KCl with declining slope beyond that concentration. Ca^{2+} activated the ATPase with a maximum effect at 1–9 mM, while Mg^{2+} caused a slight activation with a maximum effect at 0.1 mM. The activity was depressed with EDTA for all concentrations between 0.1–1 mM, but in a different manner from the ATPase of skeletal muscle myosins. PCMB did not affect either Ca^{2+} -ATPase or EDTA-ATPase. These profiles of the squid myosin ATPase were compared with those of the myosin ATPases from other sources. The squid myosin ATPase was found to be very different from the skeletal muscle myosin ATPases and, in some detailed points, distinct from the myosin ATPases from invertebrate and nonmuscular sources.

In the authors' program to elucidate the protein constitution of the obliquely striated muscle of squid mantle, isolation and characterization of physico-chemical properties of the myosin have been reported in the previous papers.^{1,2)} For characterizing the properties of myosin in relation to the contractile function of muscle, description of the ATPase activity profiles is necessary. With the ATPase activity of the invertebrate myosins, information is not so abundant as those of the vertebrate myosins due to the presence of paramyosin in the invertebrate muscles which is hardly removed from the myosin preparation and might interfere with the ATPase profile. Works have been reported by RÜEGG³⁾ and BÁRÁNY⁴⁾ on *Pecten* myosin, by DE VILLAFRANCA⁵⁾ on *Limulus* myosin, by NISHIDA⁶⁾ and HASHIMOTO^{7,8)} on *Pecten* and lobster myosins, by HIGASHI⁹⁾ on abalone myosin, and by YAMAGUCHI¹⁰⁾ on *Ascaris* myosin which has an obliquely striated muscle structure similar to the squid mantle, respectively. With the squid muscle proteins, ATPase activity of actomyosin has been studied by DE VILLAFRANCA and the present authors.¹²⁾

The present paper deals with the characteri-

zation of the ATPase activity of the squid myosin isolated intact and almost free of paramyosin by the method reported previously.¹¹⁾

Materials and Methods

Material

Fresh squid, *Ommastrephes sloani pacificus*, was purchased at the Tokyo Central Wholesale Fish Market.

Squid Myosin

Myosin was prepared from the squid mantle muscle according to the method developed by the authors.¹¹⁾

Carp Myosin

Myosin was prepared from dorsal lateral muscle of carp, *Cyprinus carpio*, according to the method reported previously.¹⁴⁾

Determination of ATPase Activity

The activity was measured at 25°C under the following conditions: Protein, 0.2 mg/ml; KCl, 50 or 600 mM; Tris-maleate buffer (pH 7.0), 20 mM;

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ATP, 1 mM; either of MgCl_2 (1 mM), CaCl_2 (5 mM) or EDTA (1 mM). Reaction time, 5 min. The phosphate liberated was determined by FISKE and SUBBAROW's method.¹³⁾

Unless otherwise stated the methods used were the same as reported previously.^{1,2,12,14)}

Results

ATP splitting in the Function of Reaction Time

ATPase activity was measured at various times of reaction in the presence of either Ca^{2+} (5 mM), Mg^{2+} (1 mM) or EDTA (1 mM) resulting in Fig. 1. The ATPase was activated markedly with Ca^{2+} , and slightly with Mg^{2+} , while it was depressed by EDTA. These patterns coincided with those of the squid actomyosin¹²⁾ and resembled those of the *Pecten* myosin.^{3,4)}

Fig. 1. Effect of Ca^{2+} , Mg^{2+} and EDTA on the time course of Pi liberation from ATP by squid myosin ATPase.

Conditions: protein, 0.2 mg/ml; KCl, 600 mM; Tris maleate buffer (pH 7.0), 20 mM; ATP, 1 mM; either of Ca^{2+} (5 mM), Mg^{2+} (1 mM) or EDTA (1 mM). ○—○ 5 mM Ca^{2+} , ×—× 1 mM EDTA, ●—● 1 mM Mg^{2+} , □—□ Control.

ATPase Activity at Various pH's

The ATPase activity was found to vary with the change in pH as shown in Fig. 2. At both 50 mM and 600 mM KCl and in the presence of

Ca^{2+} (5 mM), there were found 2 peaks at pH 7.0 and at near pH 8.5, and a hollow at pH 8.0. Such presence of 2 peaks was similar to the case with the *Pecten* myosin,^{3,4)} though the pH values of each peak and hollow did not agree precisely between the both cases. However, the pattern of the curve with the squid myosin made much difference from that of the rabbit myosin where a peak is found at pH 6.4 and the activity keeps rising beyond pH 8.0.¹⁵⁾ The results in the presence of Mg^{2+} (1 mM) were not illustrated here, because there were but little variation with pH due to the low level of the activity.

Fig. 2. ATPase activity of squid myosin at various pH's in the presence of Ca^{2+} added on 50 mM (a) and 600 mM (b) KCl. Conditions: Same to Fig. 1.

Dependence of ATPase on Ionic Strength

The ATPase activity in the presence of Ca^{2+} (5 mM) varied in a function of KCl concentration as shown in Fig. 3. While the ATPase activity

Fig. 3. ATPase activity of squid myosin at various concentrations of KCl with Ca^{2+} added. Conditions: Same to Fig. 1.

of the skeletal myosins decreases linearly with increasing ionic strength,¹⁵⁾ that of the squid myosin gave a curve with a peak at near 0.2 M KCl. The latter is identical with the case in the squid actomyosin¹²⁾ and similar to the case in the *Pecten* myosin.⁴⁾

Effect of Ca^{2+} and Mg^{2+} ions

The ATPase activity at various concentrations of Ca^{2+} and Mg^{2+} ions was illustrated in Fig. 4. At both the low and high ionic strengths, a maximum was found at 10^{-8} – 10^{-2} M Ca^{2+} , while a slight peak was found at 10^{-4} M Mg^{2+} which was much less marked as compared with the case in the squid actomyosin.¹²⁾ Further detailed study at 10^{-4} – 10^{-2} M Ca^{2+} and Mg^{2+} was carried out

Fig. 4. ATPase activity of squid myosin at various concentrations of Ca^{2+} and Mg^{2+} added on 50 mM (a) and 600 mM (b) KCl. Conditions: Same to Fig. 1. ○—○, Ca^{2+} , ●—● Mg^{2+} .

Fig. 5. ATPase activity of squid myosin at various concentrations between 0.1–9 mM of Ca^{2+} and Mg^{2+} added on 600 mM KCl. ○—○, Ca^{2+} , ●—● Mg^{2+} .

as shown in Fig. 5. Thus, the maximum value was attained between 1–9 mM, while the curve with Mg^{2+} was flat.

Effect of EDTA

Effect of various amounts of EDTA on the ATPase activity was studied parallelly on the squid myosin as well as on the carp myosin for reference as shown in Fig. 6. While the activity of the carp myosin ATPase, like that of the rabbit myosin ATPase,¹⁷⁾ was elevated with the raised EDTA concentration, that of the squid myosin was depressed with EDTA irrespective of the EDTA concentration. Such inactivation with EDTA, was found also in the squid actomyosin,¹²⁾ and in the myosins of *Pecten*⁴⁾ and *Plasmodium*.¹⁸⁾

Fig. 6. ATPase activity of squid myosin at various concentrations of EDTA added on 600 mM KCl. ○—○ Squid, ○---○ Carp.

Effect of PCMB

The activity of Ca^{2+} -ATPase and EDTA-ATPase of the squid myosin as a function of of PCMB (*p*-chloromercuribenzoate) was studied resulting in Fig. 7. Whereas the Ca^{2+} -ATPase of the skeletal muscle myosins is activated with PCMB below 0.3–0.4 $\mu\text{M}/\text{mg}\cdot\text{protein}$ and inactivated beyond that threshold condition,¹⁹⁾ that of the squid myosin did not show any effect, either accelerative or decelerative, on the elevated activity of the Ca^{2+} -ATPase and on the depressed activity of the EDTA-ATPase at any quantity of added PCMB. Such behavior of the squid myosin ATPase was also distinct from the Ca^{2+} -ATPases of the myosins of smooth muscle²⁰⁾ and *Plasmodium*¹⁸⁾ which show a linearly decreasing curve with the increasing PCMB. In the case of the EDTA-ATPase, the depressive effect of EDTA was not affected by PCMB added on.

Discussion

The present results on the ATPase activity of

Fig. 7. Effect of PCMB on ATPase activity of squid myosin. Myosin was preincubated with PCMB for 10 min at 0°C. ATPase activity was measured under the following conditions: Protein, 0.2 mg/ml; KCl, 600 mM, Tris-maleate buffer (pH 7.0), 20 mM; CaCl₂, 5 mM, or EDTA, 1 mM; ATP, 1 mM. ○—○ Ca²⁺-ATPase, ×—× EDTA-ATPase.

the squid myosin are summarized in Table 1 together with those of rabbit,¹⁶⁾ carp,²¹⁾ *Pecten*,⁴⁾ lobster⁸⁾ and *Plasmodium*¹⁸⁾ for reference. As found in the Table, the squid myosin appears distinct from the myosins of other origins in some respects. The squid myosin is similar to the *Pecten* myosin with respect to the modes of dependence on pH and on ionic strength. However, myosin of another Molluscs, abalone, shows properties similar to those of the skeletal muscle myosins in the modes of dependence on pH and on ionic strength. Myosin of lobster, an Arthropod, belongs to the latter group. Thus, it seems difficult to define an ATPase profile to cover the characteristics of the molluscan myosins.

That the squid myosin ATPase is activated, though to little extent, with Mg²⁺ is a feature which is not found in any myosin of other origins so far studied and, thus, characteristic to the squid myosin.

Also, in the presence of EDTA added, the squid myosin ATPase was depressed in contrast to the case with the skeletal muscle myosin

ATPases which are activated. Such depression with EDTA might be related with that the squid myosin ATPase was activated by Mg²⁺ to some extent contrary to the skeletal myosin ATPases which are inactivated by Mg²⁺.

The effect of EDTA might involve the dissociation of a light chain from the squid myosin molecule as proposed by SZENT-GYÖRGYI *et al.*²²⁾ Nevertheless, the recent data of the present authors* disproved such dissociation as well as the change in Ca-sensitivity of the squid myosin B by EDTA treatment. Thus, the present results of the EDTA-depressed ATPase should represent some property inherent to the squid myosin ATPase.

In the effect of PCMB on the ATPase activity, also, the squid myosin is different from the myosins of either skeletal muscle or smooth muscle. Though the squid myosin ATPase appears similar to that of *Pecten* with respect to the modes of dependence on pH and ionic strength, data on the effect of PCMB are lacking for the *Pecten* myosin.

The different sensitivity of the ATPases of the skeletal muscle myosins and the squid myosin to added PCMB might suggest a possible difference in the conformation involving SH-groups near the active site. This view is compatible with the data that the content of unaccessible SH-groups is higher in the squid myosin than in the myosins of other origins.²⁾

The present data altogether suggest that the squid myosin has ATPase profiles which are distinct from any myosins of vertebrate, invertebrate or nonmuscular origin so far studied, though it seems most similar to the *Pecten* myosin.

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Table 1. Properties of ATPase activity of squid, *Pecten*, lobster, carp, rabbit and *Plasmodium*

	Squid		<i>Pecten</i>		Lobster		Carp		Rabbit		<i>Plasmodium</i>	
Ionic strength	L	H	L	H	L	H	L	H	L	H	L	H
Effect of												
Ca ²⁺	+	+	+	+		+	+	+	+	+	+	+
Mg ²⁺	+	+	—	—		—	—	—	—	—	—	—
EDTA	—	—	—	—		+	—	+	—	+	—	—
Dependence on pH												
number of peaks		2		2		2		1		1		—
Dependence on KCl concn.		peak at 0.2 M KCl				decreasing with increasing KCl						

* Unpublished data.

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