Studies on the Properties of Fish Actomyosin—V. Activation of Fish Actomyosin ATPase by Lecithin

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Previously, it was observed that the actomyosin preparation from fish muscle ordinarily contains lecithin^{1,2)}. In view of the specific activation of the microsomal ATPase from rabbit muscle by lecithin or lysolecithin^{3,4)}, the activity of ATPase might directly involve the importance of lipid-protein interactions in ATP hydrolysis process. However, the correlation between actomyosin ATPase activity and lipid has not yet been reported.

The previous report⁵⁾ showed that the actomyosin ATPase from fish muscle was activated by lecithin. In particular it was suggested from the fact that the affinity of actomyosin for ATP increased by the addition of lecithin, that the effect of lecithin could be explained in terms of an allosteric mechanism.

In an attempt to gain further insight into the properties of fish actomyosin, this investigation was made to elucidate the activation mechanism of the actomyosin ATPase by lecithin.

Materials and Methods

Preparation of actomyosin. The actomyosin was prepared from flatfish muscle by the method as described in the previous paper²⁾.

Preparation of lecithin. Purified egg lecithin was prepared by the method of Pangborn⁶⁾.

ATPase assay. The ATPase assay was carried out by the procedure as previously reported⁵⁾. The reaction mixture (2 ml) consisted of histidine buffer (pH 7.0) 100 μ moles, $MgCl_2$ 2 μ moles, KCl 100 μ moles, ATP 10 μ moles, and 0.7–1.0 mg of protein was incubated at 25°C for 5 minutes, unless otherwise stated. The ATPase activity was expressed as μ moles of Pi liberated per mg of protein per minutes.

Treatment with phospholipase C. The reaction mixture contained, in a total volume of 25 ml, histidine buffer (pH 7.0) 100 μ moles, KCl 2 mmoles, $CaCl_2$ 2.5 μ moles, phospholipase C 10 mg, and 70 mg of protein. The procedure was similar to that for the

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Abbreviations used: ATP, adenosine triphosphate; pCMB, p-chloromercuribenzoate; NTP, p-nitrothiophenol; NEM, N-ethylmaleimide.

treatment as previously reported⁵⁾, except that the mixture was incubated for 50 minutes.

Other treatments. The actomyosin was treated with pCMB and NTP respectively. The pCMB actomyosin solution (6.7 mg/ml) containing 0.2 M KCl, 0.06–0.5 mM pCMB, pH 7.2, and the NTP actomyosin (6 mg/ml) containing 0.2 M KCl, 0.6–1.0 mM NTP, pH 6.8, were allowed to stand in the cold room overnight.

Light scattering assay. The basal medium was 0.6 M KCl–1 mM MgCl₂–25 mM histidine buffer (pH 7.1) and the protein (0.2 mg/ml), if indicated, containing lecithin (0.01 mg/ml). The intensity of light scattering of the mixture was measured in Shimadzu photometer (PG-21 type) at the wave length of 546 m μ with R_{90°}, within one minute after the addition of ATP. The change in the actomyosin complex with ATP was expressed as the relative intensity difference.

Protein concentration. Protein concentration was determined by the biuret method modified by UMEMOTO⁷⁾.

Chemicals. Phospholipase C from *Clostridium Welchii* was obtained from Sigma Chemical Co.. ATP was obtained from Sigma Chem. Co., and used without further treatment. p-Chloromercuribenzoate and p-nitrothiophenol were purchased from Tokyo Kasei Kogyo Co. Ltd.

Results and Discussion

Light scattering studies. The indication as to changes in the size and shape of the actomyosin complex has been previously provided by the method of light scattering^{8,9)}. The decrease of light scattering (R_{90°}) intensity of actomyosin with ATP could be interpreted as indicating a loosening of the actomyosin complex through detailed studies of these fields.

Table 1. Light scattering intensity of the actomyosin-lecithin system with ATP.

Assay system	Change of relative intensity
0.5 mM ATP	– 26.4 $\pm$ 0.2
0.5 mM ATP + lecithin	– 44.9 $\pm$ 3.7

Standard solution: 0.6 M KCl–1 mM MgCl₂–25 mM histidine buffer (pH 7.1), actomyosin (0.2 mg/ml). Added lecithin: 0.01 mg/ml. Temperature: 21°C.

Table 1 shows the effect of lecithin on the decrease of light scattering intensity of actomyosin with ATP. The system containing lecithin led to a marked degree of drop in the relative intensity comparing with control system, so it appears that the structural transition induced by ATP is facilitated by the addition of lecithin. This result suggests that the dissociation of actomyosin in the presence of ATP is promoted by the addition of lecithin, as reported previously⁵⁾.

Effect of phospholipase C on substrate concentration-ATPase activity curve. To know the role of lecithin for the actomyosin ATPase activity, the effect of phospholipase C on the substrate concentration-actomyosin ATPase activity curve was investigated.

Fig. 1. Effect of phospholipase C on the actomyosin ATPase activity-substrate concentration curve.

A: The actomyosin treated with phospholipase C.

B: Lecithin (1 mg/mg protein-N) was added to the treated actomyosin.

Fig. 1 shows the typical examples. It is well known that the substrate concentration-actomyosin ATPase activity gives a hyperbolic curve of the Michaelis type.

As seen from the figure, by the treatment with phospholipase C the substrate concentration curve showed the sigmoidal shape. When lecithin 1 mg per mg protein-N was added to the treated actomyosin, the substrate-concentration curve slightly approached the

hyperbolic form. The most possible interpretation of sigmoidal shape seen in allosteric enzyme is that cooperative subunit interactions due to ligands are assumed to occur.¹⁰⁾ If it is assumed that the actomyosin ATPase shows characteristic of subunit interactions as well as allosteric enzyme, the sigmoidal shape in Fig. 1 suggests that there may be cooperation among ATP molecules in their binding to the protein.

Activation by lecithin. As discussed previously⁵⁾, the activating effect of lecithin on the actomyosin ATPase could be regarded as act by the apparrent allosteric mechanism.

To examine the effect of ageing on the activation of actomyosin by lecithin, the preparation was stored at 2°C. Table 2 shows the activation of actomyosin ATPase by lecithin during storage. Although the original activity of actomyosin ATPase was little affected by storing, the effect of lecithin disappeared as the preparation became older. It is suggested from the result that the binding site of lecithin differs from that of ATP and is unstable.

Table 2. Effect of lecithin on the ATPase activity of the actomyosin stored at 2°C.

Storage time (days)	ATPase activity (Pi μ moles/mg of protein/min)	
	Without lecithin	With lecithin*
0	0.669	1.040
2	0.661	0.798
4	0.664	0.703

* 2 mg per mg of protein-N

Furthermore, in an attempt to gain greater understanding of the mechanism of activation by lecithin, the effect of some reagents on the activation by lecithin was investigated. In Fig. 2, the activity of actomyosin ATPase is plotted against the concentration of pCMB with which the actomyosin was treated. Though pCMB higher than 10^{-4} M inhibited the ATPase activity, the activation by lecithin was unaffected throughout the range of concentration of pCMB used. It appears to be clear that the SH groups of the protein is not involved in the reaction of activation of the actomyosin ATPase by lecithin, since NEM also showed a similar effect to pCMB on the activation by lecithin.

As shown in Table 3, the activation by lecithin was inhibited almost completely without lowering in the original ATPase activity, when the actomyosin was treated with NTP. The inhibiting effect of NTP on the activation by lecithin may be due to the deformation by modification of the protein by NTP.

Fig. 2. Effect of pCMB on the activation of actomyosin ATPase by lecithin.

○: without added lecithin.
●: with added lecithin (1.5 mg/mg protein-N).

Table 3. Effect of NTP on the activation of actomyosin ATPase by lecithin.

Concentration of NTP (μmoles/mg protein-N)	ATPase activity (Pi μmoles/mg of protein/min)	
	Without lecithin	With lecithin*
0	0.556	0.637
0.6	0.565	0.601
1.0	0.558	0.550

* 1 mg per mg of protein-N

These results suggest that the site related to lecithin on the actomyosin ATPase is quite different from that to ATP. However, the role of lecithin for the actomyosin can not be decided conclusively from these results whether lecithin on the actomyosin affects directly the link between myosin and actin, or affects the active myosin interacting with actin. The further study is now being attempted to obtain more information concerning the site interacting with lecithin in fish actomyosin.

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Summary

To clarify the mechanism of activation by lecithin of the actomyosin ATPase from fish muscle, the effect of lecithin on the interaction of actomyosin with ATP was examined.

1) The marked structural change of actomyosin induced by the addition of lecithin was observed in the presence of ATP, as judged by a more drop in the relative intensity of light scattering at 90° .

2) By the treatment of actomyosin with phospholipase C, the substrate concentration curve-the ATPase activity showed the sigmoidal shape.

3) The enhancing effect of lecithin on the actomyosin ATPase activity was lost as a result of storage of the preparation and treatment with NTP, without loss of the original enzyme activity. On the other hand, although pCMB inhibited the ATPase activity, the ATPase was susceptible to lecithin. From these results, it was assumed that the sensible site to lecithin on the actomyosin preparation presumably is different from that to ATP.

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