Activation Mechanism of Carp Muscle Myosin B ATPase by Lecithin

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To elucidate the activation mechanism of fish muscle myosin B ATPase by lecithin, lecithin-inactive myosin B was prepared from carp muscle. The myosin B ATPase from muscle treated with 0.1 M K_2HPO_4 -0.5 M KCl was not activated by added lecithin. During the preparative procedure, the component required for the activation by lecithin was found to be in the precipitate. It was confirmed that the activation of myosin B ATPase by lecithin is proportional to the amount of added component. This activation was enhanced in the presence of 1 mM Mg^{++} .

In the previous paper¹⁾, it was reported that about 50% of lecithin contained in carp muscle myosin B is removed selectively from it, without loss of the original enzymatic activity of the protein. However, the degree of stimulation of the myosin B ATPase by added lecithin was somewhat reduced after removal of lecithin. In view of these observations, it was suggested that the activation of myosin B ATPase by lecithin may require the presence of an unknown factor.

In the present paper, attempts were made to prepare the lecithin-inactive myosin B ATPase from carp muscle and to elucidate the activation mechanism of myosin B ATPase by lecithin. The requirement for a component for the activation of the ATPase by lecithin is reported also.

Materials and Methods

The muscle of carp *Cyprinus carpio* LINNAEUS was used for all experiments in this paper. The preparation of myosin B from carp muscle has been described in the previous paper¹⁾.

The preparation of lecithin-inactive myosin B is as follows. A carp was anesthetized with meta aminobenzoic acid ethylester methanesulfonate. Then the dorsal muscle was excised, immediately immersed in ice and minced. The minced muscle was treated with 0.5 M KCl-0.1 M K_2HPO_4 for 10 min under careful stirring, and centrifuged at $12,000 \times g$ for 20 min. After the resulting precipitate was allowed to stand several hours at 2°C, myosin B was prepared from the precipitate. In Fig. 1, the preparative procedure of the lecithin-inactive myosin B from carp muscle is summarized.

The ATPase assay was done by the procedure as previously reported¹⁾. The reaction mixture (2 ml) consisting of 100 μ moles histidine buffer (pH 7.5), 2 μ moles $MgCl_2$,

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Fig. 1. Preparative procedure of lecithin-inactive myosin B from carp muscle.

70 μ moles KCl, 10 μ moles ATP, and 0.7–1.0 mg of protein was incubated at 25°C for 2 min in the presence and in the absence of added lecithin unless otherwise stated. The lecithin added was obtained from egg by the method of PANGBORN²⁾. The activity was expressed as μ moles of Pi liberated per mg of protein per min. Protein concentration was determined by the biuret method modified by UMEMOTO³⁾.

Results and Discussion

The myosin B ATPase from fish muscle is activated by the addition of lecithin^{1,4,5)}. In order to elucidate the activation mechanism of myosin B ATPase by lecithin, an attempt was made to prepare lecithin-inactive myosin B from carp muscle without loss of the enzymatic activity. The myosin B ATPase obtained by the preparative procedure given in Fig. 1 was unaffected by the addition of lecithin.

Furthermore, in the process of preparing lecithin-inactive myosin B ATPase, three fractional components (“Supernatants I and II”, and “Precipitate”) as shown in Fig. 1 were obtained and dialyzed against the same buffer as the myosin B. Then the effects of the fractional components on the activation of the myosin B ATPase by lecithin were examined. As shown in Table 1, though the activation of the ATPase by lecithin was

Table 1. Effects of the fractional components on the activation of myosin B ATPase by lecithin.

Reaction system		ATPase activity (Pi μ moles/min/mg protein)	
		Without lecithin	With lecithin
Myosin B		0.268	0.477
Lecithin-inactive myosin B		0.357	0.362
"	+ "Supernatant-I"	0.339	0.350
"	+ "Supernatant-II"	0.344	0.357
"	+ "Precipitate"	0.274	0.459

For "Supernatants I and II", and "Precipitate" see Fig. 1. Each component added: 0.3–0.5 mg/mg of the myosin B. Lecithin added: 1.0–1.3 μ moles/mg protein-N

Table 2. ATP hydrolysis of "Precipitate".

		ATP hydrolysis (Pi μ moles/min/mg protein)	
		Without lecithin	With lecithin
"Precipitate"		0.257	0.255
Aq. extracts from "Precipitate"		nil	nil

The reaction mixture was the same as in the case of myosin B ATPase. For "Precipitate" see Fig. 1.

not observed by the addition of "Supernatant I or II", the addition of "Precipitate" to the ATPase resulted in a marked activation by lecithin. This implies that the component required for the activation of myosin B ATPase by lecithin exists in "Precipitate".

However, there is the question as to whether "Precipitate" itself has the capability of ATP hydrolysis dependent upon lecithin. "Precipitate" was thoroughly dialyzed against deionized water at 0°C and extracted with water for 30 min at 0°C. The ATP hydrolysis of "Precipitate" and the aq. extracts was examined under the same condition as the myosin B ATPase. The results is given in Table 2. "Pre-

Fig. 2. Effect of the component from "Precipitate" on the activation of the myosin B ATPase by lecithin. Lecithin added was 1.0–1.3 μ moles/mg protein-N.

precipitate" showed a hydrolysis of ATP independent upon lecithin, whereas the aq. extracts did not hydrolyze ATP regardless of the addition of lecithin. The water soluble component from "Precipitate" was found necessary for the activation by lecithin.

Furthermore, the effect of the component on the activation of the myosin B ATPase by lecithin was examined. The activation by lecithin was expressed as the ratio of activity in the presence of lecithin to that in the absence of lecithin. As shown in Fig. 2, the degree of activation of the ATPase by lecithin was increased with the amount of added component to a certain extent. From these data it would be confirmed that the enhanced activity of myosin B ATPase by added lecithin is caused by the existence of the component in myosin B.

The requirement for Mg^{++} or Ca^{++} for the activation of myosin B ATPase by lecithin is shown in Table 3. As seen in the table, the activation by lecithin of the ATPase containing the component was markedly enhanced by the addition of 1 mM Mg^{++} , whereas the addition of 1 mM Ca^{++} only slightly activated the ATPase system. The inducement to the activation by lecithin appears to require at least the presence of Mg^{++} (e.g. 1 mM).

Table 3. ATPase activity of the myosin B in the presence of the component from "Precipitate".

System		ATPase activity Pi μ moles/min/mg protein)	
		Without lecithin	With lecithin
1 mM Mg^{++}	Myosin B	0.347	0.358
	Myosin B + the component	0.265	0.520
1 mM Ca^{++}	Myosin B	0.316	0.374
	Myosin B + the component	0.307	0.346

The component/myosin B=0.8

From the results of these experiments, it is concluded that the possible mechanism of activation of the myosin B ATPase by lecithin is dependent upon the presence of the component from "Precipitate". The action of the component for the myosin B ATPase may be involved in interacting with Mg^{++} . The purification of the component required for the activation by lecithin will be reported in the next paper.

References

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