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The Ca^{2+} sensitivity of actomyosin in solution

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SUMMARY

Actomyosin dissolved in 0.6 M KCl showed a response to Ca^{2+} in the presence of MgATP and native tropomyosin (troponin–tropomyosin complex); the Mg^{2+}-ATPase activity was inhibited by some 20% by ethyleneglycol-bis(aminoethylether)-*N,N'*-tetraacetic acid (EGTA), the recovery of the viscosity decreased by ATP was delayed by EGTA and the same tendency was observed in dynamic rigidity measurements. These facts suggest that there exist some interactions between the myosin monomer and F-actin filaments in the presence of ATP and 0.6 M KCl which are regulated by Ca^{2+} under the influence of the troponin–tropomyosin complex.

It has been well known that the so-called Ca^{2+} sensitivity of actomyosin is observed under some limited conditions, *e.g.* appropriate MgATP and KCl concentrations[1]. Above certain KCl concentrations, where myosin cannot form A-filament-like aggregates[2], superprecipitation does not occur. Under such conditions, the arrow-head structure of actomyosin is formed[2], and ATP dissociates myosin monomers from F-actin filaments[1,3].

Recent work[4,5], however, has clearly indicated that complexes of F-actin with H-meromyosin or even Subfragment I possess Ca^{2+} sensitivity: the Mg^{2+}-activated ATPase activity is controlled by free Ca^{2+} provided that the troponin–tropomyosin complex[1] is present. The analogy of gross structure between acto-H-meromyosin and actomyosin in solution suggests the occurrence of Ca^{2+} sensitivity in the latter, too. The present study showed that this was indeed the case.

Myosin, actin and native tropomyosin[1] were prepared from rabbit skeletal muscle according to the routine procedure of this laboratory[6]. ATPase measurements were performed at pH 7.2 and 25° (ref. 6). Viscosity was measured at 25.0° using an Ostwald-type viscometer (flow time of water, 30 sec). Dynamic rigidity was determined in the apparatus (Sanki Eng. Ltd.) described before[7].

Abbreviation: EGTA, ethyleneglycol-bis(aminoethylether)-*N,N'*-tetraacetic acid.

The ATPase activity of myosin in the presence of 0.6 M KCl and 1 mM $MgCl_2$ was very low (about 3 nmoles/mg per min). When actin or native tropomyosin was added, the activity was slightly but definitely increased to 4–5 nmoles/mg per min (Table I). The significant point was that only actomyosin with native tropomyosin showed the 20% inhibition by 1 mM ethyleneglycol-bis(aminoethylether)-*N,N'*-tetraacetic acid (EGTA) due to Ca^{2+} sensitivity. Eisenberg and Kielley[4] reported that the inhibition of acto-H-meromyosin by EGTA depends upon KCl concentration. We have confirmed this: the percent inhibition by 1 mM EGTA was 79, 59, 20 and 11% at 0.02, 0.10, 0.3, 0.6 M KCl, respectively. With actomyosin, the EGTA inhibition was certainly greater (30–40%) at 0.3 M than at 0.6 M KCl. Fig. 1 shows the results at 0.3 M KCl, where no superprecipitation takes place. As shown in Fig. 1, the inhibition became evident as the amount of F-actin was increased to more than twice that of myosin by weight.

TABLE I

EFFECT OF EGTA AND Ca^{2+} ON THE ATPase ACTIVITY OF ACTOMYOSIN IN 0.6 M KCl

Conditions: 0.6 M KCl, 10 mM Tris buffer, pH 7.2, and 1 mM $MgCl_2$. When added, the concentration of EGTA and $CaCl_2$ was 1 mM and 0.1 mM, respectively. F-actin, 6 mg; myosin, 1.2 mg; native tropomyosin, 3 mg. Total volume, 3.0 ml. Incubated at 25° for 1 h.

	μmoles P_i split		*Inhibition by EGTA (%)*
	+Ca^{2+}	*+EGTA*	
Myosin	0.252	0.232	8
Myosin + native tropomyosin	0.389	0.368	5
Actomyosin	0.308	0.292	5
Actomyosin + native tropomyosin	0.432	0.352	19

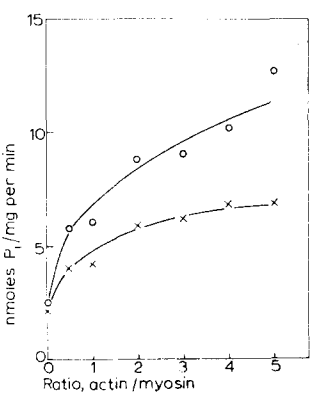

Fig. 1. Effect of increasing the amounts of F-actin and native tropomyosin on the Ca^{2+}-dependent ATPase activity of actomyosin at 0.3 M KCl. Conditions: 0.3 M KCl, 10 mM Tris buffer, pH 7.2, 1 mM $MgCl_2$. Myosin, 1.2 mg. Total volume, 3.0 ml. Incubated for 1 h. Native tropomyosin was added in the same amount as that of actin as shown on the abscissa. ○, 0.1 mM Ca^{2+}; ×, 1 mM EGTA.

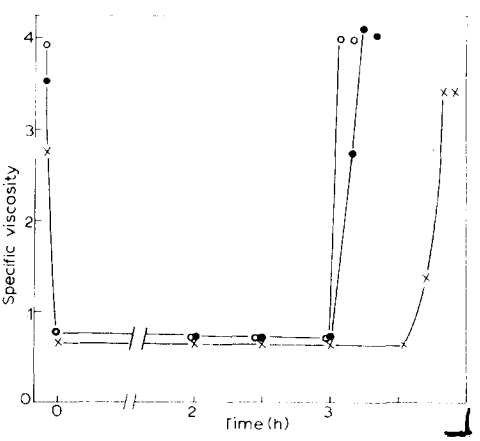

Fig. 2. Viscosity change of actomyosin solution under the influence of native tropomyosin. Conditions: 0.6 M KCl, 10 mM Tris buffer, pH 7.2, 1 mM $MgCl_2$. Myosin, 2 mg/ml; F-actin, 1 mg/ml; native tropomyosin, 0.5 mg/ml. Total volume, 2.0 ml. When added, the concentration of ATP was 2 mM. 25°. ○, control; ●, 0.1 mM $CaCl_2$; ×, 1 mM EGTA.

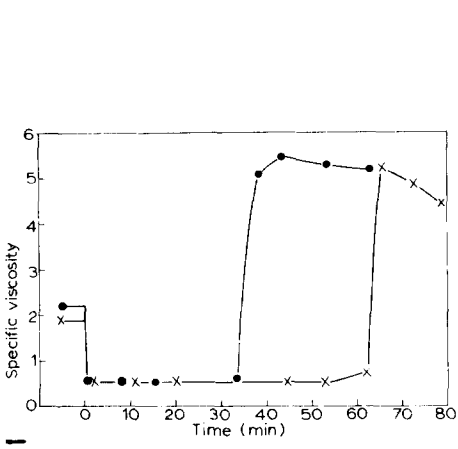

Fig. 3. Effect of EGTA on the viscosity change of acto-H-meromyosin at 0.1 M KCl. Conditions: as in Fig. 2 except for 0.1 M KCl and H-meromyosin. ●, 0.1 mM $CaCl_2$; x, 1 mM EGTA.

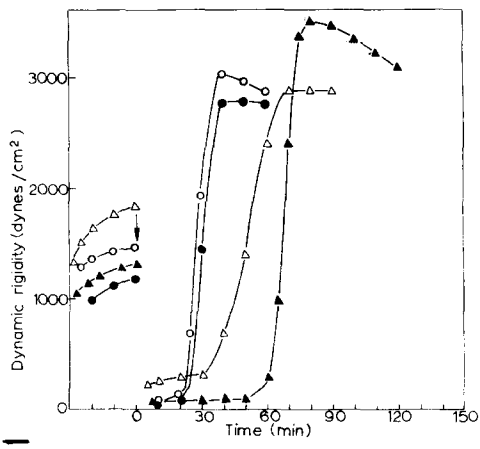

Fig. 4. Effect of $CaCl_2$ and EGTA on the dynamic rigidity of actomyosin at 0.6 M KCl under the influence of native tropomyosin. Conditions: 0.6 M KCl, 10 mM Tris buffer, pH 7.2, 1 mM $MgCl_2$. F-actin, 4 mg/ml; myosin, 3 mg/ml; native tropomyosin, 2 mg/ml. When added, the concentration of ATP was 0.5 mM (arrow). Total volume, 4 ml. 25°. Frequency, 10 Hz; amplitude of oscillation, 10 μ. ○, 0.1 mM $CaCl_2$; ●, 1 mM EGTA; △, 0.1 mM $CaCl_2$ and native tropomyosin; ▲, 1 mM EGTA and native tropomyosin.

The recovery process of the ATP-decreased viscosity of actomyosin was examined. As seen in Fig. 2 the recovery was slightly retarded by EGTA, which could well be explained by a small inhibition of the ATPase activity by EGTA (Table I). There was no difference between the conditions with and without 0.1 mM $CaCl_2$. In Fig. 3 a more remarkable effect of Ca^{2+} on the recovery process of acto-H-meromyosin in 0.1 M KCl is shown. As described before, the ATPase inhibition was as large as 60%. It is to be noted that the viscosity after recovery was much larger than the initial value before the addition of ATP. This phenomenon was discussed in our paper dealing with dynamic rigidity measurements of acto-H-meromyosin[7]. Dynamic rigidity measurements were also applied to the actomyosin solution in order to observe the Ca^{2+} effect. As shown in Fig. 4, without native tropomyosin, no effect of Ca^{2+} was observed, but with native tropomyosin, the retardation of recovery of dynamic rigidity was clearly demonstrated. The very high rigidity after recovery[7] was observed irrespective of Ca^{2+} concentration and of the presence or absence of native tropomyosin.

The present study shows that there must be such an interaction of the myosin monomer with F-actin in the presence of MgATP and 0.6 M KCl so as to result in activation of the Mg^{2+}-ATPase activity of myosin, as in the ATP–acto-H-meromyosin delate system[8]. And the interaction is regulated by Ca^{2+} under the influence of the troponin–tropomyosin complex, just as in the contraction–relaxation cycle at lower ionic strength[1]. The Ca^{2+} effect at 0.6 M KCl was much smaller than at lower KCl concentrations, which was the case with H-meromyosin. Therefore, this must be due to a property of the ATPase site on the myosin molecule, not directly related to its ability to form a special aggregate at lower ionic strength.

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