The Regulatory Effects of Tropomyosin and Troponin-I on the Interaction of Myosin Loop Regions with F-actin*

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The N terminus of skeletal myosin light chain 1 and the cardiomyopathy loop of human cardiac myosin have been shown previously to bind to actin in the presence and absence of tropomyosin (Patchell, V. B., Gallon, C. E., Hodgkin, M. A., Fattoum, A., Perry, S. V., and Levine, B. A. (2002) Eur. J. Biochem. 269, 5088–5100). We have extended this work and have shown that segments corresponding to other regions of human cardiac β-myosin, presumed to be sites of interaction with F-actin (residues 554–584, 622–646, and 633–660), likewise bind independently to actin under similar conditions. The binding to F-actin of a peptide spanning the minimal inhibitory segment of human cardiac troponin I (residues 134–147) resulted in the dissociation from F-actin of all the myosin peptides bound to it either individually or in combination. Troponin C neutralized the effect of the inhibitory peptide on the binding of the myosin peptides to F-actin. We conclude that the binding of the inhibitory region of troponin I to actin, which occurs during relaxation in muscle when the calcium concentration is low, imposes conformational changes that are propagated to different locations on the surface of actin. We suggest that the role of tropomyosin is to facilitate the transmission of structural changes along the F-actin filament so that the monomers within a structural unit are able to interact with myosin.

One of the outstanding problems of muscle is to define in protein structural terms how actin interacts with myosin. This interaction enables the conversion of myosin from an enzyme that in the resting muscle hydrolyzes its substrate, MgATP, at a very low rate to one with the high rate associated with contraction. These features are intrinsic to the regulatory process. Crystals of actomyosin or the myosin head (S1) complexed with actin are not yet available to permit the determination of the high resolution structure of the myosin motor domain bound to actin. Nevertheless, modeling of the interaction using the known structures of actin and myosin S1, mutation studies, and a variety of experimental approaches have highlighted a number of regions on the myosin motor domain that may be involved in the interaction (1–15). It was concluded from the original structural studies on chicken skeletal myosin S1 that the interface with actin is likely to involve at least three exposed segments common to members of the myosin family (1, 2). Implicated in making contact with a single actin subunit were the regions comprising chicken skeletal myosin residues 403–416 (the so-called “cardiomyopathy” loop), the helix-turn-helix region, residues 529–558, and residues 626–647 (loop 2, at the protease-sensitive junction between the 50- and 20-kDa domains). Another surface loop, residues 567–578, has been modeled as interacting with an adjacent actin monomer (1, 2, 4).

The short cardiomyopathy loop of myosin is resolved as a well defined surface protrusion that is modeled as docking onto actin upon strong binding of myosin (4, 7, 8). This loop region is clearly significant for actomyosin interaction because residue deletion results in the loss of the strong binding of actin to myosin (3). The residue change Arg → Gln in this loop of the β-chain of human cardiac myosin is associated with familial hypertrophic cardiomyopathy (16, 17) and has been reported to result in altered kinetic properties of the actin-activated myosin ATPase (18, 19).

We here use a peptide mapping approach to probe the sequence determinants of the interaction of surface loops of myosin with F-actin. Peptides comprising the cardiomyopathy loop of human cardiac β-myosin (hcβM (398–414)) and the N terminus of striated muscle myosin light chain 1 interact with F-actin (20–23) and are able to modulate the actin-activated MgATPase of myosin (23, 24). The affinity of these peptides for F-actin was observed to increase slightly in the presence of tropomyosin, whereas their interaction was prevented by the binding of the inhibitory region of troponin I to actin (22). We have extended these observations to peptides representing the other presumptive binding regions of myosin, and we have investigated their individual actin-binding properties. Our results indicate that three loops on the surface of the myosin molecule, human cardiac β-myosin residues 398–414, 554–584, and 623–660, interact with distinct sites on the actin molecule. These interactions provided the opportunity to explore the regulatory effects of tropomyosin and troponin-I. In the case of each myosin peptide, the nature of the residues making contact with F-actin was unaltered by tropomyosin. Interaction of the inhibitory region of human cardiac troponin-I with F-actin in the absence or presence of tropomyosin displaced all three myosin contacts. Some aspects of this work have been briefly reported in abstract form elsewhere (25).

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The abbreviations used are: hcβM, human cardiac β-myosin; hcTnI, human cardiac troponin-I; rTnI, rabbit cardiac troponin-I; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; IAEDANS,(((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid.
**Peptides**—The peptides of human cardiac β-myosin and human cardiac troponin-I (hcTnI) used in this study (Table I) were synthesized with acetylated N- and amidated C-terminal residues (Alta Biosciences, Birmingham University) and purified as described previously (22). The composition and purity of all peptides were confirmed by mass spectral and NMR analyses. Amino acid analysis was used to determine the concentration of each of the stock peptide solutions used for the fluorescence and NMR studies.

**Muscle Proteins**—Actin was isolated freeze-dried from rabbit skeletal muscle using the method of Spudich and Watt (26). Measurement of the absorbance of G-actin at 290 nm was used to determine the concentration of the G-actin in the solution. The actin concentration was calculated using the empirical observation that absorbance at 290 nm of a 1 mg/ml solution is equal to 0.63 units (26). F-actin was prepared in a solution containing 2 mM MgCl₂ and 50 mM KCl as described previously (22) and dialyzed overnight against several changes of 5 mM phosphate buffer, pH 7.0, in H₂O for fluorescence measurements or 25 mM Tris-HCl, pH 7.4, buffered with 25 mM Tris-HCl for the NMR titration studies.

The F-actin-tropomyosin complex was made by adding G-actin to a stock solution of 1 mg/ml rabbit skeletal tropomyosin in 50 mM Tris-HCl, pH 7.6, 100 mM KCl, to give a final concentration of 2.5 mg/ml actin, 0.5 mg/ml tropomyosin. The complex was dialyzed into several changes of 5 mM phosphate buffer, pH 7.0, in H₂O for fluorescence measurements or H₂O for the NMR titration studies.

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G-actin labeled at Cys-374 with IAE-DANS was prepared according to the method of Miki et al. (27) as reported previously (22). The extent of labeling was normally 0.8–0.9 mol/mol G-actin. This preparation of labeled G-actin was polymerized using 50 mM KCl, 2 mM MgCl₂, and aliquots were stored frozen for subsequent use.

**Fluorescence Measurements**—All fluorescence emission spectra were obtained using a PerkinElmer Life Sciences LS580B fluorescence spectrometer. Emission fluorescence intensity values (IAEDANS excitation at 340 nm) were corrected for solvent emission fluorescence and any contribution from unreacted donors. Emission fluorescence intensity values (IAEDANS excitation at 340 nm) were corrected for solvent emission fluorescence and any contribution from unreacted donors.

**NMR Studies**—All spectra were obtained using a Bruker 500-MHz DRX spectrometer equipped with a cryogenically cooled 5-mm triple-resonance cryoprobe. The residue-specific assignment of peptide resonances was carried out using standard protocols following the acquisition of total correlation spectroscopy and NOESY spectra using samples in 90% H₂O solution. Titration of the myosin peptides (concentration range used 40–300 μM) with F-actin was typically carried out by sequential addition of small (1–5 μl) volumes of the peptide solution.

The fractional change in fluorescence observed was assumed to be directly proportional to the fraction of the complex formed. The dissociation constant was derived using a nonlinear regression procedure fitting the fluorescence data obtained in separate titrations to a 1:1 binding curve.

**RESULTS**

The identification of contact residues on a small peptide interacting with a much larger protein can be undertaken by the use of NMR spectroscopy to detect the broadening of the signals from residues of the peptide at the molecular interface. This broadening is a consequence of the much faster relaxation rate in the bound state and the dynamic process of exchange between free and bound states of the target peptide (28–30). The interface with F-actin can be characterized by the progressive perturbation of specific peptide resonances during titration that directly reflects the population of the free and bound states of each peptide. The use of a cryogenic probe for such experiments permits titrations to be carried out using concentrations in the low micromolar range thereby reducing the probability of nonspecific interaction. Titrations monitored by both NMR and fluorescence have been used to confirm that binding is saturable and of defined stoichiometry. The directness of this experimental approach has allowed us to investigate the F-actin and actin-tropomyosin binding characteristics of different regions of the human β-cardiac myosin head and the modulation of these actin associations by the inhibitory region of human cardiac troponin-I.

**Multiple Contacts of Residues 398–414 of Human Cardiac β-Myosin with F-Actin Involve a Preference for a Looped Conformation**—In view of the importance of the cardiomyopathy loop region in the activation of the myosin ATPase (e.g. Refs. 3 and 7) and the ability of the cardiac myosin peptide hcM-(398–414) (Table I) to competitively inhibit actin-activated myosin ATPase (23), we have defined the nature of this interaction in detail by carrying out titrations with F-actin by using a range of concentrations of the peptide (40–300 μM). As shown in Fig. 1, the presence of sub-stoichiometric concentrations of F-actin resulted in extensive signal broadening with spectral line widths dominated by the bound ligand cross-relaxation that results from the substantial increase in correlation time of the peptide bound to F-actin. The progressive line broadening observed with increasing concentration of F-actin (Fig. 1) reports on the relative population of free and bound states of the peptide, and the interaction interface can be robustly defined by inspection of the progressively broadened resonances. Notably affected at low ratios of complexed to free peptide are the side chain signals of Gly-407 and Thr-412 increasing in the presence of higher concentrations of F-actin (Fig. 1). The observation of line width perturbation for all of these uniquely resolved peptide signals in the presence or absence of tropomyosin indicates that the hcM-(398–414) lies close to the actin surface along most of its length.

Of significance with regard to the extensive nature of the association between hcM-(398–414) and F-actin was the NOE-based evidence that the sequence Val-406 to Asn-408 necessarily adopt a β-turn conformation. Clearly resolved in the amide region of the proton NOESY spectrum is the (i → i + 2) NOE cross-peak that identifies the time-average proximity of the side chain methyl group(s) of Val-406 and the backbone NH of Asn-408 (Fig. 2). The relatively strong intensity of this (i → i + 2) cross-peak compared with the variety of interresidue (i → i + 1) NOE detected suggests the presence of a significant population of the turn conformation of the central region of the peptide. This conclusion is supported by the further evidence provided by the observation of peptide residue-specific NOE interactions with F-actin in the presence of tropomyosin suggesting the presence of complexed to free peptide are the side chain signals of Gly-407 and Thr-412 increasing in the presence of higher concentrations of F-actin (Fig. 1). The observation of line width perturbation for all of these uniquely resolved peptide signals in the presence or absence of tropomyosin indicates that the hcM-(398–414) lies close to the actin surface along most of its length.

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ther observation of the nonequivalence of the resonances of the two Cα protons of Gly-407 (Fig. 2) that is indicative of a preferred rotamer population (30).

Because the sequences flanking the central turn region of hcβM-(398–414) contain charged residues, we proceeded to investigate the sensitivity of the interaction of F-actin with the peptide to ionic strength. These spectral studies showed that the addition of salt did not significantly alter the structured peptide to ionic strength. These spectral studies showed that the interaction is salt concentration-dependent, predisposition of the central region of the peptide, and we observed that the interaction is salt concentration-dependent, with the actin-induced perturbation of the different residues along the peptide chain virtually undetectable in 50 mM KCl.

Progressive broadening is observed during titration (A–C) for signals of CaH backbone and side chain protons. Signals unaffected by the presence of F-actin are derived from buffer impurities and are indicated by a star.

Experimental conditions are as follows: T = 293 K, 25 mM Tris-2HCl in 2H2O solution, pH 7.4, A, 80 µM βheM-(398–414), in the presence of 5 mM deuterated dithiothreitol. B, as A but in the presence of 15 µM F-actin. C, as A but in the presence of 30 µM F-actin. Resonances from residues in different regions of the sequence of βheM-(398–414) are labeled. Progressive broadening is observed during titration (A–C) for signals of CaH backbone and side chain protons. Signals unaffected by the presence of F-actin are derived from buffer impurities and are indicated by a star.

Experimental conditions are as follows: T = 293 K, 25 mM Tris-2HCl in 2H2O solution, pH 7.4, A, 80 µM βheM-(398–414) in the presence of F-actin at a molar ratio of 2.5:1 peptide to F-actin. B, as A but in the presence of 24 mM KCl. C, as A but in the presence of 50 mM KCl.

Marked spectral perturbation occurred for the extreme C-terminal residues of 15 µM F-actin. Perturbation of residues 633–646 in the overlapping region was observed with both peptides in the presence of F-actin. Although neither peptide was found to interact with tropomyosin in control titrations, comparable spectral broadening in the case of each peptide occurred at lower F-actin:peptide molar ratios when tropomyosin was present. These observations indicated that tropomyosin had increased the actin-binding affinity of each peptide but had not altered the nature of the residues involved in the association with F-actin.

In the case of hcβM-(622–646), a very restricted subset of peptide residues was observed to interact with F-actin (Fig. 4). Marked spectral perturbation occurred for the extreme C-terminal residues Gly-641, Phe-644, Gln-645, and Thr-646. Resonances of residues at the N-terminal of hcβM-(622–646), Tyr-624, Ala-629, Ile-631, and Glu-632 remained virtually un-
affected and even retained fine structure (Fig. 4B). These observations indicated that the N-terminal region of the bound peptide extended away from the actin surface and retained segmental mobility independent of F-actin.

Inspection of the lysine eCH₂ side chain resonance that is derived from the cluster of five basic residues adjacent to the C-terminal of hcM-(622–646) showed that this composite signal was only slightly altered upon association with F-actin (Fig. 4B). The comparably small perturbation detected for Ala-638 and the clear demarcation observed between the actin-bound C-terminal residues and those at the mobile N terminus of hcM-(622–646) suggested that the actin-induced spectral alteration in the composite lysine resonance could have resulted from the involvement in complex formation of Lys-633 and/or Lys-640 at the C terminus of the basic cluster. The interaction of hcβM-(622–646) with F-actin was, however, relatively stable to increasing ionic strength with complex formation virtually abolished only in the presence of 0.2 M KCl (Fig. 4C). Notably, acetylation of the lysine residues of hcβM-(622–646) resulted in the complete loss of actin binding with complex formation undetectable even when F-actin was present in solution at a 2.5 M excess (Fig. 4D).

The actin affinity of hcβM-(622–646) was determined by monitoring the change in the fluorescence emission of IAE-DANS-labeled F-actin (Fig. 5A) under solution conditions corresponding to the NMR experiment shown (Fig. 4B). Saturation of the quenching of the emission of the IAE-DANS label attached to actin Cys-374 was observed with an apparent dissociation constant of 1 ± 0.5 μM derived for 1:1 complex formation. Taken overall, the observations are indicative of a specific mode of docking involving the C-terminal residues of hcβM-(622–646). These residues occur in the central region of hcβM-(633–660) whose interaction with F-actin showed that the composite lysine side chain signal was relatively unperturbed by complex formation (Fig. 5B), again suggestive of a fluctuating interaction with the surface of actin by a restricted subset of the cluster of basic residues at the N-terminal of loop 2 of human cardiac β-myosin. The association of hcβM-(633–660) with F-actin involved residues Phe-644, Val-647, His-651, Arg-652, Met-659, and Thr-660 downstream of the basic N terminus (Fig. 5B).

Specificity of Interaction of the Myosin Loop Peptides with F-actin Is Demonstrated by the Failure of Other Surface-exposed Regions of Myosin S1 to Associate with Actin—Inspection of the published structures of the myosin head shows two other regions that jut away from the surface of the molecule in a location close to the cardiomyopathy loop and loop 2 protrusions. The corresponding sequences of human cardiac β-myosin, hcβM-(291–311) and hcβM-(365–388) (Table I), were therefore synthesized for study of their potential actin-binding properties. No detectable spectral changes resulted upon titration of either peptide with F-actin or with F-actin-tropomyosin (Fig. 6). Resonances of hcβM-(291–311) (e.g. Lys-293, Lys-294, Thr-304, Tyr-308, Tyr-310, and Ala-311) retained fine structures even when present at a concentration equivalent to an equimolar ratio of peptide:F-actin (Fig. 6, A and B). Similarly, no detectable actin-induced spectral perturbation was observed for signals of residues along the sequence of hcβM-(365–388) (Lys-365, Lys-367, Leu-366, Leu-387, Thr-368, Arg-369, and Tyr-386; Fig. 6, C and D). These experiments using hcβM-(291–311) and hcβM-(365–388) indicated the complete absence of any interactions with the surface of actin and provided robust evidence for the specificity of complex formation with F-actin observed with the cardiomyopathy loop and loop 2 regions that are also located on the same face of the myosin head.

Concurrent Interaction of Three Different Myosin Loop Peptides with F-actin Indicates That They Possess Distinct Binding Sites—In order to study actin-binding site selectivity, equimolar mixtures of different myosin peptides were titrated with actin and actin-tropomyosin. As well as the human cardiac β-myosin cardiomyopathy loop and loop 2 peptides, we made use of a peptide comprising human cardiac β-myosin residues 554–584 also located on the same surface of the myosin head and modeled to be an actin-binding region (1, 2). The latter peptide, hcβM-(554–584), was observed to interact with actin and was used in binding titrations in conjunction with hcβM-(398–414) (Fig. 7A) and hcβM-(622–646) (Fig. 7B). Addition of
actin or actin-tropomyosin led to the simultaneous perturbation of resonances corresponding to both of the peptides in each mixture with line broadening effects typical of those observed with the individual peptides at comparable peptide:F-actin molar ratios. The detection of these perturbations in the spectra of the peptide mixtures was enabled by the unique resonance positions of signals derived from residues specific to each peptide sequence (e.g., His-401, Arg-403, Gly-407, Val-311, and Thr-412 of the cardiomyopathy loop) resolved clearly from signals of His-556, His-576, His-581, Phe-563, Phe-577, and Ile-580 of hçM-(554–584); Fig. 7A).

Spectra acquired during titrations of combinations of myosin peptides with actin and actin-tropomyosin showed that comparable, progressive spectral broadening for each peptide occurred at a lower molar ratio of F-actin:peptide when in the presence of tropomyosin. These observations indicated that, as found from experiments using the individual peptides as actin substrates, tropomyosin had enhanced the apparent actin affinity of each peptide. The tropomyosin-mediated enhancement of the actin affinity of the peptides is illustrated in Fig. 7, A and B, where the broadening of signals of hçM-(554–584) (e.g., His-556, His-576, His-581, and Ile-580) are clearly comparable and are observed to have occurred at a concentration in solution equivalent to the peptide:actin molar ratio of 3:1 (Fig. 7A) but 12:1 when in the presence of tropomyosin (Fig. 7B).

Also shown in Fig. 7B is the retention of the fine structure of the signals of the N-terminal residues of hçM-(622–646), indicating that these remain disposed away from the surface of actin with docking involving the same C-terminal residue interactions as was observed in the absence of tropomyosin (above). Together these observations indicate that the nature of the different myosin residues in contact with F-actin was unaltered by tropomyosin and suggest that tropomyosin acts to reduce the dissociation rate of the myosin peptide from the surface of actin. Furthermore, because there was no evidence of competition between the myosin peptides for actin binding, these observations also indicate that each of the three myosin segments studied possesses a distinct binding site on actin. It was therefore possible to use these interactions to monitor the regulatory effect of cardiac troponin-I on association with a variety of locations on the surface of F-actin.

The Binding of the Inhibitory Region of Human Cardiac Troponin-I to F-actin Results in the Displacement of Myosin Peptides Bound to Different Locations on Actin—The modulation of the F-actin-binding properties of the different myosin peptides by the inhibitory region of human cardiac troponin-I, hçTnI-(128–147) (Table I), was studied by monitoring its effect on complex formation. As illustrated in Fig. 7A, titration with the hçTnI inhibitory region up to a concentration equivalent to a 1:1 molar ratio of hçTnI-(128–147):F-actin resulted in the marked reduction in the degree of association of hçM-(398–414) and hçβM-(554–584) with actin even though the concentration of each of these peptides was at an ∼4-fold excess over F-actin. The ability of hçTnI-(128–153) to cause the dissociation of both these myosin peptides from actin is demonstrated by the observed reduction in their actin-induced spectral perturbations that correlate with the simultaneous decrease in the population of the actin-bound state of each myosin peptide. The specificity of the antagonism of myosin peptide binding by the inhibitory region of troponin-I was studied in analogous com-
petition experiments using another basic residue-rich region of troponin-I shown previously to bind to actin (22, 31). The binding to actin by this peptide, rCtnI-(164–184) (Table I), however, did not result in any alteration in the association of the three myosin peptides with actin even when present at a concentration equivalent to the myosin peptides (data not shown).

The inhibitory region of human cardiac troponin-I was also found to cause the dissociation of the myosin peptides from actin in the presence of tropomyosin. Fig. 7B shows that the addition of hCtnI-(128–153) up to a concentration equivalent to a 1:1 molar ratio to actin in the presence of tropomyosin led to the virtually complete loss of actin binding by hCtactn (molar ratio 1:1.9). hCtactn peptide:actin-each of the myosin loop peptides). D, as for C but in the presence of 17 mU Ca4. TnC (concentration equimolar to hCtnI-(128–147). Signals originating from hCtactn-(398–414) are labeled in italic font.

The studies using NMR provided information on the nature of the association of defined myosin loop regions with F-actin and gave a broad idea of the linear epitopes contributing to these interactions. Our initial probe of myosin contacts with F-actin composed residues 398–414 of human cardiac β-myosin, a peptide that competitively inhibits actin-activated myosin ATPase (23). The segment corresponding to residues 398–414 protrudes from the surface of the upper 50-kDa subdomain of the myosin head and lacks intramolecular contacts with the rest of the molecule (1, 4). Interaction of this segment of intact myosin with F-actin appears to be nucleotide-dependent and a critical contribution to the strong binding of myosin to actin (3, 4, 7–9). This region of myosin further contains sites of mutation (residues 403, 404, and 411) that are associated with hypertrophic cardiomyopathy (18, 32). However, care should be exercised in extrapolating the results obtained with isolated fragments to properties associated with the intact myosin head.

Our studies of the actin-binding properties of hCtactn-(398–414) identified its predisposition to a looped conformation and the occurrence of extensive dynamic contacts with F-actin. Although the contacts identified need not be identical to those in the S1-actin complex, these observations on the isolated peptide correlate with data showing that deletion of residues at the looped tip abolished both actin-catalyzed activation and the characteristic response of Cys-374-pyrene-labeled actin to rigor-like association (3). The latter defect is consistent with the C-terminal location on actin subdomain 1 modeled as the rigor site of interaction of this region of the myosin head (1, 4) (Fig. 9).

A different actin interaction site is indicated by our studies of the association with F-actin by residues of the loop 2 region of myosin. Mutagenesis and other approaches have identified the contribution of basic residues, specifically Lys-639 and Lys-
640, to actin binding by loop 2, an interaction that is correlated with the progression from the weak to strong binding states of myosin (6, 33). Our studies of overlapping peptides of the loop 2 region of human cardiac β-myosin indicate the intimate association with F-actin by a segment downstream of Lys-639 and Lys-640 that is modeled to be in the vicinity of Asp-24 and associated with F-actin by a segment downstream of Lys-639 and the myosin head as the cardiomyopathy and loop 2 regions did not bind actin, the peptide comprising residues 554–584 was found to interact with F-actin. No interdependence of actin binding by hcβM-(554–584) was observed in the presence of hcβM-(398–414) and hcβM-(622–646), indicating the existence of a third site on F-actin distinct from that occupied by the cardiomyopathy and loop 2 regions. It is not surprising that the individual affinities of the isolated segments are different from that of the intact molecule in view of the fact that each are contributing to the overall expanse of the myosin head involved in the interaction with actin. It is also likely that the affinity of each actin-binding region will depend on the involvement of other sites on the intact myosin head. Overall, the studies described in this and earlier reports (22) therefore provide evidence that peptides comprising these three regions of the myosin heavy chain have distinct sites of binding to F-actin that, in the intact molecule, are mapped to be some distance apart on the modeled actomyosin rigor interface (Fig. 9).

One feature of the interactions studied is that the peptides of myosin proved capable of association with actin in the presence or absence of tropomyosin. A further feature associated with all the myosin and troponin I peptides shown to bind to actin is that their affinity is increased in the presence of tropomyosin. This perhaps is to be expected for it has long been known that tropomyosin increased the affinities of intact myosin (34) and troponin (35) for F-actin. In all cases the myosin peptides dissociate from F-actin and F-actin-tropomyosin in the presence of the inhibitory peptide. This does not occur in the presence of the peptide, rctNl-(164–184), that represents a second actin-binding site on troponin I (22, 31). The observations are best explained by concluding that, independently of tropomyosin, on binding of the inhibitory region of troponin I the F-actin monomer undergoes a conformational change that renders its surface markedly less available to stable association with the myosin peptides at their individual binding locations. Caution must be shown in correlating the observations made with peptide sequences and those applying to intact myosin in its interaction with actin, but this ability of the inhibitory region of troponin-I to modulate the surface activity of actin is likely to play an important mechanistic role in the communication through the actin structure.

Reconstructions from electron microscopic studies have concluded that in the blocked state tropomyosin lies close to the regions on actin with which the binding sites of myosin are considered to interact (38). In the light of our findings it is unlikely that in the blocked state present in resting muscle tropomyosin alone prevents the interaction of myosin with actin responsible for the contractile response. It is possible in view of the currently limited resolution of the actomyosin interaction that sites of myosin other than those studied here are involved in the association with F-actin and are blocked by tropomyosin. However, our results lead us to conclude that the binding the inhibitory region of troponin-I to F-actin that occurs during relaxation in muscle, when the calcium concentration is low, leads to conformational changes that are propagated to different locations on the actin surface. We postulate that, in the blocked state of resting muscle, troponin-I imposes a constraint on F-actin that prevents the interaction with myosin required for activation of the MgATPase. On stimulation and the concomitant rise in Ca\(^{2+}\) concentration, the part of the troponin-I molecule that binds to actin is detached by binding to troponin-C F-actin then reverts to the conformational state in which it can activate the MgATPase. We suggest that tropomyosin facilitates the transmission of structural change along the F-actin filament from one actin monomer to its neighbors so that all the actin monomers within a structural unit are unable to interact with myosin.

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