Studies on Myosin of Spiny Lobster—II

Enzymatic Properties

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Purified myosin from the spiny lobster was analyzed for enzymatic properties.

The ATPase activity of lobster myosin was enhanced by Ca^{2+} and EDTA, and was inhibited by Mg^{2+} and TBS. Modes of effect of these and some other modifiers on the ATPase activity of this myosin were essentially the same as for rabbit myosin. In addition, Ca^{2+} -ATPase activity of the lobster myosin decreased nearly proportionally with increasing KCl concentration, as in the case of rabbit myosin.

On the other hand, the pH-activity curve of this myosin showed two maxima, around pH 6 and 8, and both maxima were shifted to about pH 7 and 8.5-9 in the presence of Ca^{2+} . In these respects as well as in exhibiting generally low ATPase activity under various conditions, spiny lobster myosin was more comparable with scallop myosin than with rabbit myosin.

Since we succeeded in preparing the spiny lobster myosin of high purity as reported in the preceding paper,¹⁾ we intended this time to study its enzymatic properties. The results obtained showed that spiny lobster myosin was essentially comparable to other myosins so far reported, though clearly species-specific in some aspects.

Experimental

Spiny lobster myosin The purified myosin was prepared from the abdominal muscle of spiny lobster, *Panulirus japonicus*, as reported previously.¹⁾ The myosin preparations thus obtained were stored in iced water and all the analyses were completed within a few days after initiation of storage. In some experiments, the aggregated myosin of the lobster which was obtained in the course of preparation of the monomeric myosin,¹⁾ was also assayed for comparison.

Measurement of ATPase activity The ATPase activity of myosin was measured almost as usual. The standard assay mixture contained 40 mM Tris-HCl of pH 8.0~8.5, 0.5 M KCl, 1~2 mM ATP, 1~4 mg myosin/ml. After 5 min-incubation at 25°C, the reaction was stopped by adding 10% TCA and the amount of Pi (inorganic phosphate) liberated was determined by the method of ROCKSTEIN *et al.*²⁾

Under the above conditions, effects of the following modifiers on the ATPase activity of this myosin were examined: Ca^{2+} , Mg^{2+} , EDTA, TBS,^{*3} NTP,^{*4} and iodine (I_2). In

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^{*3} 2, 4, 6-Trinitrobenzenesulfonate.

^{*4} p-Nitrothiophenol.

addition, the Q_p value was estimated at pH 9.1 by PERRY's method.³⁾ Effects of pH (5~10.5) and KCl concentration (0.065~0.5 M) on the ATPase activity were also examined.

The protein concentration was determined by the modified biuret method of UMEMOTO.⁴⁾

Determination of amounts of modifiers bound by myosin The amounts of TBS, NTP, and I_2 bound by the myosin were measured essentially according to the method of KUBO *et al.*,⁵⁾ KITAGAWA *et al.*,⁶⁾ and HASHIZUME *et al.*,⁷⁾ respectively.

Throughout the present experiment, analytical grade reagents and deionized glass-distilled water were used.

Results and Discussion

Time course of Pi liberation In Fig. 1 are presented the time courses of Pi liberation from the spiny lobster myosin-ATP system with or without Ca^{2+} or Mg^{2+} . As clearly seen, the myosin was activated by Ca^{2+} and inhibited by Mg^{2+} . Considering those results and also the reproducibility of data in some preliminary experiments, the time of reaction was fixed on 5 min as mentioned above.

Under the conditions adopted here, the ATPase activity of spiny lobster myosin was estimated at about 0.03 μ mole Pi/mg protein/min in the absence of either modifier. For

Fig. 1. Effects of Ca^{2+} and Mg^{2+} on the time course of Pi-liberation from spiny lobster myosin-ATP system. Conditions of ATPase assay: 20 mM Tris-HCl buffer, pH 8.0; 0.5 M KCl; 0.7 mg myosin/ml; 1 mM ATP; 25°C. Dotted line for aggregated myosin.

Fig. 2. Effect of Ca^{2+} on spiny lobster myosin ATPase activity. Conditions of ATPase assay: 40 mM Tris-HCl buffer, pH 8.5; 0.5 M KCl; 2.0 mg myosin/ml; 2 mM ATP; 25°C.

rabbit myosin, 0.037⁸⁾ and 0.088⁹⁾ $\mu\text{mole Pi/mg protein/min}$ have been reported as the activity under similar conditions.

Effects of modifiers on ATPase activity The activity was increased hyperbolically with increasing Ca^{2+} concentration in the range of 0~10 mM (Fig. 2). At the saturating level of Ca^{2+} , the activity was ca. 0.15 $\mu\text{mole Pi/mg protein/min}$. Under similar conditions, rabbit myosin has been reported to exhibit the activity of 0.13~0.28 $\mu\text{mole Pi/mg protein/min}$.^{10,11)}

The ATPase activity was drastically inhibited by Mg^{2+} between 0~0.1 mM and then remained at a constant level of 0.004 $\mu\text{mole Pi/mg protein/min}$ between 0.1 and 0.5 mM, as seen in Fig. 3. Above 0.5 mM, the activity was decreased again, though slowly this time. The Mg^{2+} concentration dependence of such a type has been reported for rabbit myosin.¹²⁾ In passing, Woods *et al.*¹³⁾ reported briefly the inhibition of ATPase activity of lobster myosin by Mg^{2+} . The ATPase activity of spiny lobster myosin was 0.003 $\mu\text{mole Pi/mg protein/min}$ at 2 mM Mg^{2+} . This value is significantly lower than 0.05 $\mu\text{mole Pi/mg protein/min}$ for rabbit myosin⁸⁾ under similar conditions.

The effect of EDTA on the ATPase activity is presented in Fig. 4. The activity was sharply increased with the concentration of EDTA between 0 and 0.5 mM. Above 0.5 mM, the activity was still increased, but much more slowly. The shape of curve, as a whole,

Fig. 3. Effect of Mg^{2+} on spiny lobster myosin ATPase activity. Conditions of ATPase assay: 40 mM Tris-HCl buffer, pH 8.5; 0.5 M KCl; 2.0 mg myosin/ml; 1 mM ATP; 25°C.

Fig. 4. Effect of EDTA on spiny lobster myosin ATPase activity. Conditions of ATPase assay: 40 mM Tris-HCl buffer, pH 8.5; 0.5 M KCl; 1.3 mg myosin/ml; 2 mM ATP; 20°C.

resembles that of rabbit myosin.¹⁴⁾ The ATPase activity at 1 mM EDTA was approximately 0.2 μ mole Pi/mg protein/min, about one third of 0.57 μ mole Pi/mg protein/min for rabbit myosin determined under similar conditions.

The effects of TBS and NTP on the ATPase activity are given in Fig. 5. The activity was sharply decreased with the increasing concentration of TBS and a complete inhibition was effected at 7×10^{-5} M. The ATPase activity of aggregated myosin, however, remained unaffected over the whole concentration range of TBS. On the contrary, NTP increased the ATPase activity to some extent: At 6×10^{-5} M the activity was increased to about 120% compared to that of the control. The initial burst¹⁵⁾ of Pi liberation in the presence of Mg^{2+} was completely extinguished by addition of NTP. Those effects of TBS and NTP for spiny lobster myosin are reminiscent of the results for rabbit myosin reported by TONOMURA *et al.*^{16,17)}

The amounts of TBS and NTP bound by spiny lobster myosin were calculated to be 3.9 and 1.2 moles/ 10^6 g myosin, respectively. The rabbit myosin has been reported to bind 1.1 moles of TBS¹⁶⁾ and 0.85–0.94 mole of NTP⁶⁾ per 10^6 g myosin. Unlike the monomeric myosin, the aggregated myosin was not affected by TBS at all (Fig. 5). In this connection, KUBO *et al.*¹⁸⁾ also observed a similar phenomenon with rabbit myosin and interpreted that this could be attributed to a kind of masking for the active lysyl residue as

Fig. 5. Effects of TBS and NTP on ATPase activity of spiny lobster myosin and aggregated myosin.
Conditions of ATPase assay: 40 mM Tris-HCl buffer, pH 8.0; 0.5 M KCl; 1.3 mg protein/ml; 1 mM ATP; 6 mM Ca^{2+} ; 25°C.

Fig. 6. Effect of I_2 on Ca^{2+} -ATPase activity of spiny lobster myosin.
Conditions of ATPase assay: 40 mM Tris-HCl buffer, pH 8.5; 0.5 M KCl; 1.3 mg myosin/ml; 2 mM ATP; 5 mM Ca^{2+} ; 25°C.

caused by the intermolecular aggregation.

In the next place, the effect of I_2 on the ATPase activity was studied. As seen in Fig. 6, the Ca^{2+} -ATPase activity was strongly inhibited by I_2 . The activity was completely inhibited at 1.3 mM. The EDTA-ATPase was inhibited similarly. This inhibition by I_2 may be caused by oxidation of free sulfhydryl groups, as has already been suggested by BAILEY *et al.*¹⁸⁾

Qp values The Q_p values of monomeric and aggregated forms of spiny lobster myosin were calculated to be 714–834 and 857–975 μ l/mg protein/hr, respectively. These values are much smaller than 2000–5000 μ l/mg protein/hr for rabbit myosin,³⁾ though somewhat larger than the values for another kind of lobster, 376–713 μ l/mg protein/hr.¹³⁾

Effect of pH on ATPase activity In Fig. 7 are given the pH-activity curves of spiny lobster myosin in the absence and presence of some modifiers. The myosin exhibited two pH optima, about 6 and 8, when no modifier was added. The rabbit myosin has also been reported to possess two pH optima at about 6 and above 9.5.^{9,13)} It should be noted that

Fig. 7. PH-activity curves of spiny lobster myosin ATPase in the presence and absence of some modifiers.

Conditions of ATPase assay: 100 mM Tris-HCl buffer; 0.5 M KCl; 0.7 mg myosin/ml; 2 mM ATP; 20°C.

spiny lobster myosin did not exhibit ATPase activity any more at as high a pH as 9.5, as seen in the figure.

In the presence of 5 mM Ca^{2+} , the ATPase activity was greatly enhanced almost all over the pH range. The pH-activity curve was shifted to the right, the two optimum pH's being located around 7 (ATPase activity: ca. 0.4 $\mu\text{mole Pi/mg protein/min}$) and 8.5–9 (activity: ca. 0.55 $\mu\text{mole Pi/mg protein/min}$). The Ca^{2+} -ATPase activity for rabbit myosin has been found optimal at pH 6 (activity: ca. 0.4 $\mu\text{mole Pi/mg protein/min}$) and about 9.5 (activity: >0.9 $\mu\text{mole Pi/mg protein/min}$) under similar conditions.¹⁹⁾ BÁRÁNY *et al.*¹⁹⁾ have reported that scallop myosin has two pH optima, about 6.5 (activity: ca. 0.3 $\mu\text{mole Pi/mg protein/min}$) and 8.5 (activity: ca. 0.3 $\mu\text{mole Pi/mg protein/min}$). Hence, spiny lobster myosin is more comparable to scallop myosin rather than rabbit myosin in this respect. Spiny lobster myosin, however, clearly differs from scallop myosin in the ratio of intensities of activity at both pH optima: With spiny lobster myosin, the activity is significantly higher at the alkaline optimum pH than at the acid to neutral pH, while with scallop myosin, the activities at both optima are almost equal.

In the presence of 3.3 mM EDTA, the activity was to a large extent enhanced all over the pH range and a single activity maximum appeared at about pH 7.5 instead of the two maxima mentioned above. NIHEI *et al.*¹²⁾ observed a comparable phenomenon for rabbit myosin.

Fig. 8. Effect of KCl concentration on Ca^{2+} -ATPase activity of spiny lobster myosin. Conditions of ATPase assay: 20 mM Tris-HCl buffer, pH 7.0; 1.2 mg myosin/ml; 10 mM Ca^{2+} ; 2 mM ATP; 20°C.

Effect of KCl concentration on ATPase activity Results obtained are given in Fig. 8. With the increase of KCl molarity, the ATPase activity was decreased almost straightly: *e. g.*, the activity at 0.4–0.5 M KCl (activity: 0.25–0.3 $\mu\text{mole Pi/mg protein/min}$) was half or less the activity at 0.1 M KCl (activity: 0.6 $\mu\text{mole Pi/mg protein/min}$). A comparable KCl-concentration dependence has also been reported for rabbit myosin.¹⁹⁾ On the other hand, BÁRÁNY *et al.*¹⁹⁾ have shown that the ATPase activity of scallop myosin was highest at 0.2 M KCl (activity: 0.6 $\mu\text{mole Pi/mg protein/min}$), being twice as high as that at either 0.1 M or 0.3–0.4 M KCl.

Kinetic constants of myosin ATPase
In Fig. 9 is given the ATP concentration dependence of the activity. As seen in the figure, the ATPase activity was increased hyperbolically with the

Fig. 9. Activation of spiny lobster myosin ATPase by the substrate. Conditions of ATPase assay: 40 mM Tris-HCl buffer, pH 8.0; 0.5 M KCl; 0.7 mg myosin/ml; 25°C.

Fig. 10. Lineweaver-Burk plots of spiny lobster myosin ATPase. Conditions of ATPase assay: 40 mM Tris-HCl or Tris-maleate buffer; 0.5 M KCl; 20°C.

Fig. 11. Lineweaver-Burk plots of spiny lobster myosin ATPase in the presence of Ca^{2+} and EDTA at pH 6.5. Conditions of ATPase assay: 40 mM Tris-maleate buffer, pH 6.5; 0.5 M KCl; 20°C.

Table 1. *K_m* and *V_{max}* values of spiny lobster myosin in 0.5 M KCl at 20°C

Conditions		<i>K_m</i> ($\times 10^{-4}$ M)	<i>V_{max}</i> ($\mu\text{mole} \cdot \text{g}^{-1} \cdot \text{sec}^{-1}$)
Modifiers	pH		
None	6.5	1.1	0.20
"	7.5	1.7	0.16
"	8.5	0.95	0.29
5 mM Ca ²⁺	6.5	1.1	2.65
5 mM EDTA	6.5	33	35

increase of ATP concentration up to 3 mM. Above that concentration, however, the activity was decreased. A similar phenomenon has been found in rabbit myosin.¹²⁾

The ATPase activity of spiny lobster myosin was determined under various conditions at 0.133–1 mM ATP. The Lineweaver-Burk plots of the experimental data were found to fit straight lines, as seen in Figs. 10 and 11, demonstrating that the ATP hydrolyzing reaction proceeds according to the Michaelis equation under any of those conditions. The *K_m* and *V_{max}* values calculated are collectively given in Table 1. The *K_m* values of spiny lobster myosin were fairly close to those of rabbit myosin, whereas the *V_{max}* values of the former were only one third those of rabbit myosin, under corresponding conditions.^{10, 13, 20–23)}

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