INHIBITION OF Mg^{++}ATPase ACTIVITY OF ACTIN-ACTIVATED ACANTHAMOEBA MYOSIN BY MUSCLE TROPONIN-TROPOMYOSIN: IMPLICATIONS FOR THE MECHANISM OF CONTROL OF AMOEBA MOTILITY AND MUSCLE CONTRACTION

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SUMMARY: Troponin-tropomyosin is known to inhibit the Mg^{++}ATPase activity of muscle actomyosin in the absence, but not in the presence, of Ca^{++}. In contrast, we have now found that muscle troponin-tropomyosin inhibits the Mg^{++}ATPase activity of muscle actin-activated Acanthamoeba myosin both in the presence and the absence of Ca^{++}. Addition of purified tropomyosin and troponins-I, C and T demonstrated that it is troponin-T that acts differently in the two systems which differ only in the source of the myosin. These data suggest that myosin, as well as actin, plays a role in the troponin-tropomyosin control of muscle contraction and make it unlikely that control proteins identical to troponin-tropomyosin function in this amoeba.

INTRODUCTION

Acanthamoeba castellanii, like many other non-muscle cells, is now known to contain both actin and myosin (1). By analogy to their role in muscle contractility the amoeba proteins are assumed to be the molecular basis of cell motility. Acanthamoeba actin is very similar to muscle actin in its chemical composition and physical properties (2,3) and is organized as filaments in the amoeba cytoplasm (4) probably attached to the plasma membrane (5). Acanthamoeba myosin (6), however, differs from muscle myosin in its much lower molecular weight (about 180,000 daltons) and chemical composition and does not seem to be organized into filaments in the amoeba. The ATPase activities of Acanthamoeba myosin and of muscle myosin are very low in the presence of Mg^{++} and are activated by either muscle or Acanthamoeba actin

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(2,6,7). However, unlike muscle myosin the activation by actin of the Mg^{++} ATPase of Acanthamoeba myosin requires the presence of a third protein, the cofactor, that also has been purified from the amoeba (7).

In muscle, the enzymatic activity of the actomyosin complex is controlled by a group of proteins, the troponins and tropomyosin, which, acting in concert, inhibit the Mg^{++}ATPase, by dissociation of the actin and myosin (8,9), except in the presence of Ca^{++}. Thus, under physiological conditions, the concentration of Ca^{++} regulates the ATPase of muscle actomyosin (9). Previous work in this laboratory (10) has shown that the hybrid complex of Acanthamoeba actin and muscle myosin interacts with the muscle regulatory proteins in a similar manner so that its Mg^{++}ATPase activity becomes dependent on Ca^{++}. We also have shown (7) that the amoeba cofactor protein that is required for actin-activation of Acanthamoeba myosin Mg^{++}ATPase has none of the properties of the regulatory proteins from muscle.

With this as background, it was of interest to investigate the effects of the muscle regulatory proteins on Acanthamoeba myosin both to determine if similar regulatory proteins might be present in the amoeba and also for information such experiments might provide on the mechanism of the regulation of the muscle actomyosin system. In the experiments to be described muscle actin was used to activate the Acanthamoeba myosin because it is more readily obtainable than Acanthamoeba actin and, as described before, the two actins are very similar in their activations of myosin ATPase. Control experiments were carried out with actin-activated muscle myosin subfragment-1.

RESULTS

Calcium ions alone had no effect on the actin-activated Mg^{++}ATPase of crude or purified preparations of Acanthamoeba myosin (Tables I and II), indicating that the crude preparations were free of Ca^{++}-sensitive regulatory proteins and that the purified Acanthamoeba myosin lacked the intrinsic Ca^{++}-sensitivity possessed by molluscan muscle myosins (14). As anticipated, unfractionated muscle troponin-tropomyosin strongly inhibited the

Table I. Effect of Muscle Troponin-Tropomyosin on Actin-Activated ATPase Activity of Myosin

	Mg^{++}ATPase Activity[a] μmoles P_i/min/mg myosin			
	Muscle myosin subfragment-1[b]		Acanthamoeba myosin with cofactor[c]	
Added proteins	EGTA	Ca^{++}	EGTA	Ca^{++}
None	< 0.10	< 0.10	0.14	0.20
Muscle F-actin[d]	3.70	4.52	1.13	1.21
Muscle F-actin and troponin-tropomyosin[e]	0.39	5.50	0.23	0.35

[a]ATPase activity was assayed in 2 mM ATP, 1.4 mM $MgCl_2$, 30 mM KCl, 7.5 mM imidazole chloride, pH 7.0 with either 1 mM EGTA (a chelator of Ca^{++}) or 0.5 mM $CaCl_2$ for 20 minutes at 29°C. Inorganic phosphate released from γ-P^{32}-ATP was measured by the method described elsewhere (6).

[b]Rabbit muscle myosin subfragment-1, 0.01 mg/ml, prepared by papain digestion (11).

[c]Acanthamoeba myosin, 0.017 mg/ml, prepared by agarose adsorption and hydroxylapatite chromatography (6) plus a crude preparation of cofactor protein, about 0.01 mg/ml, from the same hydrosylapatite column.

[d]Rabbit muscle F-actin, 1.0 mg/ml, prepared by a modification of the method of Spudich and Watt (12).

[e]Rabbit muscle troponin-tropomyosin, 1.3 mg/ml prepared by the method of Hartshorne and Mueller (13).

actin-activated Mg^{++}ATPase of Acanthamoeba myosin in the absence of Ca^{++} but, in striking contrast to the control experiments with actin-muscle myosin subfragment-1, this inhibition was maintained even in the presence of Ca^{++} (Table I). The same results were obtained with crude unfractionated mixtures of Acanthamoeba myosin and cofactor protein, and with recombined mixtures of purified Acanthamoeba myosin and purified cofactor.

To determine which of the several components of the troponin-tropomyosin complex were responsible for this Ca^{++}-insensitive inhibition of Acanthamoeba myosin, we compared the effects of combinations of purified tropomyosin and troponin-I, troponin-C, and troponin-T on the actin-activation of Acanthamoeba myosin and of muscle myosin subfragment-1 (Table II). In agreement with other reports (15,16), tropomyosin alone did not inhibit

Table II. Effect of Purified Tropomyosin and Troponin Components on Actin-Activated ATPase Activity of Myosin

	Percent of Mg^{++}ATPase Activity[a]					
	Muscle subfragment-1[b] with actin		Crude *Acanthamoeba* myosin and cofactor[c] with actin		Purified *Acanthamoeba* myosin and cofactor[d] with actin	
Proteins added[e]	EGTA	Ca^{++}	EGTA	Ca^{++}	EGTA	Ca^{++}
1. None	100	102	100	100	100	90
2. Tropomyosin	89	150	96	-	52	61
3. Tropomyosin + Troponin-I	10	7	6	3	10	0
4. Tropomyosin + Troponins-I + C	92	183	73	47	56	46
5. Tropomyosin + Troponins-I + C + T	22	219	5	6	14	20

[a]ATPase activity was assayed in 2 mM ATP, 1.4 mM $MgCl_2$, 27 mM KCl, 8.3 mM imidazole chloride, pH 7.0, for 15 or 30 minutes at 29°C as described in Table I. All samples contained muscle F-actin, 0.1 mg/ml. The low level of ATPase activity in the absence of actin is subtracted from the rates given here.

[b]Muscle myosin subfragment-1, 0.15 mg/ml. 100% activity was 0.56 μmoles/min/mg subfragment-1.

[c]Crude *Acanthamoeba* myosin with cofactor, 0.8 mg/ml, obtained after ammonium sulfate fractionation (6). 100% activity was 0.012 μmoles/min/mg protein.

[d]Purified *Acanthamoeba* myosin (6), 0.015 mg/ml, with "G-150" cofactor (7) 0.065 mg/ml. 100% activity was 0.29 μmoles/min/mg myosin.

[e]Rabbit muscle tropomyosin, 0.05 mg/ml, troponin-I, 0.021 mg/ml, troponin-C, 0,057 mg/ml, and troponin-T, 0.029 mg/ml were prepared by the method of Eisenberg and Kielley (9); (manuscript in preparation).

the Mg^{++}ATPase of actin-activated muscle myosin subfragment-1; addition of troponin-I caused Ca^{++}-insensitive inhibition of Mg^{++}ATPase; addition of troponin-C blocked the effect of troponin-I so that, as with tropomyosin alone, there was a variable activation of Mg^{++}ATPase in the presence of Ca^{++}; and addition of troponin-T restored inhibition which could then be reversed by Ca^{++}. Thus, tropomyosin and the three troponins, I, C, and T, were all necessary for the Ca^{++}-sensitive inhibition of the Mg^{++}ATPase activity of actin-activated muscle myosin subfragment-1.

In the actin-Acanthamoeba myosin system (Table II), tropomyosin alone inhibited the actin-activated Mg^{++}ATPase variably up to 50%, an effect that occurs with muscle myosin subfragment-1 only in the presence of excess Mg^{++} (E. Eisenberg and W.W. Kielley, in preparation). The inhibition by tropomyosin of the Acanthamoeba enzyme was more marked at higher concentrations of Mg^{++} and lower concentrations of KCl than those used in the experiments in Table II. Troponin-I and troponin-C had effects on the Acanthamoeba myosin similar to their effects on actin-activated muscle myosin subfragment-1: troponin-I inhibited the enzymatic activity and this inhibition was reversed by troponin-C. However, reconstitution of the complete system by addition of troponin-T inhibited the actin-activated Acanthamoeba myosin Mg^{++}ATPase not only in the absence of Ca^{++} but also, in contrast to the results with muscle myosin, in the presence of Ca^{++}. The results with the purified components, then, confirm those reported in Table I for the unfractionated troponin-tropomyosin complex and suggest further that it is specifically troponin-T that interacts differently with the amoeba and muscle systems.

DISCUSSION

Although these results might be just a consequence of some unique feature of the amoeba system (for example, the interaction of Acanthamoeba myosin and cofactor might be blocked by troponin-T), the difference in response of actin-activated Acanthamoeba myosin and actin-activated muscle myosin subfragment-1 to the muscle regulatory proteins may offer some insight into

the mechanism of action of the troponin-tropomyosin complex in muscle. These data support the idea that the control exerted by the muscle regulatory proteins in muscle is not mediated by events on the actin filaments alone but that muscle myosin is also an active component of the regulatory mechanism (17-20). Moreover, the data in Table II suggest that this effect of myosin might involve in some way the action of troponin-T.

It also seems probable from these observations that, unlike Physarum polycephalum (21, 22) movement of Acanthamoeba is not controlled by proteins identical to the troponin-tropomyosin complex nor, perhaps, even by Ca^{++}. Regulatory proteins may be present in the amoeba but if so they must differ from their counterparts in muscle for otherwise they would just inhibit irreversibly the functional interaction of Acanthamoeba actin and myosin.

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