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INTERACTION OF MYOSIN AND PARAMYOSIN

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The interaction of myosin and paramyosin was investigated by enzymological and ultrastructural techniques. The actin-activated Mg^{+2} ATPase of rabbit skeletal muscle myosin can be inhibited by clam adductor paramyosin. Both proteins must be rapidly coprecipitated to form filaments for this inhibition. Slowly formed cofilaments are fully activatable by F-actin. In both cases, the cofilaments possess unique structural characteristics when compared to homofilaments.

The mode of inhibition appears to be competitive when different concentrations of paramyosin and F-actin are compared. The apparent affinity of the myosin heads for actin is reduced by the presence of paramyosin within rapidly reconstituted thick filaments. These results suggest that paramyosin may serve as part of a relaxing mechanism within invertebrate muscles. It is unlikely that paramyosin plays a role in the initiation and maintenance of catch within specialized molluscan muscles.

INTRODUCTION

Muscles from molluscs and other invertebrates contain thick filaments with paramyosin forming a core and myosin at the surface (1). In these studies, it was found that paramyosin could inhibit actomyosin Mg^{+2} ATPase when the soluble proteins were mixed (2). When myosin is added to paramyosin paracrystals, binding occurs without inhibition of actin-activated ATPase (3).

We have verified these observations and extended them. The interaction of paramyosin with the LMM[1] segment of myosin within the shaft of a rapidly formed cofilament impairs the association of the HMM S1 heads with F-actin. In slowly formed cofilaments this association is unaffected. A model is presented to explain the differences in properties between the two kinds of cofilaments. The possible physiological significance of the different modes of interaction between myosin and paramyosin is discussed.

METHODS

Paramyosin was purified containing 94,000 or 105,000 MW monomer forms from clam adductor muscles (4). Myosin and actin were purified from rabbit hind leg and back muscles (5, 6). LMM, HMM, and HMM S1 were prepared from rabbit myosin (7). Protein concentrations were determined by a modification of Lowry's procedure (8). The polypeptide compositions of purified proteins, prepared fragments, and assembled filaments

[1] Abbreviations: LMM, light meromyosin; HMM, heavy meromyosin; HMM S1, subfragment 1; SDS, sodium dodecyl sulfate.

were determined by SDS-polyacrylamide slab gel electrophoresis (9).

ATPase measurements were performed at 20°C in a Radiometer pHstat (10, 11). Actomyosin activities were corrected for the intrinsic myosin Mg^{+2} ATPase. Filaments were formed rapidly by diluting protein solutions containing 7–8 mg ml^{-1} protein, 10 mM potassium phosphate, pH 7.0 and 0.6 M KCl into 2 mM $MgCl_2$, 0.1 mM $CaCl_2$ at 25°C. Slowly formed filaments were formed by dialyzing 0.5 mg ml^{-1} solutions of protein against 0.1 M KCl, 10 mM $MgCl_2$, 10 mM potassium phosphate, pH 6.5. These preparations were placed upon carbon-coated Formvar grids and negatively stained with 1% uranyl acetate. The specimens were examined with a Siemens Ia Elmiskop at an accelerating voltage of 60 kV.

RESULTS

Requirements for Inhibition

Rabbit actomyosin is inhibited in a similar fashion to clam actomyosin (Epstein, Aronow, and Harris, manuscript in preparation). As shown in Fig. 1, at 0.2 mg ml^{-1} of paramyosin and 0.2 mg ml^{-1} of actomyosin (1:1), the Mg^{+2} ATPase is inhibited 60%. The hyperbolic inhibition curve is consistent with an equilibrium constant of 10^{-7} M for the complex dissociation. Table I demonstrates that myosin and paramyosin must be rapidly precipitated together and not individually for this effect. If concentrations of paramyosin as high as 0.8 mg ml^{-1} are mixed with HMM or HMM S1 under similar conditions, no inhibition is observed. LMM rapidly coprecipitated with myosin and paramyosin will completely abolish the inhibition at concentrations of 0.3 mg ml^{-1}. Thus, the LMM segment is necessary while HMM does not contain sites sufficient for this interaction with paramyosin.

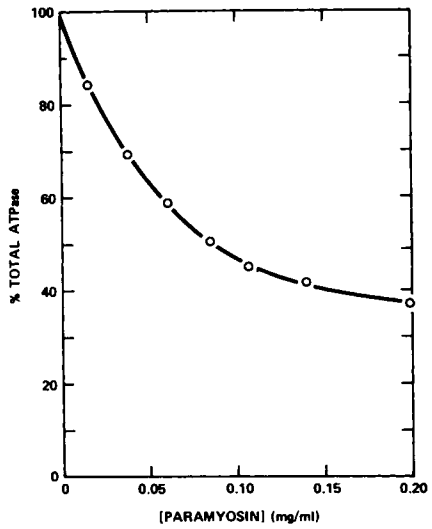

Fig. 1. Inhibition of actomyosin as a function of paramyosin concentration. Solutions contained 0.1 mg mg^{-1} myosin, 0.1 mg ml^{-1} F-actin, 30 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 0.75 mM ATP. 100% activity was 0.33 μmol ATP min^{-1} mg^{-1} for actomyosin in the absence of 94,000 MW paramyosin.

TABLE I. Requirements for Inhibition

Proteins	ATPase (μmol min^{-1} mg^{-1})
F-actin added to myosin filaments	0.295
Actomyosin added to paramyosin filaments	0.285
F-actin added to myosin-paramyosin cofilaments	0.165

All reactions were run in 30 mM KCl, 0.1 mM $CaCl_2$, 2.5 mM $MgCl_2$, and 0.75 mM ATP at 25°C and pH 7.5. Protein concentrations were actin, 0.3 mg ml^{-1}, myosin, 0.1 mg ml^{-1}, and 94,000 MW paramyosin, 0.15 mg ml^{-1}.

Structure of Cofilaments

Myosin, paramyosin, and their mixture all form filamentous structures when precipitated rapidly by dilution or slowly by dialysis to low ionic strength buffers. As shown in Fig. 2, myosin and paramyosin, when rapidly coprecipitated, formed rough-surfaced filaments with diameters of 10–20 nm and lengths between 1 and several μm. The polypeptide weight ratios were 1.0: 0.87: : myosin-paramyosin. Paramyosin by itself forms smooth-surfaced filaments of larger dimensions, while myosin filaments are rough in texture with smaller dimensions under the same conditions.

Myosin-paramyosin filaments formed by dialysis all exhibited a regular 14.5 nm axial periodicity and a unique tendency to branch (Fig. 3). No banding pattern was observed in myosin filaments. Paramyosin paracrystals showed a variety of periods. In certain portions of the cofilaments this periodicity was obscured, and protruding, head-like structures were apparent. These observations were made in filaments containing myosin to paramyosin ratios of 1:0.95 and 1:1.7. These results suggest that myosin and paramyosin can interact during the formation of filaments. At least two distinct classes of interactions between these proteins exist, as shown by the different enzymic and structural properties of rapidly and slowly precipitated cofilaments.

Competition Between Actin and Myosin

Paramyosin has no effect upon the intrinsic Mg^{+2} or Ca^{+2} ATPases of myosin (2). If Mg^{+2} ATPase activities of rapidly precipitated myosin-paramyosin or myosin filaments are compared as a function of F-actin concentration, linear reciprocal plots obeying classical Michaelis-Menten kinetics are obtained. Figure 4 demonstrates that at infinite F-actin concentrations, the activities of cofilaments formed from solutions containing two ratios of myosin to paramyosin and of myosin filaments are all equal (V_{max} = 0.33 μmol min^{-1} mg^{-1}). Thus, actin and paramyosin appear to be competitive with one another in their effects upon the catalytic properties of myosin.

Results that suggest competition are obtained when equal weights of 105,000 MW and 94,000 MW paramyosin are coprecipitated with myosin. The larger species is a better inhibitor at all concentrations of F-actin except at the intercept. Under conditions where paramyosin inhibition is significant, the F-actin is free to activate the Mg^{+2} ATPase of added HMM (Aronow and Epstein, unpublished results). Thus, paramyosin appears to decrease the affinity of HMM S1 heads and F-actin for one another.

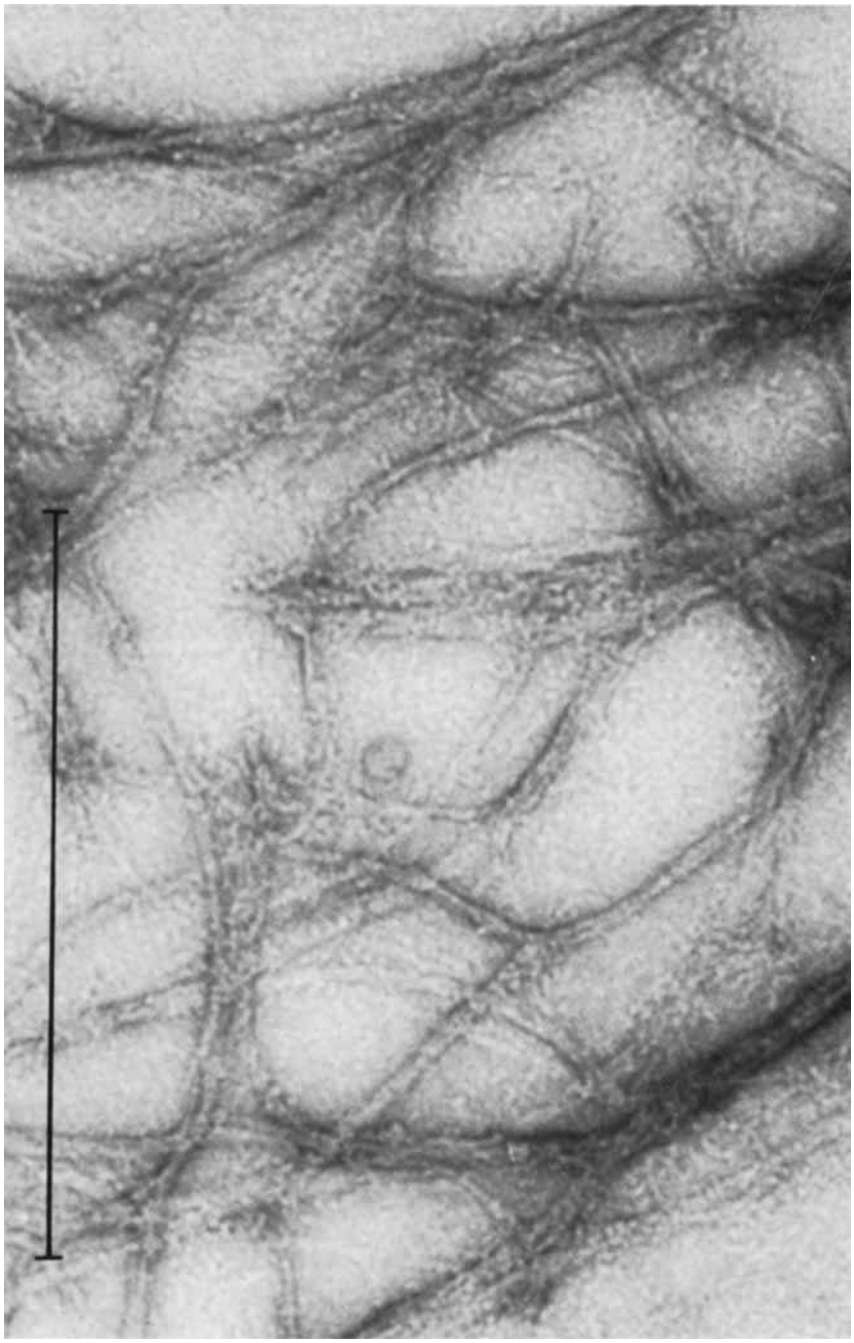

Fig. 2. Rapidly precipitated cofilaments. Myosin and 94,000 MW paramyosin were mixed 1:1 and then diluted as described in Methods. The bar denotes 0.5 μm.

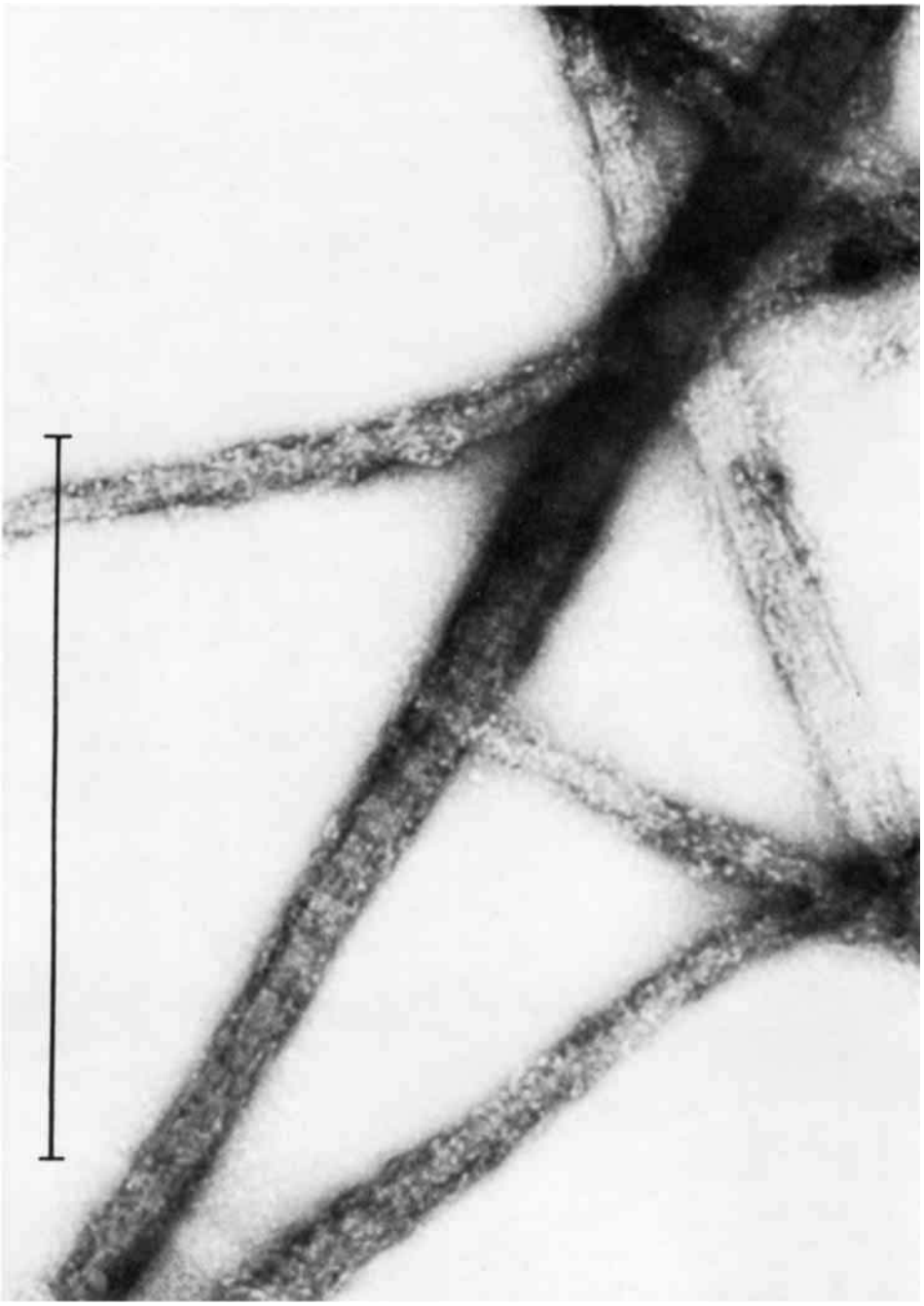

Fig. 3. Slowly precipitated cofilaments. Myosin and 94,000 MW paramyosin were mixed 1:2 and then dialyzed as described in Methods. The bar denotes 0.5 μm.

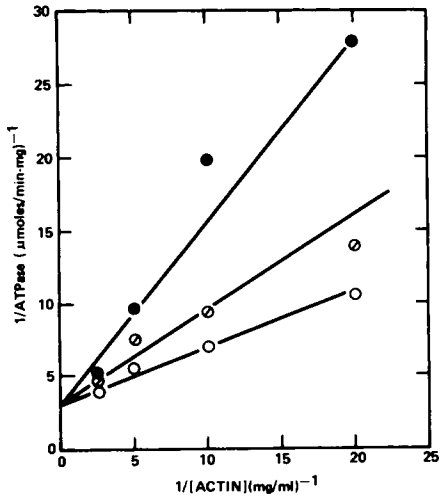

Fig. 4. Competition between actin and paramyosin. Solutions contained 0.1 mg ml^{-1} myosin, 24 mM KCl, 1 mM $MgCl_2$, and 0.75 mM ATP. Reactions were initated by F-actin. ○–○, no paramyosin; ◑–◑ and ●–●, 0.05 and 0.2 mg ml^{-1} 105,000 MW paramyosin, respectively.

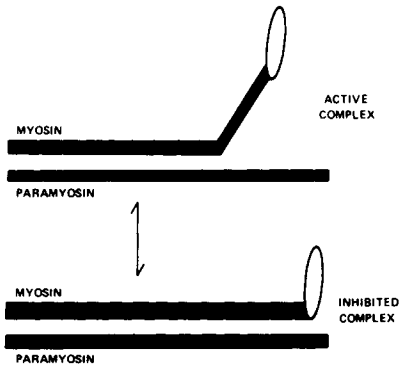

Fig. 5. Model for different myosin-paramyosin interactions. In native filaments, a reversible association between myosin crossbridges and certain portions may result in decreased affinity for F-actin. Other portions of paramyosin and LMM would bond with each other independently of the state of the crossbridges.

DISCUSSION

The studies reported here indicate that at least two classes of filaments can form from myosin and paramyosin. Aperiodic cofilaments are associated with a decreased ability to bind and be activated by F-actin. Cofilaments with regular 14.5-nm axial periods and protruding head-like structures show the same behavior towards F-actin as pure myosin filaments. We postulate that native filaments containing myosin and paramyosin may be able to alternate between these two states in vivo.

The structural basis of these states may be due to differences in the bonding between myosin crossbridges and paramyosin. LMM and paramyosin interactions are necessary for both types of filaments. The large differences in inhibitory properties of 94,000 and 105,000 MW paramyosins, both of which are longer α-helical rods than LMM, suggest that bonding with additional portions of the myosin rod may be necessary for inhibition also. Figure 5 shows a model for such a configurational equilibrium. The inhibited filaments would have the myosin crossbridges bonding to paramyosin along their entirety, thus explaining the decreased affinity for actin. The active filaments would have the crossbridges and HMM S1 heads free to bind normally to actin. Bonding between LMM segments and paramyosin would be very similar in the two states.

If our results and interpretations are correct, it is unlikely that paramyosin could be responsible for the very stable actomyosin links observed in the catch state of certain molluscan muscles (1, 2). We would postulate that paramyosin might be part of an efficient relaxing mechanism required to break the extensive actomyosin linkage formed during catch. Other myofilament components would be responsible for the initiation and maintenance of catch, while as yet unknown chemical signals are presumably regulating the entire process.

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