Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6101–6105, October 1981
Biochemistry

Conformational transition in the myosin hinge upon activation of muscle

(rabbit skeletal muscle/contraction/crossbridge movement/subfragment 2–light meromyosin/enzyme probe)

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Contributed by William F. Harrington, July 1, 1981

ABSTRACT **We have determined the rates of chymotryptic proteolysis of the myosin hinge region in glycerinated rabbit psoas fibers and myofibrils in rigor-inducing, activating, and relaxing buffers. The time course of formation of light meromyosin (LMM) provides a specific probe for cleavage within the hinge domain. In rigor-inducing and relaxing buffers proteolysis within the hinge is depressed, but on activation LMM is formed at a markedly increased rate, which is dependent on the concentration of MgATP. Peptide bond cleavage occurs at four widely separated sites spanning the length of the hinge domain. Only a trivial amount of proteolysis occurs at the head–rod swivel or within the heavy chain of the head itself (S-1 subunit) in rigor-inducing and relaxing solvents, and we find no significant change on activation. The rate of formation of LMM in rigor-inducing buffer is unchanged by addition of MgADP, P_i, or magnesium adenosine 5′-[β,γ-imido]triphosphate or in activating solvent at zero overlap between thick and thin filaments. These results provide evidence for a conformational (helix–coil) transition within the myosin hinge upon activation of skeletal muscle.**

According to the swinging crossbridge–rotating head theory of muscle contraction (1–3), force in an actively contracting muscle is produced by an angular movement (tilt) of the myosin head [subfragment 1 (S-1) subunit] while it is attached to a neighboring thin filament. According to the helix–coil theory of contraction (4–6), a region of subfragment-2 (S-2), believed to be the light meromyosin (LMM)–heavy meromyosin (HMM) hinge, melts to random coil, thus generating a retractive force when the S-2 link swings away from the thick filament surface during a crossbridge cycle. In a recent crosslinking study (7) of glycerinated myofibrils in rigor we proposed that the S-2 segment of myosin is released and swings out from the thick filament core when the pH of the bathing medium is increased over a rather narrow pH range (7.4–8.4). This behavior suggests that relatively small changes in the local ionic environment can release the cross-bridge from the thick filament surface. Outward movement of the bridge appears to be accompanied by a conformational transition in the polypeptide chains of the hinge domain to a more open, proteolytically sensitive structure. The rate of chymotryptic cleavage, as measured by formation of LMM, shows a sharp sigmoidal increase just over the pH range where the rate of crosslinking S-2 to the thick filament surface undergoes a precipitous decline. In the present study we have extended this enzyme-probe technique to actively contracting psoas muscle fibers. We find that on activation of the contractile apparatus of glycerinated fibers and myofibrils at neutral pH, the hinge region, which accounts for about one-third of the length of the S-2 segment (8), becomes markedly susceptible to cleavage by α-chymotrypsin, suggesting a structural transition within this region when the crossbridge undergoes cycling. No other region of the myosin molecule, including the head–rod junction, shows a significant change in susceptibility to enzymatic cleavage when the muscle fiber is switched on. Moreover, MgATP appears to be the only low molecular weight ligand relevant to the contractile process [including P_i, magnesium adenosine 5′-[β,γ-imido]triphosphate (Mg·p[NH]ppA), and MgADP] which can produce this effect.

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MATERIALS AND METHODS

Preparation of Glycerinated Muscle Fibers. Glycerinated rabbit psoas muscle was prepared as described by Rome (9). Fully overlapped fibers were prepared by allowing the fibers to shorten before glycerination. The preparation of myofibrils and determination of myofibrillar protein concentration were carried out as described (7).

Electrophoresis of Samples in $NaDodSO_4$-Containing Gels. We employed slab gels and a Tris/glycine buffer according to the method of Laemmli (10) or the phosphate buffer system of Weber and Osborn (11). The phosphate buffer system was used exclusively for estimation of molecular weights of polypeptide chains. To identify the LMM fragments released on proteolysis in this study, we compared their electrophoretic mobilities with the mobility of authentic M_r 75,000 LMM prepared by the method of Sutoh *et al.* (8). Coomassie brilliant blue R250 was used for staining polypeptides within the gels.

Digestion Studies. Glycerinated fibers (≈0.3 mm in diameter and ≈3 cm in length) were dissected in 50% (vol/vol) glycerol/60 mM KCl/10 mM 2-mercaptoethanol/40 mM imidazole·HCl (pH 7.1). These were fastened at both ends to thin glass rods with waterproof tape. Fibers with sarcomere lengths of about 2.1 ± 0.1 μm (fully overlapped) were used unless otherwise noted. Before digestion with α-chymotrypsin the fibers were incubated in rigor-inducing solution (60 mM KCl/5 mM $MgCl_2$/0.1 mM $CaCl_2$/40 mM imidazole·HCl, pH 7.1) containing 0.2% (vol/vol) Triton X-100 for 20 min at 4°C, then washed repeatedly with rigor solution at room temperature. Digestion conditions are described in the figure legends. Chymotryptic digestion of fibers was quenched by transferring samples at various stages of proteolysis into rigor solution containing 5 mM phenylmethylsulfonyl fluoride. Digested fibers were cut away from the glass rods and incubated in 10% (wt/vol) $NaDodSO_4$ solution (0.1 ml for each sample) overnight followed by electrophoresis on $NaDodSO_4$-containing gels ($NaDodSO_4$ gels). Digestion of myofibrils was quenched by addition of 0.05 vol of phenylmethylsulfonyl fluoride (2% in ethanol) to aliquots of the reacting system. After addition of 0.5 vol of $NaDodSO_4$ (10%, wt/vol) the suspensions were heated to 80°C for 5 min. The resulting clear solutions were examined by electrophoresis

Abbreviations: S-2, subfragment 2; S-1, subfragment 1; HMM, heavy meromyosin; LMM, light meromyosin; MHC, myosin heavy chain; Ap_5A, P^1,P^5-diadenosine pentaphosphate; p[NH]ppA, adenosine 5′-[β,γ-imido]triphosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

on $NaDodSO_4$ gels. Absorbance (525 nm) of the protein bands was determined with a gel scanner (Helena Laboratories, Beaumont, TX). The maximal loading mass of each peptide band was about 20 μg. We used the actin band (M_r 43,000) as an internal standard to calibrate other band densities on $NaDodSO_4$ gels because actin is resistant to proteolysis by α-chymotrypsin under the experimental conditions used in this study. The kinetics of digestion of fibers and myofibrils were obtained by plotting the optical density of either the myosin heavy chain (MHC) or the sum of LMM bands vs. digestion time. All the cleavage reactions showed single-exponential behavior, and rate constants were determined from linear log(optical density) vs. time plots. To analyze the rate of formation of LMM as a pseudo-first-order process, the absorbance of the LMM band(s) was determined by plotting A_{MHC} (normalized to actin) vs. A_{LMM} (normalized to actin). We found $A_{LMM}/A_{actin} = 0.80$ when $A_{MHC}/A_{actin} = 0$ by a least squares method. First-order rate constants were generally determined by this method. To minimize experimental error due to differences in fiber diameter, we averaged the results from two or three different samples at each time point.

The effect of various low molecular weight substances, including $MgCl_2$, $CaCl_2$, KCl, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), P_i, ATP, p[NH]ppA, ADP, and P^1,P^5-diadenosine pentaphosphate (Ap_5A), on the intrinsic hydrolytic activity of α-chymotrypsin was tested by measuring the rate of hydrolysis of benzoyl-L-tyrosine ethyl ester (at pH 7.1) according to the method of Hummel (12). The intrinsic activity of the enzyme was unchanged (±5%) in the presence of these substances at concentrations comparable to those used in the present study. To determine if all of the myosin molecules within our glycerinated preparations were accessible to the enzyme, fibers were digested with α-chymotrypsin (0.10 mg/ml) in 60 mM KCl/10 mM EDTA/40 mM imidazole·HCl (pH 7.1) at 25°C. We found that the head–rod junctions of all of the myosin molecules were cleaved in this solution within 30 min, as judged by the absorbances of S-1 and rod after electrophoresis of the digest on $NaDodSO_4$ gels.

To establish the sites of cleavage within the heavy chains of myosin, digested fibers were transferred to a small volume (1 ml) of rigor solvent (ionic strength $I = 0.6$ M) and denatured by addition of ethyl alcohol (70%, vol/vol) as described by Szent-Györgyi *et al.* (13). The precipitate was dialyzed vs. concentrated salt solution to dissolve any LMM, rod, and S-2 produced by proteolysis. Electrophoresis of the insoluble residue showed only a trace of bands corresponding in size to the LMM fragments (M_r 70,000–90,000). After dialysis of the supernate vs. low ionic strength buffer, the resulting precipitate was examined by electrophoresis on $NaDodSO_4$ gels. Only trace amounts of rod were detected with the LMM species.

RESULTS

In the present study we have employed an enzyme-probe technique to investigate the conformational state of the LMM–HMM hinge region of myosin in glycerinated rabbit psoas fibers and myofibrils immersed in rigor, activating, and relaxing buffer solutions. We monitored the rate of α-chymotryptic cleavage within this region by measuring the rate of decay of the myosin heavy chain as well as the rate of formation of LMM. Enzymatic cleavage was terminated at various stages of the reaction, and the digestion products were examined after electrophoresis of the digest on $NaDodSO_4$ gels. When our preparations of glycerinated fibers were digested with α-chymotrypsin (0.20 mg/ml) for 30 min (22°C) at neutral pH in either a rigor (Fig. 1, lanes b and e) or relaxing buffer system (Fig. 1, lanes c and f), the rate of clipping within the LMM–HMM hinge region was relatively slow, judging from absorbance scans of the LMM bands. Nonoverlapped fibers were used in the relaxing buffer system. Digestion of the myosin heavy chain under these conditions occurs primarily within the hinge domain, yielding LMM frag-

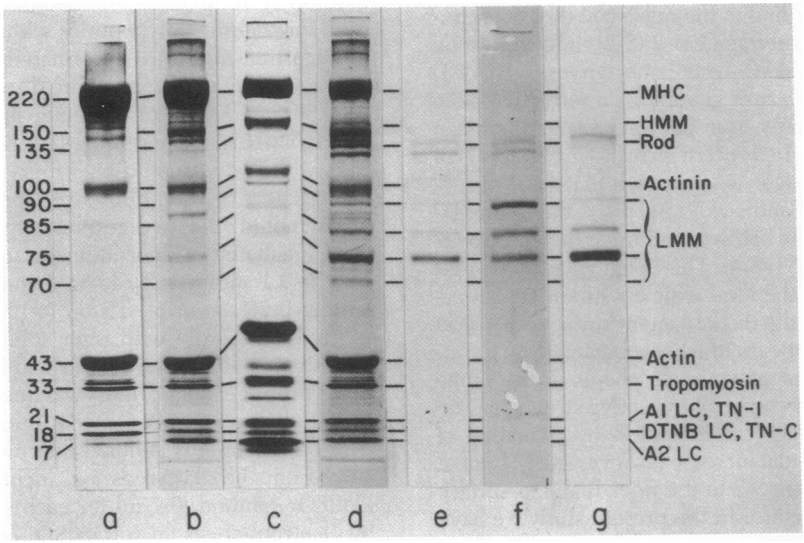

FIG. 1. Electrophoresis of muscle proteins on $NaDodSO_4$ gels after α-chymotryptic digestion of glycerinated fibers. A Tris (12.5 mM)/glycine (90 mM) buffer system was used according to the method of Laemmli (10), employing a stacking gel (6%) and two discrete separation gels (8.5% and 13%). Gel concentration is expressed as acrylamide concentration (wt/vol), and methylene bisacrylamide is 0.8:30 (wt/wt) to acrylamide. Glycerinated muscle fibers were digested with α-chymotrypsin (0.20 mg/ml) at pH 7.1 and 22°C for 30 min in rigor, relaxing, and activating solutions (lanes b–d). To identify LMM bands, digested fibers were treated with alcohol according to the method of Szent-Györgyi *et al.* (13), and the dissolved fractions after alcohol denaturation were dialyzed against low ionic strength solution ($I = 0.05$ M) at pH 6.8. The insoluble fraction at low ionic strength is shown (lanes e–g). Some HMM and S-1 (if any) fragments may be lost in the digestion process. Lane a, glycerinated rabbit psoas fibers used in this study. Lane b, fibers digested in rigor buffer (40 mM imidazole·HCl/60 mM KCl/5 mM $MgCl_2$/0.1 mM $CaCl_2$). Lane c, fibers digested in relaxing buffer ($CaCl_2$ in rigor solution was replaced by 4 mM EGTA and 2 mM MgATP). In order to avoid contraction of muscle due to proteolytic cleavage of troponin, nonoverlapped muscle fibers were used in this case. Lane d, fibers digested in activating buffer (4 mM MgATP plus rigor solution). Lanes e, f, and g contain insoluble fragments of myosin at low ionic strength of the samples in lanes b, c, and d, respectively. M_r values $\times 10^{-3}$ are indicated on the left of the figure. LC, light chain; TN, troponin.

ments with molecular weights ranging between 70,000 and 90,000. A very small amount of cleavage was observed at the rod–S-1 junction. Optical density scans of the rod band showed that the amount of this species approximates 10% LMM. Although no significant cleavage within the S-1 heavy chain was detected, cleavage does occur in the DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] light chain in all the buffer systems. Small amounts of myosin rod (Fig. 1, lanes e–g), observed after 30-min digestion, are likely due to the well-known loss of protection of the head–rod swivel (14, 15) that results from splitting within these light chains.

When rigor fibers were digested under activating conditions (rigor solution + 4 mM MgATP) rapid proteolytic cleavage within the hinge region of the cycling crossbridges was observed (Fig. 1, lanes d and g). The striking shift in cleavage rate can be seen in Fig. 2 where the relative absorbance of the heavy chain band, normalized to the absorbance of the (undigested) actin band for each fiber, is plotted vs. digestion time.

Kinetics of Digestion of Muscle Fibers in the Presence and Absence of MgATP. The time course of formation of LMM on activation of glycerinated rigor fibers in the presence of α-chymotrypsin is presented in Fig. 3 *B* and *C*. Like the cleavage of the heavy chain (Fig. 3*A*), the process is pseudo-first-order under our experimental conditions over at least 30 min and shows a strong dependence on the concentration of MgATP in the activating medium (Fig. 3*B*, Table 1). The hinge region is susceptible to enzymatic attack at four widely spaced sites as shown by the appearance of four discrete LMM fragments with M_r 70,000, 75,000, 85,000, and 90,000 on NaDodSO$_4$ gels. In 2 mM MgATP the LMM fragment with M_r 75,000, corresponding to a cleavage site near the COOH terminus of the hinge domain, is formed most rapidly (k = 0.43 hr^{-1}). Two LMM fragments with M_r 70,000 and 90,000 resulting from cleavage at either end of the hinge region are formed at relatively slow rates (k = 0.05 hr^{-1}). The LMM fragment with M_r 85,000, corresponding to a cleavage site near the center of the hinge domain is released at an intermediate rate (k = 0.18 hr^{-1}).

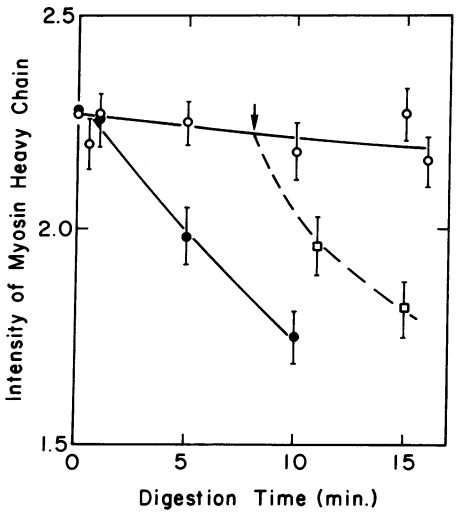

FIG. 2. Time course of myosin heavy chain digestion in rigor and activated fibers. Fibers were incubated in rigor solution containing α-chymotrypsin (0.10 mg/ml) in the presence or absence of 5 mM MgATP at pH 7.0 (25°C). Products were subjected to electrophoresis in NaDodSO$_4$ gels and stained. Absorbance of intact myosin heavy chains (M_r ≈220,000) normalized to that of actin (M_r 43,000) is plotted against incubation time. Bars indicate the range of experimental values. The composition of the rigor buffer solution is described in the legend to Fig. 1. ○, Rigor solution; ●, activating solution; □, fibers activated by adding MgATP (5 mM) after incubation in rigor solution for 8 min as indicated by the arrow.

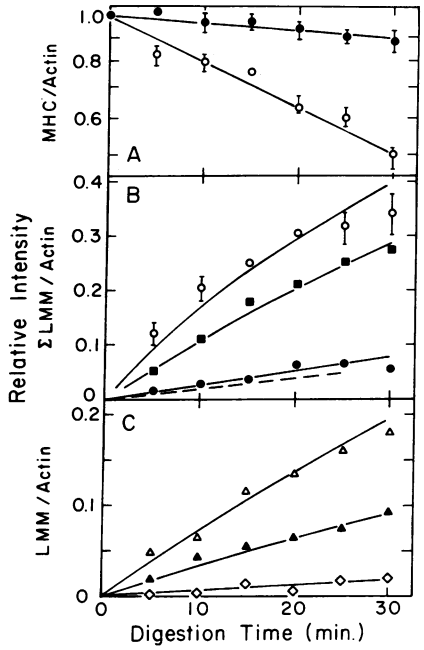

FIG. 3. Kinetics of digestion of muscle fibers in the presence and absence of MgATP. Fibers were incubated in rigor solution containing α-chymotrypsin (0.20 mg/ml) at 4°C for 5 min, then transferred to various solutions containing the enzyme (0.20 mg/ml) at 22°C and pH 7.1. Digestion products were analyzed on NaDodSO$_4$ gels. (*A*) Relative absorbance of myosin heavy chain band (on a logarithmic scale) vs. digestion time. ●, Rigor (solution is the same as described in the legend to Fig. 1); ○, activated (4 mM MgATP plus rigor solution). (*B*) Absorbances of LMM bands (normalized to actin) vs. digestion time. Solid lines show theoretical first-order curves for the formation of LMM (rate constants are given in Table 1), broken line shows the rate for "relaxed" fibers. ●, Rigor; 4 mM (○) and 2 mM (■) MgATP were added to rigor solution. (*C*) Absorbance of various LMM bands formed by addition of 2 mM MgATP to rigor solutions. The M_r 75,000, 85,000, and 70,000 bands are shown (top to bottom) with theoretical first-order rate constants of 0.43, 0.17, and 0.05 hr^{-1}, respectively. Plot of M_r 90,000 band (with rate constant of 0.05 hr^{-1}) is not shown. Bars indicate the range of experimental values.

To investigate the rate of proteolysis at the rod–S-1 junction, myosin fragments insoluble in low-salt solutions were extracted from activated fibers at various stages of digestion (*Materials and Methods*). The density of the rod band ($M_r \approx 135{,}000$) relative to the LMM bands was determined after electrophoresis of the fragments on NaDodSO$_4$ gels. We found the rate of formation of the myosin rod to be much lower than that of LMM ($k_{LMM}/k_{rod} = 14 \pm 4$). Electrophoresis of the fragments soluble in low-salt solutions gave an S-2 band of low optical density on NaDodSO$_4$ gels. These results provide evidence for a very low rate of clipping at the rod–S-1 junction.

The rate constant for digestion of the myosin heavy chain is the same as that for the appearance of the LMM fragments (summed over the LMM band) within experimental error (±10%) in the rigor and activating solutions, but the rate of cleavage of the heavy chain was appreciably higher than the rate of formation of LMM in relaxed fiber bundles. This difference is mainly the result of some additional cleavage at the head–rod swivel. In the low-Ca^{2+} environment of the relaxing medium, the DTNB light chain was digested more rapidly in the relaxing solution than in the rigor and activating solutions. Additionally, the regulatory protein, troponin, is very susceptible to α-chymotrypsin, thus allowing the fibers to be activated. Both of the above effects make the time course of proteolytic cleavage within the hinge difficult to measure in the relaxing buffer. The rate of cleavage of the hinge in this system (Fig. 3*B*) was therefore estimated from the rate of formation of LMM fragments

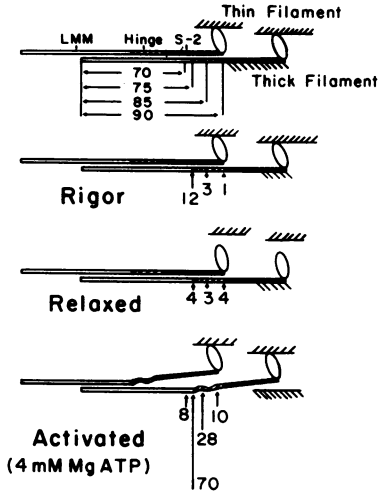

FIG. 4. Schematic representation of the arrangement of two myosin molecules (myosin dimer) along a row of crossbridges in muscle in rigor, relaxed, and activated states. The top diagram shows sizes of LMM fragments per single polypeptide chain ($M_r \times 10^{-3}$) formed by a α-chymotryptic cleavage within the hinge. In the lower three diagrams the rate of cleavage at each susceptible site of LMM, based on first-order rate constant of formation of fragment as described in Fig. 3, is indicated by length of the arrow. Numbers under arrows indicate magnitude of first-order rate constants ($\times 100$ hr^{-1}).

obtained from nonoverlapped (stretched) fibers.

The chymotryptic cleavage sites in the hinge region as well as the relative rates of cleavage in rigor, relaxed, and activated fibers are shown schematically in Fig. 4.

Effect of Ligands on Chymotryptic Proteolysis Within the Hinge in Fibers and Myofibrils. Table 1 summarizes the effect of various ligands on the first-order rate constant of chymotryptic proteolysis within the hinge region of glycerinated fibers and myofibrils. We found that addition of P_i, p[NH]ppA, ADP, or ADP + P_i had no significant effect on the rate of cleavage of myofibrils in standard (rigor) buffer. In the case of ADP and the ADP/P_i mixture, the residual adenylate kinase activity in the myofibrils was inhibited by addition of Ap_5A to prevent reformation of ATP (16). In the absence of this inhibitor the system was activated, resulting in rapid proteolytic cleavage.

Activation of Glycerinated Fibers. As was noted earlier, the rate of proteolysis within the hinge of glycerinated fibers showed a strong dependence on the concentration of MgATP in the activating medium.

Glycerinated fibers stretched to zero overlap between thick and thin filaments showed, however, no significant increase in the rate of proteolysis within the hinge when these fibers were exposed to activating solvent conditions (standard solution + 4 mM MgATP). Replacement of MgATP by CaATP (no Mg^{2+}) in the nonoverlapped fiber system actually depressed the rate of cleavage in the hinge region, although in this solvent the rate of ATP splitting increases by about 25-fold. In all experiments in which proteolyses of fibers and myofibrils were examined under identical environmental conditions, the rate constants of cleavage in the myofibrillar system were invariably higher. This effect is likely the result of differences in accessibility of the enzyme to the contractile machinery in the two preparations.

Activation of myofibrils results in rapid contraction of these elongated structures into globules, and it was important to establish whether such drastic morphological changes influenced proteolysis of the heavy chain. Myofibrils that had undergone contraction to the globular state in the presence of MgATP were dialyzed exhaustively against rigor buffer to remove residual ligand and then digested with α-chymotrypsin. The cleavage rate of heavy chain in these precontracted myofibrils was unaffected by this treatment. As was observed previously (14), α-chymotryptic cleavage within the hinge region in aggregated myosin rod filaments (Table 1) is much slower than with native thick filaments, suggesting that this region becomes susceptible to the enzyme only in the presence of the S-1 subunits.

Table 1. Digestion of glycerinated muscle fibers and myofibrils with α-chymotrypsin under various conditions

Condition	Digestion rate constant of myosin, hr^{-1}	
	Fibers	Myofibrils
Rigor		
Standard solution	0.19 ± 0.05	0.70 ± 0.10
Precontracted*		0.94 ± 0.15
Phosphate (10 mM)		0.88 ± 0.10
Mg-p[NH]ppA (1 mM)	0.25 ± 0.10	0.82 ± 0.10
MgADP (1 mM), Ap_5A (40 μM)		0.65 ± 0.15
MgADP (1 mM), Ap_5A (40 μM), and P_i (10 mM)		0.85 ± 0.15
Activated		
MgATP (0.2 mM)	0.27 ± 0.04	
(1 mM)	0.56 ± 0.08	
(2 mM)	0.88 ± 0.08	3.1 ± 0.3
(4 mM)	1.4 ± 0.2	
(10 mM)	2.1 ± 0.2	
MgADP (1 mM)†		2.5 ± 0.3
Relaxed‡	0.14 ± 0.10	0.52 ± 0.05
Nonoverlapped§		
MgATP (4 mM)	0.26 ± 0.10	
CaATP (4 mM)	0.16 ± 0.05	
Rod filaments¶		0.0 + 0.05

Glycerinated rabbit psoas fibers or myofibrils were digested with α-chymotrypsin at pH 7.1 and 22 ± 1°C. About 10 fibers were incubated in an 80-ml reservoir containing enzyme at 0.20 mg/ml, or myofibrils (2.0 mg of myofibrillar protein per ml) were digested with enzyme at 0.05 mg/ml for up to 20–30 min. Digestion rate constants were obtained as described for Fig. 3; ± indicates range. Various ligands were added to the standard solution (40 mM imidazole·HCl/60 mM KCl/5 mM $MgCl_2$/0.1 mM $CaCl_2$). Fully overlapped samples (sarcomere length ≈2.1 ± 0.1 μm) were used unless noted.

* Myofibrils were treated with MgATP (1 mM) and then dialyzed exhaustively against standard solution to remove the residual ligand.

† Residual adenylate kinase converts ADP into ATP unless the inhibitor of this enzyme (Ap_5A) is added.

‡ Rate constant was obtained from the rate of formation of LMM. $CaCl_2$ in the standard solution was replaced by 4 mM EDTA and 2 mM MgATP. Nonoverlapped fibers were used. Initial rate of cleavage of myofibrils is given.

§ Fibers with sarcomere length of about 3.8 μm were used. $MgCl_2$ in the standard solution was replaced by $CaCl_2$ for the digestion in the presence of CaATP.

¶ Digestion of myofibrils was performed in a divalent-metal-ion-free solution (40 mM imidazole·HCl/30 mM KCl/10 mM EDTA) to cleave the junctions between heads and rods for 15 min. Digestion was then allowed to proceed in rigor buffer for another 30 min to cleave the LMM–S-2 hinge region. Digestion rate of rod is given.

DISCUSSION

The low thermal stability of the LMM–HMM hinge region of myosin is reflected in a number of physical properties. This region has been known for years to be rapidly cleaved by a variety of proteolytic enzymes (8, 17–19) in contrast to the flanking LMM and short S-2 segments of the myosin rod, which are relatively resistant to enzymatic attack. The hinge region melts at a significantly lower temperature than does isolated LMM or short S-2 (20, 21), and recent calorimetric measurements have shown that it exhibits a much lower enthalpy of melting [ΔH = 0.4–0.6 cal/g compared to 3–5 cal/g for LMM and short S-2 (1 cal = 4.184 J) (20, 22, 23)].

Our observation that proteolytic cleavage occurs at widely spaced sites within the hinge region of actively cycling crossbridges is consistent with involvement of a major fraction of this region in a conformational (helix–coil) transition when the S-2 link swings away from the thick filament surface. Attachment of the myosin head to a neighboring thin filament seems to be essential to the conformational transition within the hinge. Nonoverlapped filaments bathed in an identical activating solvent do not exhibit this phenomenon. Nor do nonoverlapped filaments in which the myosin heads are rapidly cleaving ATP (presence of 4 mM CaATP). The results presented above suggest that release of S-2 from the stabilizing environment of the thick filament surface and the proposed consequent abrupt melting (5) of the coiled coil of α-helices to random coil occur after attachment of the myosin head to a neighboring thin filament in a crossbridge cycle. Arguments have been presented in an earlier paper (6) to show that the contractile force generated by such a process is sufficient to account for the isometric tension developed in a working muscle.

Release of the S-2 link and melting of the hinge region does not seem to depend directly on the electrostatic charge of the uncleaved ATP molecule within the active site. Addition of Mg·p[NH]ppA to the rigor system has no effect on the proteolytic cleavage reaction. Moreover, neither MgADP nor P_i nor a combination of these ions is able to induce any significant opening of the hinge at neutral pH. It seems possible that a vectorially directed proton ejected during ATP cleavage may be responsible for modulating the head–hinge interaction (6, 8). Another possibility is an alteration in the local ionic environment at the interface between the lower surface of the S-1 subunit and the adjoining S-2 region in the thick filament (see Fig. 4) core that is linked to binding of the myosin head to the thin filament. We reported in an earlier paper (8) that, unlike short S-2, long S-2 self-associates at physiological ionic strengths. It seems reasonable to assume that this interaction [between the hinge region and the short S-2 segment of a neighboring molecule (see Fig. 4)] is conserved in the thick filament and serves to hold the S-1 subunit close to the thick filament core in the rigor and resting states of muscle. Transient modulation of this interaction, after attachment of S-1 to actin, may occur in a cycling crossbridge, thus releasing the S-2 segment.

Although we believe it likely that the increased rate of proteolysis on activation of rigor fibers results from partial melting of the α-helical hinge region to random coil (7), it is still possible that this effect results from a change in the steric accessibility of this region to enzymatic attack as a result of crossbridge cycling. We cannot decide conclusively between these two possibilities at the present time, but we favor the former point of view for the following reasons. (*i*) The lattice spacing remains essentially unchanged (24) or may even show a small decrease ($\approx$15% in skinned fibers) (25) when a muscle contracts isometrically, suggesting that the angular displacement of the S-2 segment from the thick filament surface would be very small. (*ii*) The bulky myosin heads, which are in close proximity to a neighboring hinge in the systematic assembly of the filament (Fig. 4), do not themselves prevent cleavage when S-2 is associated with the thick filament surface, because the rate of chymotryptic proteolysis within the hinge is actually depressed after digestion of the swivel joint and release of the S-1 subunit (Table 1, ref. 14).

Recent attempts to detect significant crossbridge rotation of the actin-attached myosin heads in actively contracting muscle have been unsuccessful. Güth (26) found no change in the degree of polarization of the intrinsic tryptophan fluorescence of glycerinated single fibers (rabbit psoas) after abrupt release of the isometrically contracting muscle. Yanagida (27) reported that the polarized fluorescence measurements of bound nucleotides (ε-ATP, ε-ADP) in isometrically contracting muscle are unchanged from their polarization in the rigor state. These results suggest that the angular orientation of S-1 subunits remain unaltered when muscle is switched on. Our finding of a conformational transition within the hinge region, but no significant structural change within the heavy chain of the S-1 subunit or within the S-1–rod junction after activation is consistent with these results and provides support for the helix–coil mechanism of force generation in skeletal muscle.

We thank Dr. S. J. Lovell for critical reading of this manuscript. This research was supported by National Institutes of Health Grant AM 04349 and was carried out during the tenure of a postdoctoral fellowship to H.U. from the Muscular Dystrophy Association. This is contribution no. 1107 from the McCollum–Pratt Institute.

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