NIH Public Access

Author Manuscript

J Biol Chem. Author manuscript; available in PMC 2005 September 9.

Published in final edited form as:
J Biol Chem. 1984 February 25; 259(4): 2617–2621.

Regulation of Molluscan Actomyosin ATPase Activity*

Joseph M. Chalovich‡, **Peter D. Chantler**§,¶, **Andrew G. Szent-Gyorgyi**§,||, and **Evan Eisenbergt**‡

‡*From the Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205 and the*

§*Department of Biology, Brandeis University, Waltham, Massachusetts 02154*

Abstract

The interaction of myosin and actin in many invertebrate muscles is mediated by the direct binding of Ca^{2+} to myosin, in contrast to modes of regulation in vertebrate skeletal and smooth muscles. Earlier work showed that the binding of skeletal muscle myosin subfragment 1 to the actin-troponin-tropomyosin complex in the presence of ATP is weakened by less than a factor of 2 by removal of Ca^{2+} although the maximum rate of ATP hydrolysis decreases by 96%. We have now studied the invertebrate type of regulation using heavy meromyosin (HMM) prepared from both the scallop *Aequipecten irradians* and the squid *Loligo pealii*. Binding of these HMMs to rabbit skeletal actin was determined by measuring the ATPase activity present in the supernatant after sedimenting actoHMM in an ultracentrifuge. The HMM of both species bound to actin in the presence of ATP, even in the absence of Ca^{2+}, although the binding constant in the absence of Ca^{2+} (4.3×10^3 m^{-1}) was about 20% of that in the presence of Ca^{2+} (2.2×10^4 m^{-1}). Studies of the steady state ATPase activity of these HMMs as a function of actin concentration revealed that the major effect of removing Ca^{2+} was to decrease the maximum velocity, extrapolated to infinite actin concentration, by 80–85%. Furthermore, at high actin concentrations where most of the HMM was bound to actin, the rate of ATP hydrolysis remained inhibited in the absence of Ca^{2+}. Therefore, inhibition of the ATPase rate in the absence of Ca^{2+} cannot be due simply to an inhibition of the binding of HMM to actin; rather, Ca^{2+} must also directly alter the kinetics of ATP hydrolysis.

During the 1970s, it was generally accepted that regulation of vertebrate skeletal muscle contraction involved a steric blocking of the binding of myosin to actin by the troponin-tropomyosin complex. This model was based on the finding that the relative position of tropomyosin on the actin filament is different in the presence of Ca^{2+} than in the absence of Ca^{2+} (2–4). However, it has been recently demonstrated that the binding of myosin to actin persists in the absence of Ca^{2+} under conditions where troponin-tropomyosin inhibits the actomyosin ATPase activity *in vitro* (5,6) and force production in skinned rabbit fibers (7,8). Apparently, a step in the hydrolysis of nucleotide which occurs after the binding of myosin to actin is inhibited in the absence of Ca^{2+}. There is also evidence that relaxation of vertebrate smooth muscle is due to inhibition of a kinetic step subsequent to the binding of myosin to actin (9) although regulation in this case is mediated by phosphorylation of the myosin rather than by Ca^{2+} binding to troponin-tropomyosin.

In the present study, we have investigated the effect of Ca^{2+} on the actin-activated molluscan HMM[1] ATPase activity and on the binding of molluscan HMM to actin in the presence of ATP. HMM was prepared from myosin isolated from the striated adductor muscle of the scallop

---

*A preliminary report of this work was presented at the 27th Annual Biophysical Meeting, San Diego, February 13, 1983 (1).

¶Supported by Grant AM 32858 from the United States Public Health Service. Established Investigator of the American Heart Association. Present address, Department of Anatomy, Medical College of Pennsylvania, 3200 Henry Avenue, Philadelphia, PA 19129.

||Supported by Grant AM 15963 from the United States Public Health Service and a grant from the Muscular Dystrophy Association.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Aequipecten irradians and from the helically smooth mantle retractor muscles of the squid *Loligo pealii*. As with vertebrate smooth muscle, relaxation of these molluscan muscles is myosin-linked (10). Here, however, rather than controlling phosphorylation of myosin, Ca^{2+} binds directly to a region of the myosin involving both the heavy and light chains (11). Our results suggest that, as with vertebrate skeletal muscle and smooth muscle myosins, regulation of the actin-activated molluscan myosin ATPase activity involves inhibition of a kinetic step which occurs after the binding of the HMM to actin.

EXPERIMENTAL PROCEDURES

Myosin was isolated from fiber bundles obtained from the striated adductor muscle of the scallop *A. irradians* and the mantle retractor muscle of the squid *L. pealii* by standard procedures (12). Myosin was finally dissolved either in 0.6 m NaCl, 10 mm phosphate, 5.0 mm $MgCl_2$, 1.0 mm $CaCl_2$, 0.1 mm EGTA, 3.0 mm NaN_3, pH 7.0, prior to HMM production, or in 0.1 m NaCl, 10 mm phosphate, 1.5 mm $MgCl_2$, 1.5 mm $CaCl_2$, 0.1 mm EDTA, 3.0 mm NaN_3, pH 7.0, prior to Ca·Mg·S-1 production. Heavy meromyosin was prepared by tryptic digestion (400:1 weight ratio) for 1 min at 23 °C as described earlier (13,14). The reaction was terminated by addition of soybean trypsin inhibitor at a 2:1 weight ratio to trypsin. Ca·Mg·S-1 was prepared by papain digestion of myosin using procedures first described by Stafford *et al.* (15). After termination of the digestion by addition of iodoacetic acid to 5 mm, S-l was separated from unreacted iodoacetic acid by further ammonium sulfate fractionation (65% saturation). Actin was prepared both by the procedure of Spudich and Watt (16) as modified by Eisenberg and Kielley (17) and by the procedure of Kendrick-Jones *et al.* (10). A representative sodium dodecyl sulfate-polyacrylamide gel of the proteins used is shown in Fig. 1. The regulatory and essential light chains of *Aequipecten* myosin migrate as a single band. The *Aequipecten* regulatory light chain is clipped during the preparation of Ca·Mg·S-l and is clearly resolved. Preparations of HMM from both *Aequipecten* and *Loligo* myosin had the same content of light chains as the original myosin. The molecular weights used for HMM, S-l, and F-actin were 350,000, 120,000, and 42,000, respectively. HMM and S-l concentrations were determined by the biuret assay (18) with crystalline bovine serum albumin as the standard. The actin concentration was determined spectrophotometrically using an absorption coefficient of 1150 cm^2/g at 280 nm.

Actin-activated ATPase Assays

Actin-activated ATPase assays were measured at 25 °C either by measuring the rate of liberation of $[^{32}P]P_i$ from $[\gamma\text{-}^{32}P]$ATP (20) or by the pH-stat method (11,21). ATPase rates were measured in a solution of the following final composition: 1 mm ATP, 1.8 mm $MgCl_2$, 10 mm imidazole, 1 mm dithiothreitol, and 0.5 mm EGTA or 0.5 mm Ca-EGTA (pCa = 4.8), pH 7.0. In assays based on the determination of $[^{32}P]P_i$, a minimum of four time points were taken to establish the rate. The parameters V_{max} and K_{ATPase} were determined by using a nonlinear least squares routine based on Marguardt's compromise (22). The approximate 95% confidence intervals for values of V_{max} and K_{ATPase} were about 10 and 20%, respectively.

Binding Assays

Binding was measured at 25 °C in the same solution used to measure actin-activated ATPase rates with the addition of 1 mg/ml of bovine serum albumin. The actoHMM was sedimented in an air-driven ultracentrifuge (6) and the free S-l or HMM concentration was determined by an ATPase assay. An NH_4^+-ATPase assay (6) was used for *Aequipecten* S-l and HMM, while an actin-activated ATPase assay with 50 μm actin was used for *Loligo* HMM. Standard curves

[1]The abbreviations used are: HMM, heavy meromyosin; S-l, myosin subfragment 1; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

for *Aequipecten* HMM and *Loligo* HMM are shown in Fig. 2 with the rates expressed as a function of the concentration of catalytic sites. The NH_4^+-ATPase rate of *Aequipecten* HMM increases linearly with the HMM concentration over a wide range of HMM concentrations; only the usual working range is shown. The addition of 8 μm actin had no effect on the NH_4^+-ATPase rate although this is more than twice the actin ever present in these assays from the supernatants of binding experiments. The NH_4^+-ATPase rate of *Aequipecten* S-l (not shown) was also linear with S-l concentration and unaffected by actin although the rate per catalytic site was only 40% of that of *Aequipecten* HMM. In contrast to *Aequipecten* HMM, the NH_4^+-ATPase activity of *Loligo* HMM was low (15% of *Aequipecten* HMM) and this low rate was activated 2-fold by the addition of 8–13 μm actin. This activating effect of actin was observed only if the actin was added to solution prior to the HMM. Because of this unusual effect of actin, the actin-activated Mg^{2+}-ATPase assay was used for *Loligo* HMM as shown in Fig. 2. Binding constants were determined by the nonlinear least squares routine with the constraint that at the limit of infinite actin concentration all of the HMM is bound. The approximate 95% confidence interval for S-l binding experiments was 20% and for all HMM experiments was within 13%.

RESULTS

We began by studying the effect of Ca^{2+} on the actin-activated ATPase rate of molluscan HMM. Fig. 3 shows double reciprocal plots of ATPase activity *versus* actin concentration for both *Aequipecten* striated muscle and *Loligo* smooth muscle HMM. As can be seen, for *Aequipecten* HMM, the maximum actin-activated ATPase rate (V_{max}) is almost 5-fold greater in the presence of Ca^{2+} (14 s^{-1}) than in the absence of Ca^{2+} (3 s^{-1}). Similarly, for *Loligo* HMM, V_{max} in the presence of Ca^{2+} (35 s^{-1}) is seven times greater than V_{max} in the absence of Ca^{2+} (5 s^{-1}). This effect of Ca^{2+} on V_{max} is not observed with *Aequipecten* S-l (data not shown). Both in the presence and absence of Ca^{2+}, the value of V_{max}, for *Aequipecten* S-l is similar to the value of V_{max} for *Aequipecten* HMM in the presence of calcium.

The double reciprocal plots in Fig. 3 also yield values for K_{ATPase}. In the case of *Aequipecten* HMM, K_{ATPase} in the absence of Ca^{2+} (2.4×10^4 m^{-1}) is only slightly weaker than in the presence of Ca^{2+} (3.1×10^4 m^{-1}). Similarly for *Loligo* HMM, the values of K_{ATPase} are 1.1×10^4 m^{-1} and 1.4×10^4 m^{-1} in the absence and presence of Ca^{2+}, respectively. The large effect of Ca^{2+} on V_{max} for molluscan HMM in conjunction with the very small effect of Ca^{2+} on K_{ATPase} suggests that Ca^{2+} regulates the ATPase activity by effecting a kinetic step in the ATPase cycle rather than by effecting the binding of HMM to actin. Before reaching such a conclusion, however, there is another possibility which must be ruled out, especially considering the relatively high ATPase activity which occurs in the absence of Ca^{2+}. This residual ATPase activity could be due to a fraction of protein which is not regulated by Ca^{2+}, in which case, our observation that the value of K_{ATPase} is the same in the presence and absence of Ca^{2+} may not be meaningful. It is still possible that in the absence of Ca^{2+}, the HMM which is Ca^{2+}-sensitive does not bind to actin at all and therefore does not contribute to the ATPase activity.

This possibility can only be ruled out by directly measuring the binding of the molluscan HMM to actin in the presence of ATP. As a control on this binding, we first studied the effect of Ca^{2+} on the association of unregulated *Aequipecten* Ca·Mg·S-1 with pure actin in the presence of ATP. Fig. 4 shows a double reciprocal plot of S-l bound *versus* actin concentration in the presence and absence of Ca^{2+}. As can be seen, the double reciprocal plot is linear and has an ordinate intercept of 1, which shows that all of the S-l binds to actin at infinite actin concentration. Despite the large error in the data at the lower actin concentrations due to a 20% correction for aggregated S-l which sedimented even in the absence of actin, it is clear that Ca^{2+} has no effect on the binding of this unregulated S-l. The best fit to the data in both the

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

presence and absence of Ca^{2+} gives a value for $K_{binding}$ of 4.4×10^4 m^{-1}. This binding constant is similar to the binding constant of rabbit skeletal S-l to actin in the presence of ATP at low ionic strength; it is orders of magnitude weaker than the binding expected in the absence of nucleotide or in the presence of ADP.

The binding of the Ca^{2+}-regulated *Aequipecten* HMM and *Loligo* HMM to rabbit skeletal actin in the presence of ATP is shown in Fig. 5. In both cases, the binding constant of HMM in the presence of Ca^{2+} (2×10^4 m^{-1}) is similar to the binding constant of *Aequipecten* S-l to actin. At the highest actin concentration used (200–250 μm), more than 70% of the HMM bound to actin. In the absence of Ca^{2+}, it is clear that HMM binds to actin but with a lower binding constant (4×10^3 m^{-1}). At the highest actin concentrations, 50–60% of the HMM from both *Aequipecten* and *Loligo* is bound. Therefore, most of the HMM is bound to actin under conditions where the ATPase rate is inhibited. At 200 μm actin, for *Aequipecten*, the amount of HMM bound in the presence of Ca^{2+} is only 40% greater than the amount bound in the absence of Ca^{2+} although the rate in the presence of Ca^{2+} is increased about 5-fold over that in the absence of Ca^{2+}. Similarly, for *Loligo*, a 7-fold increase in ATPase rate in Ca^{2+} is accompanied by only a 40% increase in the amount of HMM bound to actin. Therefore, although the binding of molluscan HMM to actin is weakened in the absence of Ca^{2+}, the major part of the inhibition of the ATPase activity must be due to inhibition of a kinetic step in the ATPase cycle, rather than to an effect on binding.

DISCUSSION

The actin activation of scallop myosin ATPase activity is regulated by direct binding of Ca^{2+} to the myosin molecule. The data presented in this paper suggest that a major part of this Ca^{2+} regulation involves inhibition of a kinetic step which occurs in the ATPase cycle after the binding of the myosin·ATP complex to actin. The key data supporting this view show that, in the absence of Ca^{2+} under conditions where more than 50% of the scallop HMM is bound to actin, the ATPase activity is only about 20% of its value in the presence of Ca^{2+}.

The exact amount of inhibition of the kinetic step which occurs in the absence of Ca^{2+} is difficult to determine because the overall regulation in this system is relatively low; removing Ca^{2+} only inhibits the ATPase activity about 80%. In contrast, myofibrils or myosin preparations are inhibited by more than 95%. As we pointed out under "Results," this relatively low Ca^{2+} sensitivity would occur if a small fraction of the HMM were insensitive to Ca^{2+}. Therefore, the true amount of inhibition of the kinetic step could be greater than the 80% which we observe.

The residual ATPase activity of the Ca^{2+}-insensitive HMM may also obscure the true value of K_{ATPase} which occurs in the absence of Ca^{2+}. Our results show that K_{ATPase} is not affected by Ca^{2+} although $K_{binding}$ becomes 5-fold weaker in the absence of Ca^{2+}. While it is possible that, in the absence of Ca^{2+}, K_{ATPase} is stronger than $K_{binding}$, an alternative explanation is that we are measuring the K_{ATPase} value of only a small fraction of Ca^{2+}-insensitive HMM, and the true effect of Ca^{2+} on K_{ATPase} parallels its effect on $K_{binding}$. In this regard, it is of interest to compare the values of $K_{binding}$, K_{ATPase}, and V_{max} in the molluscan system with the values obtained for these parameters in muscle systems with other regulatory mechanisms. In Table I, values of these parameters obtained with the molluscan S-l and HMM are compared to values of these parameters obtained with the troponin-tropomyosin system of rabbit skeletal muscle and the Ca^{2+}-dependent myosin phosphorylation system of turkey gizzard muscle. In each of the three types of regulatory systems, the removal of Ca^{2+} has a measurable weakening effect on $K_{binding}$, an effect which ranges from about a factor of 2 for rabbit skeletal muscle S-l to about a factor of 5 for the molluscan proteins.[2] Furthermore, in all three systems, Ca^{2+} affects V_{max} more than K_{ATPase} The reduction in V_{max} is the dominant factor in determining the steady

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

state ATPase rate in the absence of Ca^{2+}. Therefore, it appears to be generally true in a number of systems that regulation of the actomyosin interaction occurs mainly through an effect on a kinetic step in the ATPase cycle (perhaps P_i release) rather than through an effect on the binding of myosin·ATP to actin.

This conclusion depends on the assumption that the binding that we are observing between myosin and actin in the presence of ATP is specific. One line of evidence supporting this assumption is the similarity between the values of $K_{binding}$ and K_{ATPase};K_{ATPase} is certainly a specific parameter. A second line of evidence is the data which show that the binding in the presence of ATP has the same ionic strength dependence as the much tighter binding in the presence of 5′-adenylyl imidodiphosphate (25). We also have data showing that the difference in the K_{ATPase} values of the two different light chain-containing species of S-l (26), namely (Al)S-l and (A2)S-1, is correlated with their different values of $K_{binding}$.[3] We believe that this is strong evidence that the weak binding observed in the presence of ATP is indeed specific.

In summary, although the regulatory apparatus of molluscan muscle differs from that of vertebrate skeletal and smooth muscle, our data suggest that, here too, a key aspect of the regulatory process is a change in rate of a kinetic step in the ATPase cycle.

References

1. Chalovich JM, Chantler PD, Szent-Gyorgyi AG, Eisenberg E. Biophys J 1983;41:153a.
2. Haselgrove JC. Cold Spring Harbor Symp Quant Biol 1972;37:341–352.
3. Huxley HE. Cold Spring Harbor Symp Quant Biol 1972;37:361–376.
4. Parry DAD, Squire JM. J Mol Biol 1973;75:33–55. [PubMed: 4713300]
5. Chalovich JM, Chock PB, Eisenberg E. J Biol Chem 1981;256:575–578. [PubMed: 6450206]
6. Chalovich JM, Eisenberg E. J Biol Chem 1982;257:2432–2437. [PubMed: 6460759]
7. Brenner B, Schoenberg M, Chalovich JM, Greene LE, Eisenberg E. Proc Natl Acad Sci U S A 1982;79:7288–7291. [PubMed: 6961408]
8. Schoenberg, M., Brenner, B., Chalovich, J. M., Greene, L. E., and Eisenberg, E. (1984) in *Contractile Mechanisms in Muscle* (Sugi, H., and Pollack, G. H., ed) Vol. 1, pp. 269–284, Plenum Publishing Corp., New York
9. Sellers JR, Eisenberg E, Adelstein RS. J Biol Chem 1982;257:13880–13883. [PubMed: 6128340]
10. Kendrick-Jones J, Lehman W, Szent-Gyorgyi AG. J Mol Biol 1970;54:313–326. [PubMed: 4250215]
11. Chantler PD, Szent-Gyorgyi AG. J Mol Biol 1980;138:473–492. [PubMed: 6251228]
12. Chantler PD, Szent-Gyorgyi AG. Biochemistry 1978;17:5440–5448. [PubMed: 215199]
13. Craig R, Szent-Gyorgyi AG, Beese L, Flicker P, Vibert P, Cohen C. J Mol Biol 1980;140:35–55. [PubMed: 6997502]
14. Chantler PD, Sellers JR, Szent-Gyorgyi AG. Biochemistry 1981;20:210–216. [PubMed: 6110441]
15. Stafford WF, Szentkiralyi EM, Szent-Gyorgyi AG. Biochemistry 1979;18:5273–5280. [PubMed: 160245]
16. Spudich JA, Watt S. J Biol Chem 1971;246:4866–4871. [PubMed: 4254541]
17. Eisenberg E, Kielley WW. J Biol Chem 1974;249:4742–4748. [PubMed: 4276966]
18. Gornall AG, Bardawill CJ, David MM. J Biol Chem 1949;177:751–766. [PubMed: 18110453]
19. Laemmli UK. Nature (Lond) 1970;227:680–685. [PubMed: 5432063]
20. Chock SP, Eisenberg E. J Biol Chem 1979;254:3229–3235. [PubMed: 155064]
21. Eisenberg E, Moos C. J Biol Chem 1967;242:2945–2951. [PubMed: 4226122]

[2]Recent reports by Wagner and Giniger (23) and Wagner and Stone (24) stated that rabbit skeletal muscle HMM shows a much larger Ca^{2+} effect on binding than we have reported for S-l. Since up to the present time we have been unable to duplicate these results (J. M. Chalovich and E. Eisenberg, unpublished data), we must leave the question of this effect open at this time.

[3]J. M. Chalovich, L. A. Stein, L. E. Greene, and E. Eisenberg, unpublished observation.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

22. Bevington, P. R. (1969) *Data Reduction and Error Analysis for the Physical Sciences*, pp. 235–240, McGraw-Hill, New York
23. Wagner PD, Giniger E. J Biol Chem 1981;256:12647–12650. [PubMed: 6458606]
24. Wagner PD, Stone DB. Biochemistry 1982;22:1334–1342. [PubMed: 6404300]
25. Greene LE, Sellers JR, Eisenberg E, Adelstein RS. Biochemistry 1983;22:530–535. [PubMed: 6838810]
26. Weeds AG, Taylor RS. Nature (Lond) 1975;257:54–56. [PubMed: 125854]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

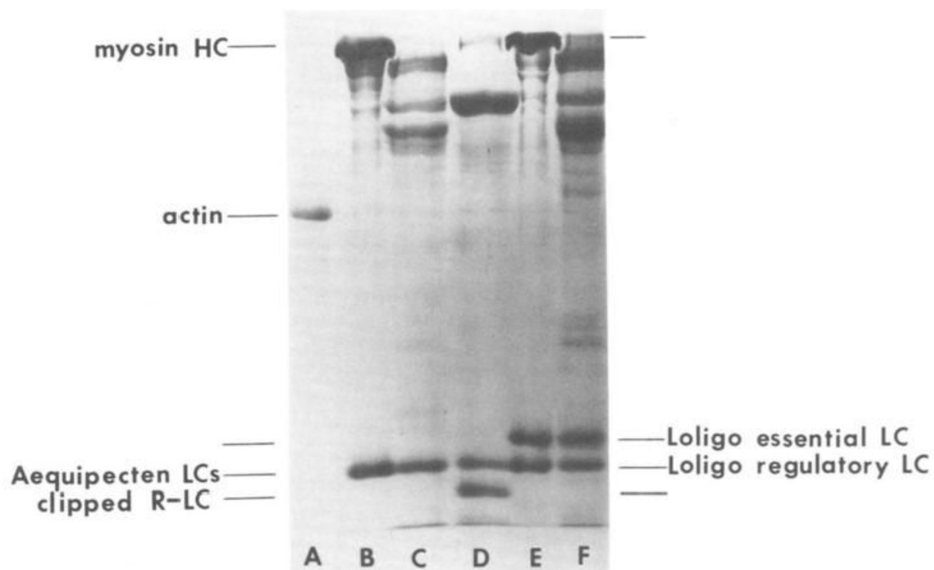

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel representative of the proteins used

Lane A, 2 μg of rabbit skeletal actin; *lane B*, 20 μg of *Aequipecten* myosin; *lane C*, 30 μg of *Aequipecten* HMM; *lane D*, 30 μg of *Aequipecten* Ca·Mg·S-1; *lane E*, 20 μg of *Loligo* myosin; *lane F*, 30 μg of *Loligo* HMM. The gel was 12.5% polyacrylamide and run using the Laemmli (19) discontinuous buffer system. *LC*, light chain; *R-LC*, regulatory light chain.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

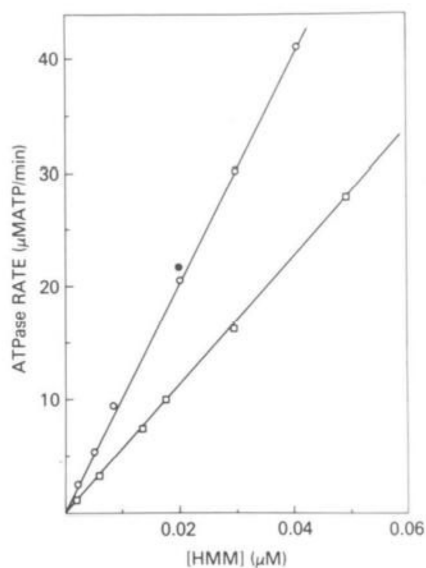

Fig. 2. Standard curves for determining the concentration of *Aequipecten* HMM and *Loligo* HMM in binding studies

The concentration of *Aequipecten* HMM (○) was determined by an NH_4^+-EDTA-ATPase assay containing 5 mM ATP, 0.4 M NH_4C1, 35 mM EDTA, and 25 mM Tris, pH 8.0. The effect of 8 μM actin on the rate is indicated (•). The concentration of *Loligo* HMM (□) was determined by an actin-activated ATPase assay containing 50 μM actin, 1 mM ATP, 1.8 mM $MgCl_2$, 10 mM imidazole, 1 mM dithiothreitol, and 0.5 mM Ca^{2+}-EGTA, pH 7.0.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Fig. 3. Double reciprocal plots of HMM ATPase activity *versus* actin concentration

A, Aequipecten HMM; *B, Loligo* HMM. The | ATPase rates were measured at 25 °C in a solution containing 1 mM ATP, 1.8 mM $MgCl_2$, 10 mM imidazole, 1 mM dithiothreitol, and either 0.5 mM EGTA or 0.5 mM Ca^{2+}-EGTA. All rates are expressed per catalytic site or per "head" and are corrected for the rate at zero actin. For *Aequipecten*, this correction is 0.3 and 0.13 s^{-1} in Ca^{2+} and EGTA, respectively, while for *Loligo*, the correction is 0.19 and 0.07 s^{-1} in Ca^{2+} and EGTA, respectively. Rates are shown in both the presence (• and ▪) and absence (○ and □) of Ca^{2+}.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Fig. 4. Double reciprocal plot of the fraction of *Aequipecten* S-l bound to actin, in the presence of ATP, as a function of the free actin concentration

Binding was measured at 25 °C in a solution containing 1 mM ATP, 1.8 mM $MgCl_2$, 10 mM imidazole, 1 mM dithiothreitol, and either 0.5 mM EGTA or 0.5 mM Ca^{2+}-EGTA; the S-l concentration in all assays was 0.04 μM. Data in both the presence (•) and absence (○) of Ca^{2+} gave an association constant of 4.4×10^4 M^{-1}.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

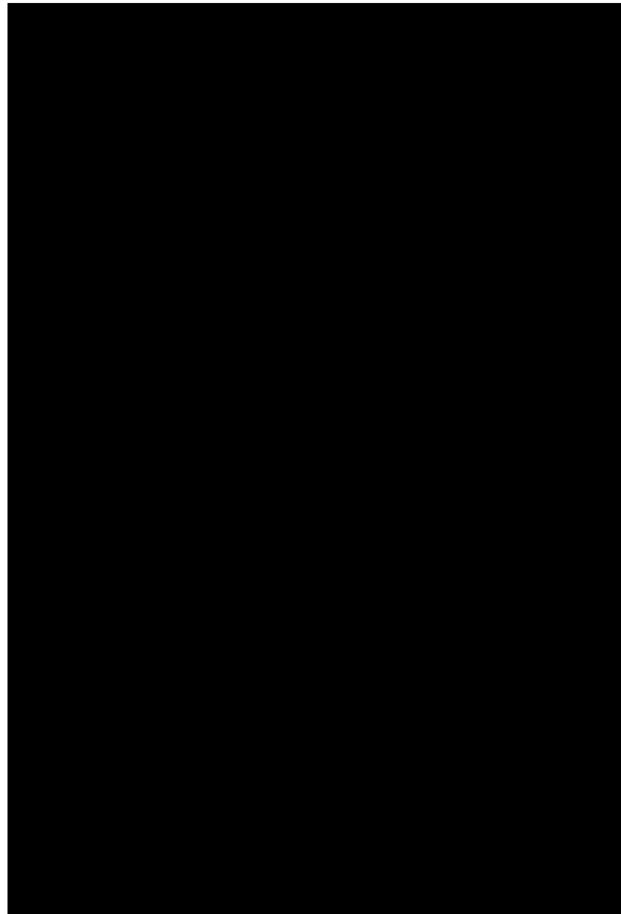

Fig. 5. Double reciprocal plots of the fraction of HMM bound to actin, in the presence of ATP, as a function of the free actin concentration

A, Aequipecten HMM; *B, Loligo* HMM. Binding was measured under the conditions described in the legend to Fig. 3 except that the HMM concentration was 0.03 μM. Binding is shown in both the presence of Ca^{2+} (• and ▪) and in the absence (○ and □) of Ca^{2+}. The binding constants for *Aequipecten* HMM are 2.1×10^4 M^{-1} and 4.1×10^3 M^{-1} in the presence and absence of Ca^{2+}, respectively, and for *Loligo* HMM, are 2.2×10^4 M^{-1} and 4.4×10^3 M^{-1}, respectively.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table I

Comparison of binding and kinetic constants for invertebrate striated, invertebrate smooth, vertebrate striated, and vertebrate smooth muscle regulatory systems

Myosin type	$K_{binding}$		K_{ATPase}		V_{max}[a]	
	Ca^{2+}	EGTA	Ca^{2+}	EGTA	Ca^{2+}	EGTA
	M^{-1}		M^{-1}		s^{-1}	
Aequipecten Ca·Mg·S-l	4.4×10^4	4.4×10^4	3.7×10^4	3.7×10^4	15	15
Aequipecten HMM	2.1×10^4	4.1×10^3	3.1×10^4	2.4×10^4	14	3
Loligo HMM	2.2×10^4	4.4×10^3	1.4×10^4	1.1×10^4	35	5
Rabbit skeletal S-l[b]	2.3×10^4	1.3×10^4	5×10^4	1.7×10^4	22	0.8
Turkey gizzard HMM,[cd]	2.2×10^4	5.9×10^3	4×10^4	9.5×10^3	0.55	0.02

[a] Maximum rate per catalytic site.

[b] Refs. 5 and 6.

[c] Ref. 9.

[d] In this case, Ca^{2+} refers to the phosphorylated form of HMM and EGTA refers to the dephosphorylated form.