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KCl Concentration Dependence on the ATPase Activity of Crab Actomyosion

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Introduction

It has been known that osmotic pressure of body fluid of a crab inhabiting in sea water is about three times higher than that of a rabbit[1] and that the living muscle isolated from a crab induces normal contraction and relaxation even at as high ionic milieu as $\mu=0.6^{2\sim4)}$. On the other hand, Sarkar[5] reported that KCl concentration dependence of the ATP-induced contraction observed on glycerol-extracted muscle fibers of a horse-shoe crab is essentially identical with that shown on the rabbit psoas fibers. This implies that the contractile element of crab muscle also cannot contract at high KCl concentrations. Therefore, these results suggest that the contractility of crab's living muscle exhibited at high ionic milieu is not explained with that of its actomyosin system.

In this communication, to confirm this point, KCl concentration dependence of the ATPase activity of myofibrils and of myosin B was studied using crab muscle.

Materials and Methods

Myofibrils and myosin B were obtained from leg muscles of a crab, *Parelithodes Camtschatica*, collected in Nemuro, the east coast of Hokkaido. The preparation of myofibrils was performed according to the procedure of Nagai *et al.*[6]. Myosin B was prepared according to Szent-Györgyi[7] and purified by the procedure of Greenstein and Edsall[8].

ATPase activity of myofibrils and of myosin B was estimated by the method of Nagai *et al.*[9]. The quantitative analysis of nitrogen was performed by micro-Kjeldahl method, and the concentration of protein was calculated taking 6.25 as the factor.

ATP used was in the form of a crystalline disodium salt obtained from the Sigma Chemical Co. Other reagents were commercial products of the best reagents grade available.

Results

KCl concentration dependence on myofibrillar ATPase activity. As shown in Fig. 1, the ATPase activity of crab myofibrils was very low without addition of bivalent cations and was affected very little by increasing the concentration of KCl. The ATPase was generally activated by addition of 100 μM Ca^{++} and the extent of the activation was relatively great at low KCl concentrations. The ATPase in the absence of other bivalent

cations was remarkably activated also by addition of 2 mM Mg^{++}; at 30 mM KCl the activity increased from 0.02 μmoles Pi/mg/min to 0.26 μmoles Pi/mg/min. The activated ATPase was decreased by increasing the concentration of KCl and at 0.5 M KCl it coincided with the activity obtained in the presence of Ca^{++}. This result indicates that the actomyosin of a crab also dissociates at high KCl concentrations and that it does almost completely at KCl concentrations higher than 0.5 M.

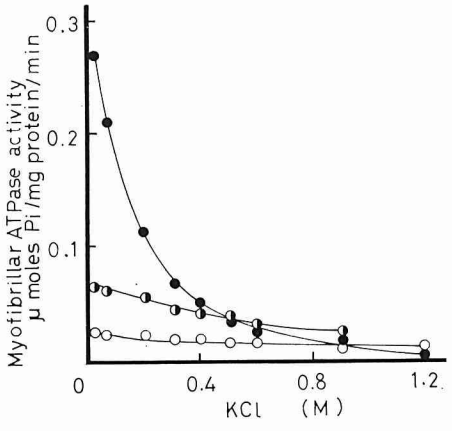

Fig. 1

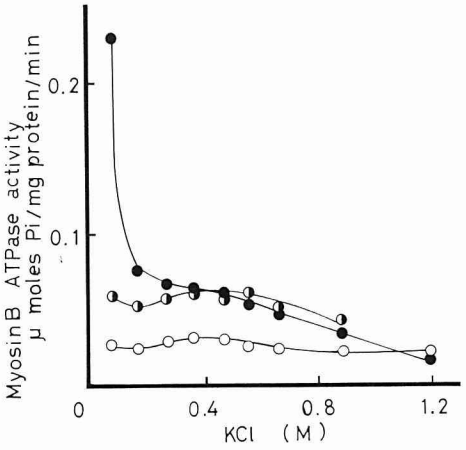

Fig. 2

Fig. 1 KCl concentration dependence on ATPase activity of crab myofibrils. Reaction mixture; 66.7 mM Tris-acetate buffer (pH 7.0), 2 mM ATP and 1.0 mg/mℓ of myofibrils. Final volume; 2 mℓ. Temperature; 20°C. Reaction time; 7 min.

○; without $MgCl_2$ and $CaCl_2$
◑; with 100 μM $CaCl_2$
●; with 2.0 mM $MgCl_2$

Fig. 2 KCl concentration dependence on ATPase activity of crab myosin B. Reaction mixture; 66.7 mM Tris-acetate buffer (pH 7.0), 2 mM ATP and 0.75 mg/mℓ of myosin B. Final volume; 2 mℓ. Temperature; 20°C. Reaction time; 10 min.

○; without $MgCl_2$ and $CaCl_2$
◑; with 100 μM $CaCl_2$
●; with 2.0 mM $MgCl_2$

KCl concentration dependence on ATPase activity of myosin B. Without addition of bivalent cations the ATPase activity of myosin B was also very low and was only slightly affected by increasing the KCl concentration in the range of 0.05 M to 1.2 M. By addition of 100 μM Ca^{++} the ATPase was about two times activated. This activation was observed at all KCl concentrations in the range of 0.05 to 0.9 M. Furthermore, the ATPase activity was markedly increased by addition of 2 mM Mg^{++} at low KCl concentrations. The activated ATPase decreased with increase of the KCl concentration. The extent of the decrease was remarkably great at 0.05 to 0.15 M KCl in comparison with that at higher than 0.15 M KCl. These results on crab myosin B are similar to those on the myofibrils.

Discussion

Our results showing the KCl concentration dependence on the ATPase activity of the crab myofibrils and its myosin B in the absence of bivalent cations (Figs. 1 and 2) are different from that obtained on rabbit myosin B by Yutasaka[10], who indicated that the KCl dependence curve has a minimum at 0.2 M KCl. Recently, Jou[11] obtained the result showing that under the same conditions the ATPase activity of rabbit myosin B increased with increasing the KCl concentration up to 0.4 M KCl and then at higher KCl concentrations gradually decreased. The present result on the crab myosin B, therefore, is also different from this result. However, the KCl dependence on the ATPase activity of the crab actomyosin system in the presence of Ca^{++} resembled to those shown on rabbit myosin B by Jou[11] and by Maruyama[12] and further the dependence in the case of myofibrils and of myosin B in the presence of Mg^{++} is in agreement with that on rabbit myofibrils obtained by Takahashi[13] and with those on rabbit myosin B obtained by Jou[11] and by Maruyama[12], respectively. Therefore, it may be reasonable to consider, as previously mentioned by Sarkar[5], that in the presence of bivalent cations, especially Mg^{++}, the actomyosin system of a crab is essentially similar to that of a rabbit.

As mentioned above, it has been reported that the living muscle of a crab is able to normally contract even at high ionic milieu. However, from the present results this contractility of crab muscle is not simply explained as a characteristics of crab actomyosin system.

In regard to the relation between the contractility of muscle and the temperature, Szent-Györgyi[14] considered that the muscle has an ability to adapt itself to the temperature of the circumstances and there is a regulating mechanism by which the muscle exerts this adaptation. Further he suggested that the mechanism may be abolished during the preparation of actomyosin system. Therefore, it may be possible that the muscle could have similar adaptation mechanism even to the ionic milieu.

On the other hand, it has been reported that frog's living muscle equilibrated in Ringer's solution, 2.5 times higher in tonicity, can induce fully caffeine contracture[15,16]. This fact implies that the muscle of frog inhabiting in fresh water also can contract at high ionic milieu under certain conditions. Accordingly, it appears to us that the discrepancy in contractility between the living muscle and the isolated actomyosin system exhibited under the hypertonic conditions is not specific only for crab muscle. Therefore, this discrepancy may have to be reduced to a general problem on the difference in contractility between the living muscle and the isolated actomyosin system rather than that on the adaptation.

Summary

KCl concentration dependence on the ATPase activity of myofibrils and of myosin B was studied using crab muscle.

1) Without addition of bivalent cations, the ATPase activities of these actomyosin systems were vrey low.

2) By addition of 100 μM Ca^{++} or 2 mM Mg^{++}, these ATPase activities were activated.

3) These activated ATPases were decreased with increasing the KCl concentration.

From these results, it was indicated that the actomyosin system of a crab is essentially similar to that of a rabbit.

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