Mechanism of Regulation of Cardiac Actin–Myosin Subfragment 1 by Troponin–Tropomyosin

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ABSTRACT: The effect of Ca^{2+} on the interaction of bovine cardiac myosin subfragment 1 (S-1) with actin regulated by cardiac troponin–tropomyosin was evaluated. The ratios of actin to troponin and to tropomyosin were adjusted to optimize the Ca^{2+}-dependent regulation of the steady-state actin-activated magnesium adenosinetriphosphatase (MgATPase) rate of myosin S-1. At 25 °C, pH 6.9, 16 mM ionic strength, the extrapolated values for maximal adenosine 5′-triphosphate (ATP) turnover rate at saturating actin, V_{max}, were 6.5 s^{-1} in the presence of Ca^{2+} and 0.24 s^{-1} in the absence of Ca^{2+}. In contrast to this 27-fold regulation of ATP hydrolysis, there was negligible Ca^{2+}-dependent regulation of cardiac myosin S-1 binding to actin. In the presence of ATP, the dissociation constant of regulated actin and cardiac myosin S-1 was 32 μM in the presence of Ca^{2+} and 40 μM in the presence of [ethylenebis(oxyethylenenitrilo)]tetraacetic acid. These dissociation constants are indistinguishable from the concentrations of actin needed to reach half-saturation of the myosin S-1 MgATPase rates, 37 μM actin in the presence of Ca^{2+} and 53 μM in its absence. Although there may be Ca^{2+}-dependent regulation of cross-bridge binding in the intact heart, the present biochemical studies suggest that cardiac regulation critically involves other parts of the cross-bridge cycle, evidenced here by almost complete Ca^{2+}-mediated control of the myosin S-1 MgATPase rate even when the myosin S-1 is actin-bound.

The contraction of striated muscles, including the heart, is produced by the cyclical interaction of myosin molecules with "regulated" actin filaments, i.e., actin filaments plus troponin–tropomyosin. A rise in intracellular Ca^{2+} concentration triggers muscle shortening by initiating the sliding of the actin and myosin filaments relative to each other. Ca^{2+} regulates this process by reversibly binding to troponin (Ebashi et al., 1969), which is bound to tropomyosin, which in turn binds to seven actin molecules along a filament. Not fully elucidated, however, are what aspects of the actin–myosin interaction are altered by troponin–tropomyosin so as to regulate contraction. It has been proposed that the troponin–tropomyosin complex achieves this regulation by preventing the binding of the myosin cross-bridge to actin in the absence of Ca^{2+}. In accordance with this proposal, binding of Ca^{2+} to troponin shifts the position of tropomyosin on the skeletal thin filament (Huxley, 1972; Haselgrove, 1972; Parry & Squire, 1973; Huxley et al., 1984). However, there are two reasons to question whether regulation of cross-bridge binding determines the contraction and relaxation of striated muscle. First, recent studies using purified proteins from skeletal muscle, as well as intact skeletal muscle fibers, suggest that other effects of troponin–tropomyosin may be more important in regulating contraction than control of cross-bridge binding. At low ionic strength, muscles may remain relaxed even though stiffness measurements (Brenner et al., 1982) and X-ray diffraction (Brenner et al., 1984) demonstrate considerable numbers of attached cross-bridges. Furthermore, Ca^{2+} does not alter the binding of skeletal muscle myosin subfragment 1 (S-1)[1] to the reconstituted thin filament in the presence of ATP (Chalovich et al., 1981; Chalovich et al., 1982; Wagner & Giniger, 1981). There is an effect of Ca^{2+} on the binding of skeletal muscle heavy meromyosin to regulated actin (Wagner & Giniger, 1981; Wagner & Stone, 1983; Wagner, 1984; Chalovich & Eisenberg, 1984), but the magnitude and importance of this effect are presently unclear. Recent data indicate Ca^{2+} may increase heavy meromyosin's affinity for actin by an order of magnitude (Wagner, 1984) or by no more than a factor of 3 (Chalovich & Eisenberg, 1984). On the basis of these results, regulation of cross-bridge binding is not the sole, and is doubtfully the primary, effect of troponin–tropomyosin in skeletal muscle. On the other hand, recent time-resolved X-ray diffraction studies of muscle fiber activation prompted reiteration of the contention that tropomyosin movement facilitates subsequent cross-bridge binding (Huxley et al., 1984). Until now, no experiments relating to this central and controversial area have been reported for cardiac muscle.

A second reason to investigate, specifically with cardiac proteins, the mechanism by which troponin–tropomyosin regulates contraction is that the properties of cardiac proteins are not predictable from the behavior of skeletal proteins. Although tropomyosin and actin are conserved between the two types of muscles (Vandekerckhove & Weber, 1978; Lewis & Smille, 1980), all three troponin subunits, troponin I, troponin T, and troponin C, have significantly different amino acid sequences in cardiac, compared to skeletal, muscle (Grand et al., 1976; van Eerd & Takahashi, 1976; Cooper & Ordahl, 1984). One functional consequence of this is that skeletal muscle troponin C binds 4 mol of Ca^{2+} whereas cardiac troponin C binds only 3 mol (Holroyde et al., 1980) and has only one low-affinity Ca^{2+}-specific site. Furthermore, acto–cardiac myosin S-1 and acto–skeletal myosin S-1 differ 25-fold in affinity for ADP (Siemankowski & White, 1984) and 5-fold in MgATPase rate (Taylor & Weeds, 1976). To better understand the regulation of cardiac contraction, we have investigated the effect of Ca^{2+} on the actin binding affinity and

[1] Abbreviations: S-1, myosin subfragment 1; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; MOPS, 4-morpholinepropanesulfonic acid; SDS, sodium dodecyl sulfate; TPCK, *N*-tosyl-L-phenylalanine chloromethyl ketone; TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; DEAE, diethylaminoethyl; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; ATPase, adenosinetriphosphatase; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; PMSF, phenylmethanesulfonyl fluoride.

actin-activated MgATPase rate of cardiac myosin S-1 in the presence of cardiac troponin–tropomyosin.

Materials and Methods

Protein Preparation. Bovine hearts were trimmed of fat and atria and used either fresh or after freezing in liquid nitrogen and storage at −70 °C. Myosin was prepared as previously described (Tobacman & Adelstein, 1984), with modification as follows. The extracted actomyosin was precipitated as previously reported and resuspended at high ionic strength, and $(NH_4)_2SO_4$ was added to 40% saturation. The resulting precipitate was pelleted and back-extracted to 33% saturation with $(NH_4)_2SO_4$ in the presence of MgATP. This was centrifuged, and the myosin-containing supernatant was dialyzed against 20 mM KCl, 10 mM MOPS (pH 7.0), 1 mM dithiothreitol, 1 mM EGTA, 10 mg/L leupeptin, 10 mg/L TLCK, 5 mg/L TPCK, 5 mg/L pepstatin A, 25 mg/L benzamidine, and 0.01% NaN_3. The resulting myosin filaments were washed with the same buffer 2–3 times and either stored in 50% glycerol or used directly to make myosin S-1.

To prepare myosin S-1, myosin was dialyzed against a buffer containing 10 mM imidazole (pH 7.0), 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, and 0.01% NaN_3. Chymotrypsin was added at a 1:300 ratio to myosin. After a 4-h incubation at 0 °C, digestion was quenched with 0.3 mM PMSF. S-1 was purified from the digest by dialysis to low ionic strength, centrifugation, and DEAE-cellulose chromatography. It was stored in 50% glycerol at −20 °C. In aqueous solution on ice the S-1 lost 5% of its actin-activated MgATPase activity per day. Therefore, it was used within 48 h of dialysis to remove the glycerol.

Cardiac troponin and tropomyosin were prepared by a modification, as follows, of several previously published procedures (Holroyde et al., 1980; Brekke & Greaser, 1976; Stull & Buss, 1977). A total of 400–500 g of ventricle was ground and then homogenized in 2 L of 39 mM boric acid, 0.3 mM sodium borate, 25 mM KCl, 1 mM dithiothreitol, 0.2% Triton X-100, 0.3 mM PMSF, 5 mg/L pepstatin A, 4 mg/L TPCK, 4 mg/L TLCK, 10 mg/L leupeptin, and 0.01% NaN_3 (pH 7.0). The pelleted myofibrils were washed twice with the same buffer but without Triton X-100 and rehomogenized briefly with the blender. Five additional washes were performed, each with about 4 L of buffer that was further modified to include 100 mM KCl. The myofibrils were then washed twice with ethanol and twice with ether and air-dried at room temperature, and the resulting powder was stored at −70 °C. To prepare native tropomyosin, 8 g of powder was extracted for 5 h at 0 °C with 200 mL of 7 mM imidazole (pH 7.0), 1 mM dithiothreitol, 0.01% NaN_3, 25 mg/L benzamidine, 10 mg/L leupeptin, 5 mg/L pepstatin A, 10 mg/L TLCK, 3 mg/L TPCK (buffer A), and 1 M KCl. Troponin was obtained from a 30–45% $(NH_4)_2SO_4$ fraction of this extract. The troponin was dialyzed overnight vs. buffer A, clarified, and then applied to a 30-mL DEAE-cellulose column equilibrated with buffer A. Approximately 30 mg of troponin was recovered by elution with a 0–0.3 M NaCl gradient. The resulting troponin was 95% pure by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970). Troponin T appeared as a doublet, which is due to true heterogeneity and not due to proteolysis (Risnik et al., 1985). It was stored at −70 °C and was stable for about 1 week after thawing. Tropomyosin was obtained from a 45–60% $(NH_4)_2SO_4$ saturation fraction of the same ether powder extract. It was purified by hydroxylapatite (Calbiochem, fast-flow) chromatography (Eisenberg & Kielley, 1974). The α and β forms of tropomyosin were not separated.

Rabbit skeletal muscle actin was prepared as previously described (Eisenberg & Kielley, 1974).

Protein concentrations were determined by using the following molecular weights and extinction coefficients: actin, $M_r = 42000$, $E_{290} = 0.62$ cm^2/mg; tropomyosin, $M_r = 68000$, $E_{280} = 0.33$ cm^2/mg; troponin, $M_r = 85000$, $E_{278} = 0.45$ cm^2/mg; S-1, $M_r = 120000$, $E_{280} = 0.75$ cm^2/mg. In each case, absorbances were corrected for light scattering by subtraction of the absorbance at 320 nm.

Binding Assays. Prior to binding experiments, S-1 was clarified by centrifugation at 50000g for 45 min at 4 °C. S-1 binding to regulated actin was determined by separating free S-1 from actin-bound S-1 by pelleting the actin at 100000g for 20 min in an Airfuge (Beckman) (Chalovich & Eisenberg, 1982). This was followed by an NH_4EDTA ATPase assay of the S-1 remaining in the supernatant. At the highest actin concentration, no more than 33% of the ATP was split during the Airfuge centrifugation itself. In the absence of ATP, 99% of the S-1 cosedimented with the actin. The excess troponin–tropomyosin stabilized the S-1 during the centrifugation and had no effect when added directly to the NH_4EDTA ATPase assays. Furthermore, the small concentration of actin carried over from the supernatants to the NH_4EDTA ATPase assays (<2 μM) had negligible effect on these assays. In control experiments, the NH_4EDTA ATPase rate was proportional to S-1 concentration and was linear with time (at least 1 h) provided no more than 15% of the ATP was hydrolyzed. At 25 °C in the presence of 25 mM Tris-HCl (pH 8), 0.4 M NH_4Cl, 35 mM EDTA, and 5 mM ATP, the k_{cat} for ATP hydrolysis was 4.4 s^{-1}.

ATPase Assays. S-1 MgATPase rates were determined at 25 °C in the presence of 6 mM imidazole (pH 6.9), 1 mM ATP, 1 mM dithiothreitol, 3.5 mM $MgCl_2$ (except Figure 1A), and either 1 mM EGTA or 0.4 mM $CaCl_2$, for an ionic strength of 16 mM. ATPase rates were constant for at least 15 min and were determined as previously described (Pollard & Korn, 1973). The rates were proportional to S-1 concentration at the low S-1:actin ratios used.

Results

Thin Filament Reconstitution. The first task was to define conditions for reconstitution of an actin filament optimally regulated by cardiac troponin–tropomyosin. To promote acto–S-1 binding, the ionic strength and $MgCl_2$ concentrations were kept as low as possible while still maintaining saturation of the actin with tropomyosin. The known inhibitory effect of tropomyosin on the actin-activated MgATPase rate of myosin S-1 was used to monitor for saturation of actin with tropomyosin. In the presence of 1 mM $MgCl_2$ in excess of ATP, tropomyosin had no effect on the ATPase rate (data not shown). With 2 mM excess $MgCl_2$ tropomyosin inhibited the acto–S-1 ATPase rate in a concentration-dependent manner (Figure 1A) that was also dependent upon the presence of $CaCl_2$. In the presence of 1 mM EGTA, approximately 3 times as much tropomyosin was required as the amount needed to achieve the same level of inhibition in the presence of 0.4 mM $CaCl_2$. Therefore, 2 mM $MgCl_2$ in excess of ATP, which was used successfully with rabbit skeletal native tropomyosin (Chalovich et al., 1981), was not a satisfactory condition for these experiments. This does not imply that the bovine cardiac proteins differ greatly from the rabbit skeletal proteins. Rather, it confirms that small changes in ionic conditions produce large changes in tropomyosin behavior (Yang et al., 1979).

Tropomyosin–actin binding and tropomyosin-induced ATPase inhibition are closely dependent upon the concentration

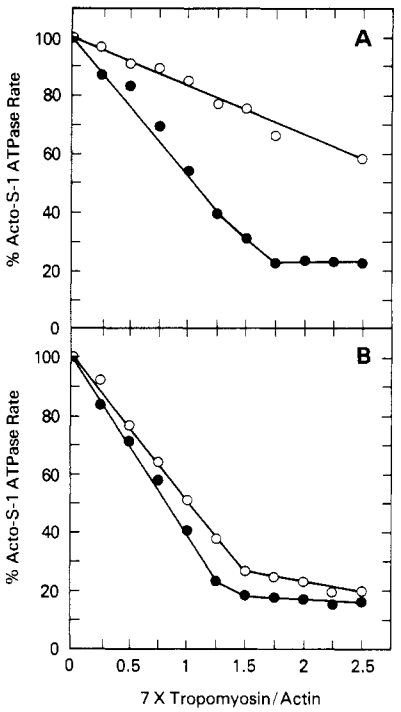

FIGURE 1: Effect of tropomyosin on the cardiac S-1 actin activated MgATPase rate. At 25 °C, 0–5 μM tropomyosin was added to 14 μM F-actin and 0.2 μM S-1 in the presence of 7 mM imidazole (pH 7.0), 1 mM ATP, 1 mM dithiothreitol, and either 1 mM EGTA (○) or 0.4 mM $CaCl_2$ (●). Panel A: 2 mM $MgCl_2$ in excess of ATP. Panel B: 2.5 mM $MgCl_2$ in excess of ATP. The MgATPase rates were determined as described under Materials and Methods and are expressed as a percentage of the rate in the absence of tropomyosin. In the absence of tropomyosin, the acto–S-1 ATPase rates were 1.9 and 1.6 s^{-1} in the absence and presence of Ca^{2+}, respectively, for panel A. For panel B, the corresponding rates were 1.3 s^{-1} in the absence of Ca^{2+} and 1.1 s^{-1} in the presence of Ca^{2+}.

of Mg^{2+} (Yang et al., 1979). The apparently large effect of $CaCl_2$ shown in Figure 1A appears to be due to the ability of Ca^{2+} to replace Mg^{2+} in promoting the tropomyosin–actin interaction, as seen by the results in Figure 1B. When the $MgCl_2$ concentration was raised from 2 to 2.5 mM in excess of ATP, $CaCl_2$ was no longer needed to promote the inhibition by tropomyosin. Furthermore, the tropomyosin titration in the presence of 2 mM excess $MgCl_2$, plus 0.4 mM $CaCl_2$, is very similar to that observed in the presence of 2.5 mM excess $MgCl_2$ and no $CaCl_2$, suggesting that Ca^{2+} and Mg^{2+} have roughly equal effects. At saturating tropomyosin, the MgATPase rate was inhibited by a factor of about 5. On the basis of these results, the tropomyosin:actin ratio was maintained at 2.25:7 in subsequent experiments and the $MgCl_2$ concentration kept at 2.5 mM in excess of ATP. Under these conditions, actin could be expected to be saturated with tropomyosin, regardless of whether Ca^{2+} was present or not.

A parallel approach was used to determine how much troponin was needed for the reconstituted regulated actin filament. In the presence of tropomyosin, troponin titrations were performed in the presence and absence of Ca^{2+} and the acto–S-1 MgATPase rates determined (Figure 2). Addition of troponin in the presence of Ca^{2+} increased the ATPase rate by an order of magnitude with a peak value at 1.5–1.75:7 troponin:actin. The shape of this curve was unchanged by quadrupling the tropomyosin concentration (data not shown). In the absence of Ca^{2+} and the presence of tropomyosin, troponin reduced the MgATPase rate by a factor of 5, with the maximum inhibition at 2 mol of troponin/7 mol of actin (Figure 2). Since tropomyosin alone decreased the rate by a factor of 5, the overall effect of troponin–tropomyosin was

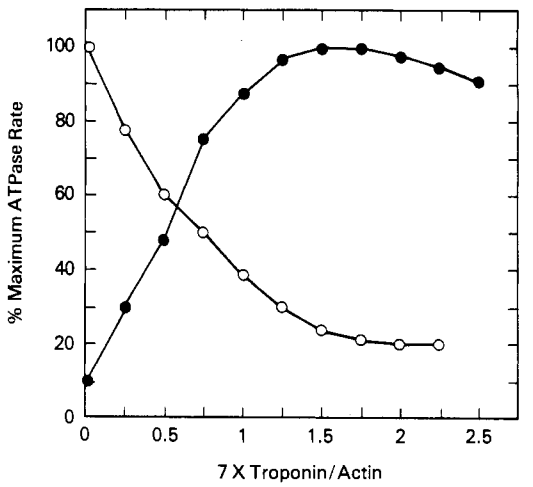

FIGURE 2: Effect of troponin on the cardiac myosin S-1 actin activated MgATPase rate in the presence of tropomyosin. Varying concentrations of cardiac troponin, between 0 and 5 μM, were added to 14 μM F-actin, 4.5 μM tropomyosin, 1 mM ATP, 3.5 mM $MgCl_2$, 1 mM dithiothreitol, 7 mM imidazole (pH 7.0), and either 1 mM EGTA (○) or 0.4 mM $CaCl_2$ (●). The rates are expressed as percentages either of the rate in the absence of troponin (EGTA) or of the rate at saturating troponin ($CaCl_2$). The myosin S-1 concentrations were 0.2 μM when $CaCl_2$ was added and 0.25 μM when EGTA was added. In the absence of troponin the absolute values for the ATPase rates were 0.3 s^{-1} in the presence of 1 mM EGTA and 0.2 s^{-1} in the presence of 0.4 mM $CaCl_2$. Note that the MgATPase rates in the presence of EGTA are normalized to 100% = 0.3 s^{-1}, whereas the MgATPase rates in the presence of $CaCl_2$ are normalized to 100% = 1.9 s^{-1}.

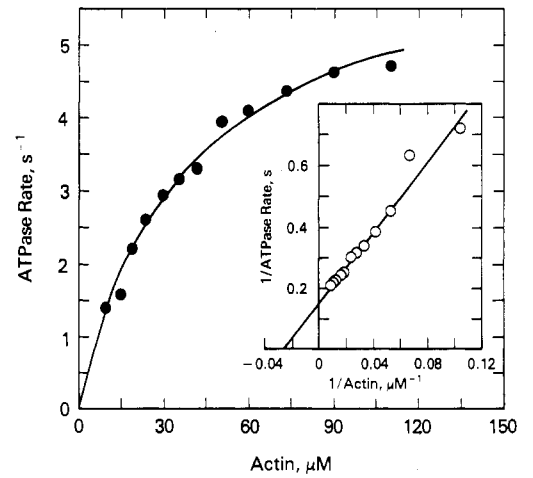

FIGURE 3: Effect of regulated actin concentration on the MgATPase rate of cardiac myosin S-1 in the presence of Ca^{2+}. Concentrated solutions of F-actin, tropomyosin, and troponin were mixed in a molar ratio of 7:2.25:2. Varying concentrations of this regulated actin were mixed with 0.2 μM myosin S-1 and 0.4 mM $CaCl_2$ under the conditions used in Figure 2. The resulting MgATPase rates (●) were fit by nonlinear regression to the Michaelis–Menten equation (—). Inset: Lineweaver–Burk plot of the same data (○) and theoretical curve.

approximately 25-fold in the absence of Ca^{2+}. Optimal actin-activated MgATPase regulation by Ca^{2+} occurred at 2 mol of troponin/7 mol of actin.

Effect of Ca^{2+} on ATPase Rate Using Regulated Actin. All of the preceding experiments were performed at a single actin concentration, 14 μM. Similar troponin and tropomyosin titrations were not performed at other actin concentrations. Instead, the optimal actin:troponin:tropomyosin ratio determined at a comparatively low actin concentration (Figures 1 and 2) was maintained as the actin concentration was increased. The exact value of the ratio varied with different protein preparations. Approximately 2 mol of troponin and

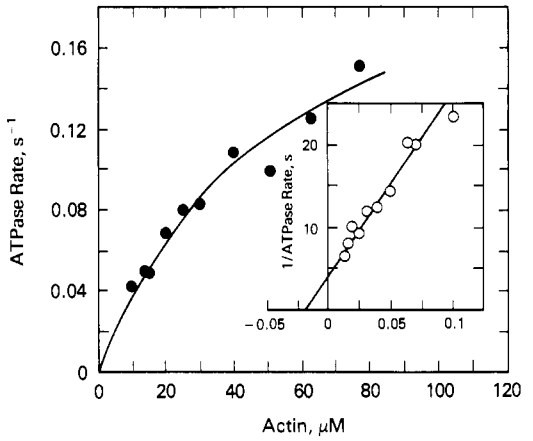

FIGURE 4: Effect of regulated actin concentration on the MgATPase rate of cardiac myosin S-1 in the absence of Ca^{2+}. Varying concentrations of regulated actin were mixed with 0.3 μM myosin S-1 as described in Figure 3, except 1 mM EGTA was substituted for $CaCl_2$. The MgATPase rate of myosin S-1 alone (0.013 ± 0.002 s^{-1}) was subtracted. (●) ATPase rates. Data analysis is as in Figure 3. Inset: Lineweaver–Burk plot of the same data (○) and theoretical curve.

2 mol of tropomyosin were usually optimal per 7 mol of actin.

Figure 3 shows the MgATPase rate of cardiac S-1 in the presence of Ca^{2+} as a function of the regulated actin concentration. The ATPase rate increased in a hyperbolic manner with increasing actin concentrations, as shown by the hyperbolic direct plot and linear double-reciprocal plot (inset). The extrapolated value of V_{max} (6.5 s^{-1}) is similar to the value previously reported for bovine cardiac myosin S-1 in the absence of regulatory proteins (Siemankowski & White, 1984; Taylor & Weeds, 1976; Smith & Cusanovich, 1984). The K_{app} of myosin S-1 for actin was 37 μM.

Figure 4 shows that the effect of Ca^{2+} on the regulated system was primarily on V_{max}, which was reduced from 6.5 to 0.24 s^{-1} by addition of EGTA instead of $CaCl_2$. Approximately 30-fold regulation of the MgATPase rate of S-1 by Ca^{2+} occurred over the entire range of actin concentrations studied. Again, the data exhibited a hyperbolic dependence upon actin concentration. Note, however, in the presence of EGTA, the K_{app} for actin was 53 μM, close to the value of 37 μM measured in the presence of $CaCl_2$.

S-1 Binding to Regulated Actin. Since the regulation has little effect on the K_{app} of the ATPase rate, one would predict little effect of Ca^{2+} on the directly measured binding of myosin S-1 to regulated actin in the presence of ATP. Figure 5 shows that this is the case. In both the presence and absence of Ca^{2+} the binding data are well-described by simple binding curves extrapolating to 100% bound S-1 at infinite actin. The dissociation constant in the presence of Ca^{2+}, 32 μM, is almost indistinguishable from that in the absence of Ca^{2+}, 40 μM. Therefore, under conditions where removal of the Ca^{2+} inhibits the acto–S-1 MgATPase rate by 96%, there is negligible alteration in the affinity of cardiac myosin S-1 for regulated actin.

Discussion

The effect of Ca^{2+} on this regulatory system is to increase by a factor of 27 the maximum rate of ATP hydrolysis, which is required for muscle contraction, but to strengthen the binding of myosin S-1 to actin by a factor of only 1.25. Thus, by these techniques, cardiac troponin–tropomyosin neither sterically nor allosterically blocks the binding of isolated cardiac myosin heads to the thin filament. Rather, marked inhibition of the ATPase cycle occurs even when the myosin

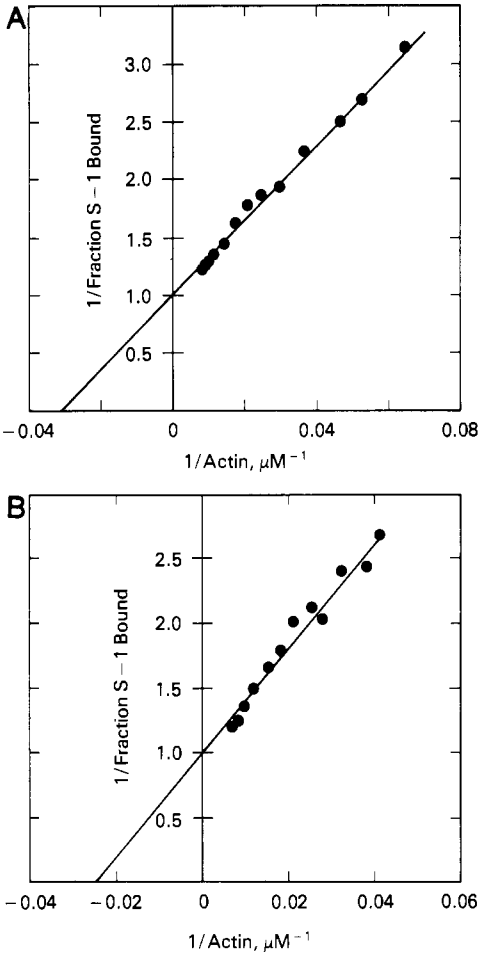

FIGURE 5: Binding of cardiac myosin S-1 to regulated actin in the presence and absence of Ca^{2+}. Precentrifuged myosin S-1 (final concentration, 0.15 μM) and regulated actin were mixed in the presence of 0.4 mM $CaCl_2$ (panel A) or 1 mM EGTA (panel B) under the same conditions used for the MgATPase determinations. Binding was determined by a sedimentation assay at room temperature as described under Materials and Methods. 1/(actin concentration) is plotted vs. 1/(fraction of S-1 bound to actin). The binding constant was determined by nonlinear regression analysis of the data before it was converted to reciprocal form.

S-1 is actin-bound. At 55 μM actin, for example, EGTA inhibits the acto–S-1 MgATPase rate 97%, but the fraction of myosin S-1 that is bound to actin decreases only slightly, from 62% to 56%.

For cardiac as opposed to skeletal muscle, these experiments also provide the first comparison in the presence of ATP between the dissociation constant of the actin–cardiac myosin S-1 complex and K_{app}, the apparent dissociation constant derived from the plot of MgATPase rate vs. actin concentration. The values of the two constants were indistinguishable in the presence of Ca^{2+}. (In the absence of Ca^{2+}, this analysis would be meaningless, because the MgATPase rates presumably involve a small fraction of the actin that is not properly regulated.) It remains to be seen if the K_{app} and the K_D of cardiac myosin S-1 and actin diverge under other conditions, with consequent implications for the mechanism of ATP hydrolysis.

Since regulation of the ATPase rate does not occur by inhibition of binding, some other step or steps in the kinetic cycle must be regulated by troponin–tropomyosin. Direct experimentation will be required to determine the exact mechanism of regulation, but some constraints are already apparent. Specifically, nucleotide binding and hydrolysis cannot be rate-limiting because they occur so rapidly in the

absence of actin (Flamig & Cusanovich, 1983) that inhibition on acto-S-1 would be bypassed by free myosin S-1. A much more likely site of myosin regulation by cardiac troponin–tropomyosin is phosphate release, which is also the suggested mechanism for rabbit skeletal muscle (Chalovich & Eisenberg, 1982). Recent studies of scallop heavy meromyosin (Wells & Bagshaw, 1984, 1985) and smooth muscle heavy meromyoson (Sellers et al., 1982; Sellers, 1985) indicate these molecules also are regulated at the level of phosphate release (and not actin binding). Therefore, regulation of this specific part of the cross-bridge cycle may be a common feature of the otherwise distinct myosin-linked and actin-linked regulatory systems.

The almost complete absence of Ca^{2+}-dependent regulation of myosin cross-bridge binding to the actin troponin–tropomyosin complex applies only to myosin subfragment 1. The affinity for actin of cardiac heavy meromyosin, myosin, or myosin heads within the intact fiber may be subject to modulation by Ca^{2+}. In the skeletal muscle system maximum Ca^{2+} sensitivity of binding requires both a two-headed myosin and the presence of both pairs of light chains (Wagner & Giniger, 1981; Wagner & Stone, 1983; Wagner, 1984). Chymotryptic cardiac myosin S-1 used in this report contains only one light chain and, of course, is single-headed. It would be worthwhile to repeat the present study using cardiac heavy meromyosin. Obtaining this molecule with intact light chains will not be straightforward, however, because cardiac myosin as isolated usually lacks a full complement of light chain 2 (L. Tobacman, unpublished data). Furthermore, digestion to produce heavy meromyosin rapidly degrades whatever light chain 2 is present. Further experiments will be required to determine if some Ca^{2+}-mediated control of cardiac myosin binding to actin indeed occurs. Nevertheless, the profound, Ca^{2+}-mediated regulation of the MgATPase rate of cardiac myosin S-1 when it is actin-bound suggests that the primary mechanism of action of cardiac troponin–tropomyosin is on other parts of the cross-bridge cycle.

Registry No. ATPase, 9000-83-3; Ca, 7440-70-2.

References

Brekke, C. J., & Greaser, M. L. (1976) *J. Biol. Chem. 251*, 866–871.

Brenner, B., Schoenberg, M., Chalovich, J. M., Greene, L. E., & Eisenberg, E. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7288–7291.

Brenner, B., Yu, L. C., & Podolsky, R. J. (1984) *Biophys. J. 46*, 299–306.

Chalovich, J. M., & Eisenberg, E. (1982) *J. Biol. Chem. 257*, 2432–2437.

Chalovich, J. M., & Eisenberg, E. (1984) *Biophys. J. 45*, 221a.

Chalovich, J. M., Chock, P. B., & Eisenberg, E. (1981) *J. Biol. Chem. 256*, 575–578.

Cooper, T. A., & Ordahl, C. P. (1984) *Science (Washington, D.C.) 226*, 979–982.

Ebashi, S., Endo, M., & Ohtsuki, I. (1969) *Q. Rev. Biophys. 2*, 351–384.

Eisenberg, E., & Kielley, W. W. (1974) *J. Biol. Chem. 249*, 4742–4748.

Flamig, D. P., & Cusanovich, M. A. (1983) *J. Biol. Chem. 258*, 977–983.

Grand, R. J. A., Wilkinson, J. M., & Mole, L. E. (1976) *Biochem. J. 159*, 633–641.

Haselgrove, J. C. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37*, 341–352.

Holroyde, M. J., Robertson, S. P., Johnson, J. D., Solaro, R. J., & Potter, J. D. (1980) *J. Biol. Chem. 255*, 11688–11693.

Huxley, H. E. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37*, 361–376.

Huxley, H. E., Kress, M., & Faruqi, A. R. (1984) *Biophys. J. 45*, 10a.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lewis, W. G., & Smille, L. B. (1980) *J. Biol. Chem. 255*, 6854–6859.

Parry, D. A. D., & Squire, J. M. (1973) *J. Mol. Biol. 75*, 33–55.

Pollard, T. D., & Korn, E. D. (1973) *J. Biol. Chem. 248*, 4682–4690.

Risnik, V. V., Verin, A. D., & Gusev, N. B. (1985) *Biochem. J. 225*, 549–552.

Seilers, J. R. (1985) *Biophys. J. 47*, 302a.

Sellers, J. R., Eisenberg, E., & Adelstein, R. A. (1982) *J. Biol. Chem. 257*, 13880–13883.

Siemankowski, R. F., & White, H. D. (1984) *J. Biol. Chem. 259*, 5045–5053.

Smith, S. J., & Cusanovich, M. A. (1984) *J. Biol. Chem. 259*, 9365–9368.

Stull, J. T., & Buss, J. E. (1977) *J. Biol. Chem. 252*, 851–857.

Taylor, R. S., & Weeds, A. G. (1976) *Biochem. J. 159*, 301–315.

Tobacman, L. S., & Adelstein, R. S. (1984) *J. Biol. Chem. 259*, 11226–11230.

Vandekerckhove, J., & Weber, K. (1978) *J. Mol. Biol. 126*, 783–802.

van Eerd, J. P., & Takahashi, K. (1976) *Biochemistry 15*, 1171–1180.

Wagner, P. D. (1984) *Biochemistry 23*, 5950–5956.

Wagner, P. D., & Giniger, E. (1981) *J. Biol. Chem. 256*, 12647–12650.

Wagner, P. D., & Stone, D. B. (1983) *Biochemistry 22*, 1334–1342.

Wells, C., & Bagshaw, C. R. (1984) *FEBS Lett. 168*, 260–264.

Wells, C., & Bagshaw, C. R. (1985) *Nature (London) 213*, 696–697.

Yang, Y.-Z., Korn, E. D., & Eisenberg, E. (1979) *J. Biol. Chem. 254*, 2084–2088.