Kawasaki Med. J. Vol. 7 : No. 1, 1-17, 1981

MODIFICATION OF ACTOMYOSIN ATPase BY PARAMYOSIN : STUDIES ON THE ISOLATED NATIVE MYOFILAMENTS

Hirohiko IWATSUKI*

Department of Biology, Faculty of Science, Okayama University, Okayama 700

Accepted for Publication on January 17, 1981

Abstract

The native myofilaments were isolated from the three different types of muscles containing different amounts of paramyosin : viz. adductor muscle of the spanish oyster (*Atrina japonica*), retractor muscle of the horseshoe crab (*Tachypleus tridentatus*) and psoas muscle of the rabbit. The content of paramyosin (expressed as a weight ratio relative to myosin) estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was 0.67 in the adductor muscle of the spanish oyster and 0.46 in the horseshoe crab muscle.

Two kinds of enzyme activities, viz. Mg^{2+}- and Ca^{2+}-ATPase activities, and the rates of superprecipitation at various KCl concentrations were compared among the native myofilaments isolated from the three types of muscle. The inhibition of Mg^{2+}-ATPase activity by KCl was reduced in the native myofilaments that contained paramyosin compared with the native myofilaments devoid of paramyosin. The paramyosin-containing myofilaments required higher concentrations of KCl for the maximum activation of the Ca^{2+}-ATPase activity than those devoid of paramyosin. At higher KCl concentrations, superprecipitation took place at faster rates in the native myofilaments containing paramyosin than in the native myofilaments devoid of it. These results suggest that paramyosin has an effect on myosin ATPase and in turn on the actin-myosin interaction.

INTRODUCTION

Paramyosin first described by Hall et al.[1] has been found in muscles from a variety of animals : e.g. mollusks[2,3], annelids[4], arthropods[5-7], pseudocoelomates[8,9] and echinoderms[10]. Recently, special attention has been given to the

*Present address : Department of Anatomy, Kawasaki Medical School, Kurashiki 701-01

Abbreviations : EGTA, ethylene glycol bis-(β-aminoethyl ether)-*N*, *N*'-tetraacetic acid ; SDS, sodium dodecyl sulfate.

岩月 宏彦

fine structure of the thick filaments containing paramyosin and it has been demonstrated that paramyosin forms a core of the thick filament which is covered by a surface layer of myosin[5,11-14]. However, very little is known concerning the functional implication of paramyosin in these filaments. It has been suggested that paramyosin might be involved in the "catch-contraction" mechanism for the reason that paramyosin is particularly abundant in molluscan adductor muscles that are characterized by the catch-contraction[15]. It has also been reported that the interaction of paramyosin with myosin results in elongation of the myosin-containing filaments[5] and that the Mg^{2+}-dependent actomyosin ATPase is activated by paramyosin at 0.1 M KCl[5] and at 0.03 M KCl[12].

It seems that the native myofilaments isolated from muscles that contain various amounts of paramyosin are the appropriate system to study the effect of paramyosin, since only a limited extent of reconstitution of the myofilaments containing myosin, paramyosin and actin is achieved after exhaustive purification of myosin, paramyosin and actin. The purpose of this study is to compare both actomyosin ATPase activity and the rate of superprecipitation among the native myofilaments isolated from the three types of muscles, viz. a) "catch"-adductor muscles of spanish oyster that have a large content of paramyosin, b) horseshoe crab muscles that contain less amount of paramyosin, and c) rabbit skeletal muscles that contain no paramyosin. As shown here the effects of paramyosin on actomyosin ATPase are clearly visualized when measurements of ATPase activity are carried out at various concentrations of KCl.

MATERIALS AND METHODS

The red adductor muscles of the spanish oyster (*Atrina japonica*), the retractor muscles of the horseshoe crab (*Tachypleus tridentatus*) and the psoas muscles of the rabbit were used for the isolation of the native myofilaments and for the extraction of the contractile proteins.

Isolation of the native myofilaments

Native myofilaments were isolated essentially according to the procedure of Huxley[16]. Ten grammes of the muscle strips stored in a glycerol solution[17] were transferred into 200 ml of a solution containing 0.1 M KCl, 1 mM $MgCl_2$ and 30 mM Tris-maleate, pH 7.2 (Solution A). They were shredded into extremely thin bundles of fibers with needles, and rinsed with Solution A. The fiber bundles were cut with scissors and homogenized with the N.S. universal homogenizer (Nihon Seiki) in 100 ml of a solution containing 0.1 M KCl, 10 mM $MgCl_2$, 5 mM EGTA and 30 mM Tris-maleate, pH 7.2 (Solution B). The homogenization was continued until nearly all the fibers were disrupted

into myofibrils monitoring with the phase-contrast microscope. Soluble proteins and small membrane fragments were removed by centrifugation at $1,500 \times g$ for 5 min. The sediments were resuspended in Solution B and washed by repeating the centrifugation one more cycle. The sediments were suspended in 100 ml of Solution A containing 0.5 % Tween-80 and incubated for 18 h to dissolve membrane components[18]. Myofibrils were sedimented at $1,500 \times g$ for 5 min and washed with Solution B by repeating homogenization and sedimentation at $5,000 \times g$ for 5 min for 4 cycles. They were resuspended in 70 ml of a solution containing 0.1 M KCl, 10 mM $MgCl_2$, 5 mM EGTA, 10 mM ATP and 30 mM Tris-maleate, pH 7.2 (Solution C), and homogenized with a Tephron homogenizer to disintegrate myofibrils into myofilaments. Bulky fibrillar components were sedimented at $650 \times g$ for 5 min. The supernatant fraction containing native myofilaments was dialyzed against 30 volumes of a solution containing 0.1 M KCl and 30 mM Tris-maleate, pH 7.2 (4×6 h). The entire process of the procedure was carried out at 4°.

Preparation of the contractile proteins

Myosin was prepared from the rabbit skeletal muscles by the method of Szent-Györgyi[17] with the modification of Ebashi and Ebashi[19]. Actin was extracted from the aceton powder of rabbit skeletal muscles prepared according to Straub[20] with the modification suggested by Ebashi and Maruyama[21], and purified as described by Mommaerts[22]. Myosin B was extracted from the three types of muscles described above with the Weber-Edsall solution according to the conventional method[23], and purified by repeating precipitation and redissolution[17]. Paramyosin was fractionated from myosin B of spanish oyster adductor muscles according to Rüegg[24], and purified by washing with a solution containing 0.5 M KCl, 5 mM $MgCl_2$, 2 mM ATP and 30 mM Tris-maleate, pH 6.3[12].

Electron microscopy

The native myofilaments in Solution C were negatively stained with 2 % uranyl acetate and examined in a Hitachi electron microscope (Type HU 12A).

Electrophoresis

SDS polyacrylamide gel electrophoresis was carried out essentially according to Weber and Osborn[25] using 5 % polyacrylamide gels containing 0.1 % SDS. After electrophoresis the gels were stained with Coomassie brilliant blue for 2 h. The gels were scanned at 570 nm by a Yamato-Asuka densitometer (Ozumor 82) with both scanning and integration circuits operating simultaneously.

The contents of paramyosin, actin and myosin in the native myofilaments were calculated from the relative areas of various peaks in the densitometrical tracing of polyacrylamide gels essentially according to the method of Levine

et al.[26]. Myosin, actin and paramyosin were identified by electrophoresing the purified individual components. Molecular weight markers were myosin heavy chain of the rabbit skeletal muscle (200,000), β-galactosidase (130,000), phosphorylase A (94,000), bovine serum albumin (68,000), catalase (60,000), trypsin (23,300), RNase (13,700) and cytochrome-C (11,700).

Measurement of ATPase activity

For the assay of ATPase activity, reaction was carried out in 2 ml of a solution containing 1 mM ATP, 30 mM Tris-maleate (pH 7.2), 0.25 mg myofilament protein/ml and various concentrations of KCl, $MgCl_2$ and $CaCl_2$ as indicated in the legends to figures at 25° for 10 min. Reaction was stopped by adding 8 ml of 10 % trichloroacetic acid, and liberated Pi was determined according to Fiske and SubbaRow[27].

Measurement of superprecipitation

Superprecipitation was initiated by adding 1 mM ATP to a solution containing 1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 30 mM Tris-maleate (pH 7.2), 0.5 mg protein/ml and various concentrations of KCl as indicated in Figure legends. The reaction was carried out at 22° and monitored by changes in the optical density of the myofilaments suspension at 550 nm essentially according to Ebashi[28].

RESULTS

Structure and composition of the native myofilaments

The electron microscopic observations on negatively stained preparations of the isolated native myofilaments used in this study reveal thick and thin filaments with little or no contamination by membrane fraction (Figs. 1-3). As described below, both the thick and the thin filaments show different properties characteristic of the source of preparations. To facilitate discussion, the native myofilaments isolated from the three types of muscles are called as follows : a) Type I sample, native myofilaments isolated from the red adductor muscles of the spanish oyster ; b) Type II sample, native myofilaments from the retractor muscles of the horseshoe crab ; and c) Type III sample, native myofilaments from the psoas muscles of the rabbit.

The thick filaments of the Type I sample are considerably larger in both length and diameter than the other types (Table 1). The thick filaments of the Type I sample show a rough appearance on their surface owing to the globular portions of myosin (Fig. 1). In spite of relatively large content of paramyosin in these filaments, the axial periodicity of 14.5 nm that is characteristic of paramyosin is scarcely visible. In some thick filaments, the projections are absent in a central zone of about 0.2 μm (Fig. 1c). Although the

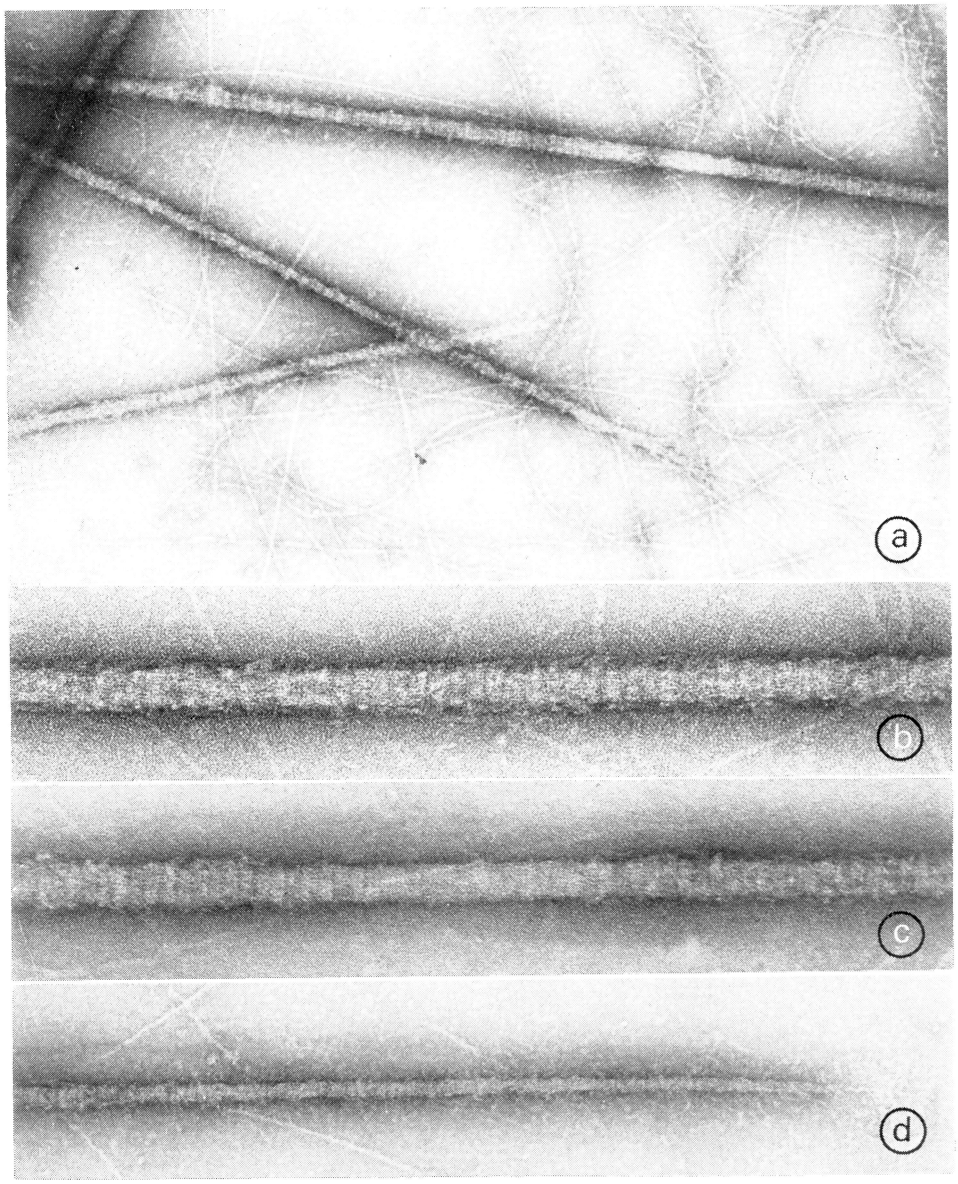

Fig. 1. Electron micrographs of the native myofilaments isolated from the red adductor muscle of the spanish oyster (Type I sample). a, General view showing a number of thick and thin filaments. ×78,000. b, Thick filament showing surface projections of myosin heads. ×170,000. c, A central portion of thick filament showing the bare zone devoid of the myosin projection. ×170,000. d, A tapered end of thick filament. ×97,000.

thin filaments of this type are longer than those of skeletal muscle (Type III sample), there are no differences in their fine structure. The thick and thin filaments of the Type II sample are also longer than those of the Type III sample. The thick filaments have again a central bare zone of about 0.2 μm

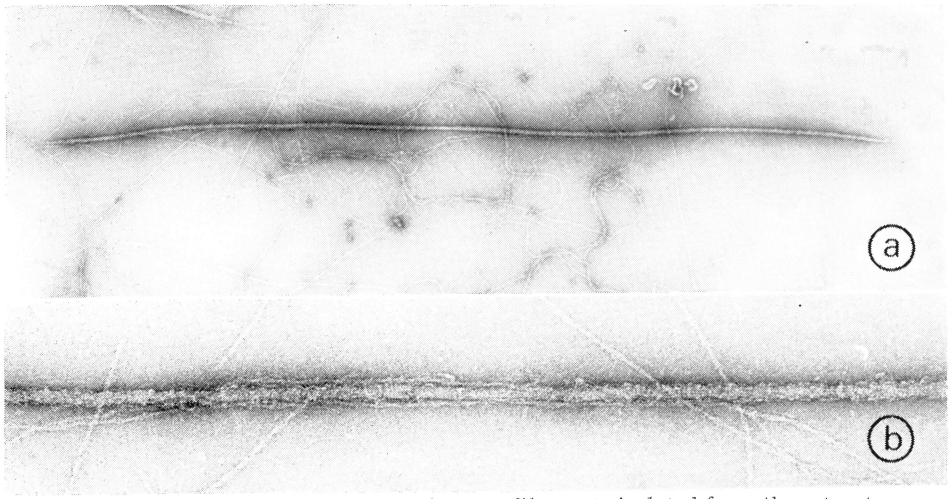

Fig. 2. Electron micrographs of the native myofilaments isolated from the retractor muscle of the horseshoe crab (Type II sample). a, A thick filament showing projections all the way along its length except for the central bare zone. Thin filaments are also visible. ×22,000. b, A larger magnification to show the central bare zone of the thick filament. ×98,000.

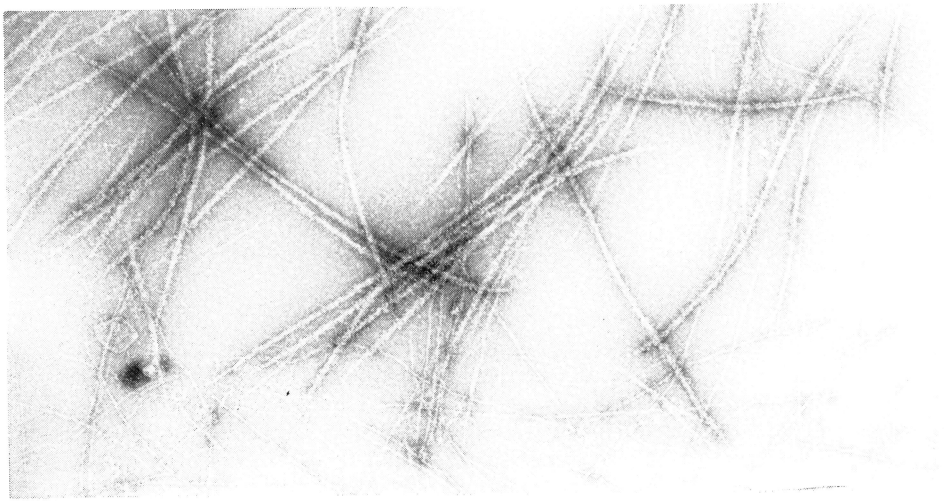

Fig. 3. Electron micrograph of the native myofilaments isolated from the psoas muscle of the rabbit (Type III sample). ×44,000.

(Fig. 2). The profile of thick and thin filaments of the Type III sample are in agreement with the previous results of Huxley[16] (Fig. 3).

The contents of paramyosin and actin relative to myosin have been investigated in the three samples of the native myofilaments by means of SDS gel electrophoresis (Table 1). The content of paramyosin in the Type I sample in terms of the weight ratio of paramyosin to myosin is 1.5-fold higher than in the Type II sample. The highest weight ratio of actin to myosin, however, is observed in the Type II sample and the ratio is 1.5-fold and 3-fold of that in the Type I and the Type III sample, respectively.

TABLE 1

Dimensions of the thick filament and amounts of paramyosin and actin relative to myosin in the three types of samples of the native myofilaments

	Thick filament dimensions		Relative contents of contractile proteins	
	Length(μm)	Diameter(nm)	Paramyosin/Myosin	Actin/Myosin
Type I sample	12.0—17.0	35—50	0.67±0.03	1.07±0.05
Type II sample	4.5— 6.0	25—30	0.46±0.02	1.46±0.05
Type III sample	1.2— 1.5	20	—	0.56±0.02

The weight ratios of paramyosin to myosin and of actin to myosin are expressed as the average ± standard error (n=20).

TABLE 2

Various proteins found in the three samples of the native myofilaments

	Type I sample	Type II sample	Type III sample
Myosin heavy chain	200,000±1,000	200,000±1,000	200,000±1,000
C-Protein	150,000±1,000	149,000±1,000	149,000±1,000
Paramyosin	100,000±1,000	104,000±1,000	—
α-Actinin	—	101,000±1,000	100,000±1,000
P-II-a	—	58,000±1,000	—
P-II-b	—	55,000±1,000	—
Actin	45,000±1,000	45,000±1,000	45,000±1,000
Troponin-T	—	—	39,000±1,000
Tropomyosin	36,000±1,000	34,000±1,000	36,000±1,000
P-II-c	—	27,000±1,000	—
Myosin light chain and/or Troponin-I	—	—	23,000±1,000
P-II-d	—	16,000±1,000	—
Myosin light chain and/or Troponin-C	—	—	14,000±1,000
P-I	11,000±1,000	—	—

The values of molecular weight are expressed as the average ± standard error (n=20). P-I, Unidentified component characteristic of the Type I sample ; P-II-a, b, c and d, Unidentified components characteristic of the Type II sample.

The proteins that constitute the native myofilaments have been investigated by means of electrophoresis on SDS–polyacrylamide gels. Tentative identification of proteins and their molecular weights are listed in Table 2. Myosin heavy chain as well as actin shows identical molecular weight among the three samples of the native myofilaments. The molecular weight of paramyosin subunit in the Type I sample is 100,000 in agreement with the reported value for other molluscan paramyosin[25,29–31]. As was reported by DeVillafranca and Haines[32], paramyosin subunit in the Type II sample has a slightly larger molecular weight (104,000) than that in the Type I sample. In order to verify this point, a mixture of the Type I and the Type II samples has been coelectrophoresed. Appreciable separation was obtained between paramyosin subunit of the Type I sample and that of the Type II sample. Troponin components and myosin light chains observed in the Type III sample are absent in the other types of samples. An unidentified component having an apparent molecular weight of 11,000 is characteristic of the Type I sample, and four components having the apparent molecular weight of 58,000, 55,000, 27,000 and 16,000 are characteristic of the Type II sample. These components are also present in myosin B from the adductor muscles of the spanish oyster or in that from the retractor muscles of the horseshoe crab.

Mg^{2+}-activated ATPase activity

It is well known that the Mg^{2+}–ATPase activity of rabbit actomyosin is influenced by KCl concentrations[33]. The role of paramyosin on the Mg^{2+}–stimulated actomyosin ATPase is discernible in terms of comparison of the KCl-dependence of the activity among the three samples (Fig. 4). At 0.04 M KCl, the Mg^{2+}–ATPase activity shows the highest value in all of the three samples. As the KCl concentration is increased from 0.1 M to 0.22 M, the activity is gradually decreased in the case of the Type I sample. At concentrations of KCl higher than 0.22 M, the activity is almost completely inhibited. Profile of KCl dependence of the Type II sample is similar to that of the Type I sample. In the case of the Type III sample that contains no paramyosin, however, strong inhibition of ATPase activity is produced at the range of KCl concentrations considerably lower than that required for inhibition of ATPase activity of the Type I and II. The ATPase activity is almost completely inhibited by the KCl concentrations higher than 0.1 M.

In further attempt to characterize the three samples of native myofilaments, the effect of Mg^{2+} concentrations on the ATPase activity has also been investigated at four different KCl concentrations, viz. 0.06, 0.1, 0.14 and 0.3 M (Fig. 5). In the case of the Type I sample, the activation by Mg^{2+} is observed at the KCl concentrations of 0.06 M, 0.1 M and 0.14 M, but not at 0.3 M.

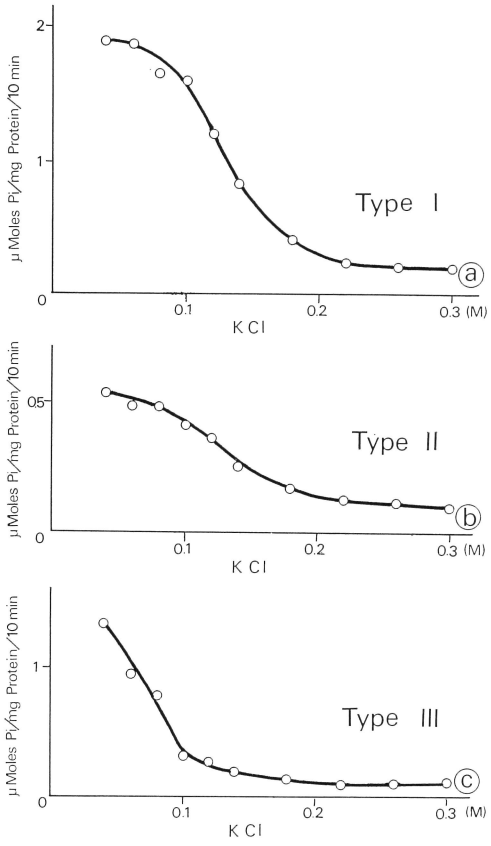

Fig. 4. Mg^{2+}-ATPase activity of the native myofilaments as a function of KCl concentration. For the ATPase assay, reaction was carried out in a solution containing 1 mM $MgCl_2$, 1 mM ATP, 0.01 mM $CaCl_2$, 30 mM Tris-maleate (pH 7.2), 0.25 mg protein/ml and various concentrations of KCl at 25° for 10 min. a, Type I sample ; b, Type II sample ; c, Type III sample.

At 0.06 M and 0.1 M KCl, maximum activation is obtained with 1 mM $MgCl_2$ and the activity is approximately twice as large as that without Mg^{2+}. At 0.14 M KCl, the degree of activation by Mg^{2+} becomes less, and maximum activation takes place at 0.1 mM $MgCl_2$. In the case of the Type II sample, Mg^{2+}-activation of ATPase is qualitatively the same as that of the Type I sample, although the extent of activation is significantly smaller. In the case of the Type III sample, however, very slight activation by Mg^{2+} is observed at 0.06 M KCl only. In the presence of 1 mM ATP, the optimum $MgCl_2$ concentration is 1mM in most cases, whereas in the presence of 10 mM ATP, 10 mM $MgCl_2$ was required for the maximun activation.

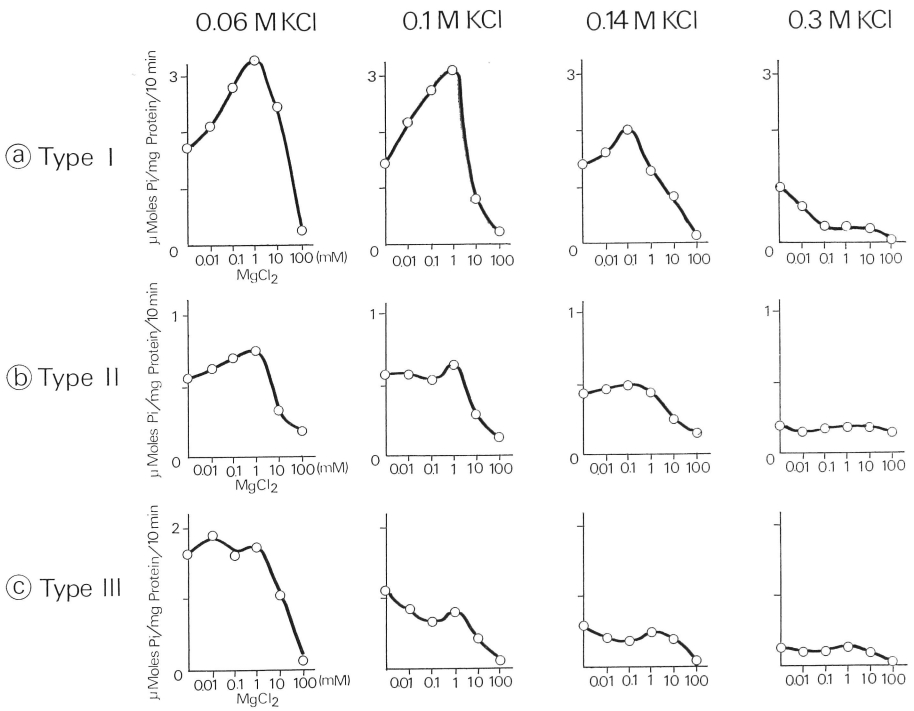

Fig. 5. Effects of Mg^{2+} and KCl concentrations on the ATPase activity of the native myofilaments. Conditions of ATPase assay were the same as those in the legends to Fig. 4 except that concentrations of $MgCl_2$ were varied. a, Type I sample ; b, Type II sample ; c, Type III sample.

Ca^{2+}-activated ATPase activity

As has been reported previously, the actomyosin ATPase is activated by Ca^{2+} and it is also influenced by KCl concentrations[33–35]. The KCl dependence of Ca^{2+}-ATPase activity has been compared between the native myofilaments containing paramyosin (Type I and II) and those containing no paramyosin (Type III) (Fig. 6). In the case of the Type I sample, the Ca^{2+}-ATPase activity is enhanced as the KCl concentration increases and reaches the maximum at 0.25–0.3 M KCl. Further increase in the KCl concentration leads to the inactivation of the ATPase activity. The KCl dependence of Ca^{2+}-ATPase activity in the other types differs considerably from that of the Type I. The optimum KCl concentration is 0.1 M in the Type II sample, and 0.05 M in the Type III sample.

Superprecipitation

In view of the reports that the superprecipitation of actomyosin depends on

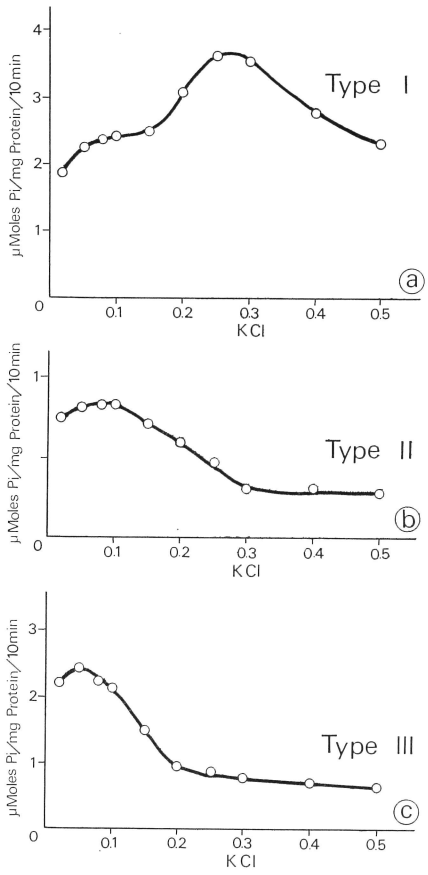

Fig. 6. Ca^{2+}-ATPase activity of the native myofilaments as a function of the KCl concentration. For the ATPase assay, reaction was carried out in a solution containing 1 mM $CaCl_2$, 1 mM ATP, 30 mM Tris-maleate (pH 7.2), 0.25 mg protein/ml and various concentrations of KCl at 25° for 10 min. a, Type I sample ; b, Type II sample ; c, Type III sample.

the concentration of KCl as well as $MgCl_2$ and ATP[36,37], it is expected that the superprecipitation of the three types of native myofilament samples shows different dependence on the concentration of KCl. As shown in Fig. 7 and 8, the Type I and II samples carry out effective superprecipitation in a wide range of KCl concentrations (0.06–0.22 M). It should be noted that maximum level of superprecipitation is attained very rapidly with the Type I and the Type II samples. In the case of the Type III sample, superprecipitation is observed only in a range of KCl less than 0.14 M, and proceeds very slowly : viz. it takes 13

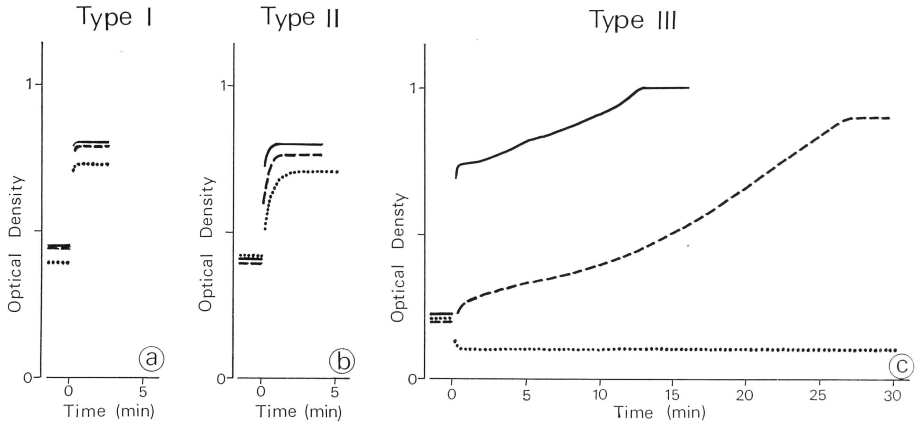

Fig. 7. Time course of the superprecipitation of the three different types of native myofilaments at different KCl concentrations. a, Type I sample ; b, Type II sample ; c, Type III sample. —, time course of the increase in optical density (550 nm) at 0.1 M KCl ; - -, that at 0.14 M KCl ; ·····, that at 0.18 M KCl.

min at 0.1 M KCl and 27 min at 0.14 M KCl to reach maximum level. These results are in agreement with the effect of KCl concentration on Mg^{2+}-activated ATPase activity of the three different samples described above.

DISCUSSION

The fundamental identity of actomyosin from molluscan catch muscles with that from skeletal muscles was suggested by Rüegg[24] and confirmed by Szent-Györgyi et al.[11]. It has been shown that the enzymic properties of the crude actomyosin preparations (myosin B) extracted from the paramyosin-containing muscle are different from those of the purified actomyosin[5,24]. This suggests that paramyosin may modify the enzymic activity of the actomyosin system. In this study, characteristics of ATPase activity and superprecipitation have been investigated with the use of preparations of the isolated native myofilaments that contain various amounts of paramyosin. It seems that the use of the isolated native myofilaments is most appropriate in this type of study, particularly since in our experience only a limited extent of reconstitution of the myosin-paramyosin composite filaments is achieved with the use of exhaustively purified myosin and paramyosin.

Both Mg^{2+}- and Ca^{2+}-ATPase activities of the Type I sample show much higher values than those of the Type II and III samples (cf. Figs. 4-6). It might be pointed out that the Type I sample contains contaminating ATPase.

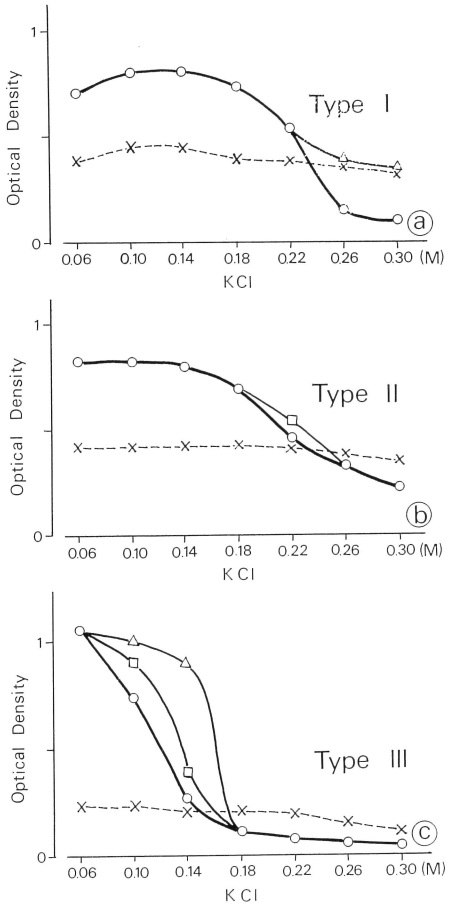

Fig. 8. Effect of KCl concentration on the superprecipitation of the three different types of native myofilaments. a, Type I sample ; b, Type II sample ; c, Type III sample. Key : ×, the optical density of suspension before the addition of ATP ; ○, at 1 min after the addition of ATP ; □, at 10 min ; and △, at 30 min.

However, at least, the possibility that the membrane ATPase might be a contamination may be ruled out by the following reason : The native myofilaments are pretreated with Tween-80 for 18 h[18]. In fact, as shown in Figs. 1-3, membrane fractions were rarely observed in both the electron micrographs and the SDS electrophoretogram.

According to recent reports[38,39], myosin light chain plays an important role in the ATPase activity of myosin in some types of muscle. Myosin light chain could not been identified in the native myofilaments containing paramyosin

(Type I and II), although the molecular weight of myosin heavy chain is the same regardless of the type of native myofilaments. This would indicate that myosin molecule in the muscles containing paramyosin is different from that in the skeletal muscles at least in terms of light chain composition. Moreover, there are some additional differences in protein composition and content among the three types of native myofilament samples. Therefore, it might be suspected that the striking differences between the ATPase activity in the native myofilaments containing paramyosin and that in the native myofilaments devoid of paramyosin are dependent on the differences in the composition and content of various proteins other than paramyosin. However, the following fact[40] supports the view that the interaction of paramyosin with myosin within the thick myofilaments is the primary factor in a production of various characteristic enzymic properties described here. Two different preparations of myofilaments could be reconstituted from myosin B (natural actomyosin containing paramyosin) of the horseshoe crab muscles. The first preparation of myofilaments showed the same profile as the isolated native myofilaments if the ionic strength of myosin B was reduced from 0.6 to 0.1 very slowly by means of dialysis. If the ionic strength was reduced rapidly by dilution, the second preparation of myofilaments was obtained in which myosin, paramyosin and actin were located in the different sets of structure, viz. short thick filaments, long filaments characterized by 14.5 nm periodicity and thin fllaments, respectively. The activation of ATPase by Mg^{2+} at 0.1 M KCl (pH 7.2) was observed in the first preparation but not in the second preparation, although the protein composition was basically the same between the two.

It has been demonstrated that the actomyosin ATPase is activated by Mg^{2+} as long as the strength of interaction between actin and myosin is maintained, but the Mg^{2+}-ATPase activity is reduced by increase of the KCl concentration presumably due to the reduced interaction between actin and myosin[37,41-43]. As shown in this paper, the stimulation of Mg^{2+}-ATPase activity and superprecipitation in the paramyosin-containing system (Type I and II) takes place at the KCl concentrations higher than the system devoid of paramyosin (Type III). It is tempting to speculate that the interaction of paramyosin with myosin would in turn favor the interaction between actin and myosin even at higher KCl concentrations.

Ozawa and Maruyama[34] have demonstrated that the Ca^{2+}-ATPase activity is intrinsic to myosin and is not influenced by actin nor by other proteins in skeletal muscles. The present result that the myofilaments containing paramyosin require higher KCl concentrations for maximum activation of Ca^{2+}-ATPase activity than those devoid of paramyosin, supports the view that paramyosin

will modify directly the myosin ATPase.

Paramyosin has been believed to play some role in the catch contraction mechanism. The actual picture of the role to be assigned, however, remains obscure. Further insight into the catch contraction mechanism would be gained by analysis of the details of the difference between the catch muscles containing paramyosin (e.g. adductor of spanish oyster) and the horseshoe crab muscles that also contain paramyosin but do not behave as "catch" muscles. So far as Ca^{2+}-ATPase activity is concerned, the effect of paramyosin is more evident in the Type I sample from the adductor muscle of the spanish oyster than in the Type II sample from the horseshoe crab muscle. This difference might reflect the differences in the molecular weight of paramyosin (cf. Table 2) or in the content of paramyosin (cf. Table 1). No appreciable difference of the effect of paramyosin is observed between the Type I sample and the Type II sample in both Mg^{2+}-ATPase activity and superprecipitation. Thus the results shown here favor the tentative view that the effect of paramyosin on myosin ATPase would be more crucial in the catch contraction mechanism than that on actin-myosin interaction.

Acknowledgements

Grateful acknowledgement is made to Prof. Siro Kawaguti (Okayama University and Kawasaki Paramedical College) and Prof. Takuji Ohkura (Kawasaki Medical School) for thier constant interests and guidances in this investigation. The author also wishes to express his thanks to Dr. Noriaki Ikemoto (Boston Biomedical Research Institute) and Dr. Yoshiaki Nonomura (Tokyo University) for many helpful suggestions throughout this work.

Thanks are also due to the Tamano Marine Laboratory of Okayama University for the supply of animals used in this study.

REFERENCES

1) Hall, C. E., Jakus, M. A. and Schmitt, F. O. : The structure of certain muscle fibrils as revealed by the use of electron stains. J. Appl. Phys. **16** : 459-465, 1945
2) Bailey, K. : Invertebrate tropomyosin. Biochim. Biophys. Acta **24** : 612-619, 1957
3) Hanson. J., Lowy, J., Huxley, H. E., Bailey, K., Kay, C. M. and Rüegg, J. C. : Structure of molluscan tropomyosin. Nature **180** : 1134-1135, 1957
4) Kominz, D. R., Saad, F. and Laki, K. : Vertebrate and invertebrate tropomyosins. Nature **179** : 206-207, 1957
5) Ikemoto, N and Kawaguti, S. : Elongating effect of tropomyosin A on the thick myofilaments in the long-sarcomere muscle of the horse-shoe crab. Proc. Japan Acad. **43** : 974-979, 1967
6) DeVillafranca, G. W. and Leitner, V. E. : Contractile proteins frcm horseshoe crab muscle. J. Gen. Physiol. **50** : 2495-2496, 1967
7) Bullard, B., Luke, B. and Winkelman, L. : The paramyosin of insect flight muscle. J. Mol. Biol. **75** : 359-367, 1973
8) Swanson, C. J. : Occurrence of paramyosin among the nematomorpha. Nature New

Biol. **232** : 122-123, 1971
9) Waterston, R. H., Epstein, H. F. and Brenner, S. : Paramyosin of *Caenorhabditis elegans*. J. Mol. Biol. **90** : 285-290, 1974
10) Obinata, T., Shirao, T. and Murakami, S. : Sea urchin paramyosin. Int. J. Biochem. **6** : 569-574, 1975
11) Szent-Györgyi, A. G., Cohen, C. and Kendrick-Jones, J. : Paramyosin and the filaments of molluscan "catch" muscles. II. Native filaments : isolation and characterization. J. Mol. Biol. **56** : 239-258, 1971
12) Nonomura, Y. : Fine structure of the thick filaments in molluscan catch muscle. J. Mol. Biol. **88** : 445-455, 1974
13) Elliott, A. : The arrangement of myosin on the surface of paramyosin filaments in the white adductor muscle of *Crassostrea angulata*. Proc. R. Soc. Lond. B **186** : 53-66, 1974
14) Camatini, M., Castellani, L. C., Franchi, E., Lanzavecchia, G. and Paoletti, L. : Thick filaments and paramyosin annelid muscles. J. Ultrastruct. Res. **55** : 433-447, 1976
15) Johnson, W. H., Kahn, J. S. and Szent-Györgyi, A. G. : Paramyosin and contraction of "catch muscles". Science **130** : 160-161, 1959
16) Huxley, H. E. : Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. J. Mol. Biol. **7** : 281-308, 1963
17) Szent-Györgyi, A. : Chemistry of muscular contraction. 2nd edn., pp. 143-155, Academic Press, New York. 1951
18) Abbott, R. H. and Chaplain, R. A. : Preparation and properties of the contractile element of insect fibrillar muscle. J. Cell Sci. **1** : 311-330, 1966
19) Ebashi, S. and Ebashi, F. : A new protein component participating in the superprecipitation of myosin B. J. Biochem. **55** : 604-613, 1964
20) Straub, F. B. : Actin. Studies Inst. Med. Chem., Univ. Szeged **2** : 3-15, 1942
21) Ebashi, S. and Maruyama, K. : Preparation and some properties of α-actinin-free actin. J. Biochem. **58** : 20-26, 1965
22) Mommaerts, W. F. H. M. : Reversible polymerization and ultracentrifugal purification of actin. J. Biol. Chem. **188** : 559-565, 1951
23) Banga, I. and Szent-Györgyi, A. : Preparation and properties of myosin A and B. Studies Inst. Med. Chem., Univ. Szeged **1** : 5-15, 1941
24) Rüegg, J. C. : The proteins associated with contraction in lamellibranch 'Catch' muscle. Proc. R. Soc. Lond. B **154** : 209-223, 1961
25) Weber, K. and Osborn, M. : The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244** : 4406-4412, 1969
26) Levine, R. J. C., Elfvin, M., Dewey, M. M. and Walcott, B. : Paramyosin in invertebrate muscles. II. Content in relation to structure and function. J. Cell Biol. **71** : 273-279, 1976
27) Fiske, C. H. and SubbaRow, Y. : The colorimetric determination of phosphorus. J. Biol. Chem. **66** : 375-400, 1925
28) Ebashi, S. : Calcium binding activity of vesicular relaxing factor. J. Biochem. **50** : 236-244, 1961
29) Woods, E. F. : Subunit structure of oyster paramyosin. Biochem. J. **113** : 39-47, 1969
30) Olander, J. : Substructure of the paramyosin molecule. Biochemistry **10** : 601-609, 1971
31) Weisel, J. W. and Szent-Györgyi, A. G. : The coiled-coil structure : Identity of the two chains of *Mercenaria* paramyosin. J. Mol. Biol. **98** : 665-673, 1975

32) DeVillafranca, G. W. and Haines, V. E. : Paramyosin from arthropod cross-striated muscle. Comp. Biochem. Physiol. **47B** : 9-26, 1974
33) Maruyama, K. and Ishikawa, Y. : Effect of magnesium and calcium on the ATPase activity of actomyosin at low ionic strength. Biochim. Biophys. Acta **77** : 682-685, 1963
34) Ozawa, I. and Maruyama, K. : Antagonistic effects of Ca and Mg ions on the ATPase activity of myofibrils, actomyosin and myosin at various KCl concentrations. Sci. Pap. Coll. Gen. Educ., Univ. Tokyo **18** : 261-277, 1968
35) Mommaerts, W. F. H. M. and Seraidarian, K. : A study of the adenosine triphosphatase activity of myosin and actomyosin. J. Gen. Physiol. **30** : 401-422, 1947
36) Szent-Györgyi, A. : Chemistry of Muscular Contraction, 2 nd edn., pp 72-82, Academic Press, New York, 1951
37) Maruyama, K. and Gergely, J. : Interaction of actomyosin with adenosine triphosphate at low ionic strength. II. Factors influencing clearing and superprecipitation : adenosine triphosphatase and birefringence of flow studies. J. Biol. Chem. **237** : 1100-1106, 1962
38) Stracher, A. : Evidence for the involvement of light chains in the biological functioning of myosin. Biochem. Biophys. Res. Commun. **35** : 519-525, 1969
39) Weeds, A. G. and Lowey, S. : Substructure of the myosin molecule. II. The light chains of myosin. J. Mol. Biol. **61** : 701-725, 1971
40) Iwatsuki, H. and Ikemoto, N. : Effect of tropomyosin A on actomyosin ATPase. Zool. Magazine **78** : 19, 1969
41) Yasui, T. and Watanabe, S. : A study of "superprecipitation" of myosin B by the change in turbidity. J. Biol. Chem. **240** : 98-104, 1965
42) Watanabe, S. and Yasui, T. : Effects of magnesium and calcium on the superprecipitation of myosin B. J. Biol. Chem. **240** : 105-111, 1965
43) Eisenberg, E. and Moos, C. : Interaction of actin and myosin during clearing. Nature **211** : 529-530, 1966