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Influence of the cardiac myosin hinge region on contractile activity

(subfragment 2/motility/sarcomere shortening/myocytes)

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ABSTRACT **The participation of cardiac myosin hinge in contractility was investigated by *in vitro* motility and ATPase assays and by measurements of sarcomere shortening. The effect on contractile activity was analyzed using an antibody directed against a 20-amino acid peptide within the hinge region of myosin. This antibody bound specifically at the hinge at a distance of 55 nm from the S1/S2 junction, was specific to human, dog, and rat cardiac myosins, did not crossreact with gizzard or skeletal myosin, and had no effect on ATPase activity of purified S1 and myofibrils. However, it completely suppressed the movement of actin filaments in *in vitro* motility assays and reduced active shortening of sarcomeres of skinned cardiac myocytes by half. Suppression of motion by the anti-hinge antibody may reflect a mechanical constraint imposed by the antibody upon the mobility of the S2 region of myosin. The results suggest that the steps in the mechanochemical energy transduction can be separately influenced through S2.**

The mechanism of muscle shortening is thought to occur by means of binding of a myosin head (S1) to actin followed by its power stroke and dissociation. The portions of myosin governing the power stroke have been the subject of many studies. Motility assays have shown that the myosin head alone (S1 or heavy meromyosin) is sufficient to move fluorescently labeled actin filaments (1, 2) or move on *Nitella* actin (3) and, more significantly, that S1 alone is sufficient to generate force in an *in vitro* system (4).

It has been proposed that the proteolytically sensitive area within the myosin tail or the hinge region between S2 and light meromyosin may also play a significant role in contraction, possibly by undergoing a helix-to-coil transition following "melting" of the S2 coiled-coil helix. This was deduced from conformational changes within S2 as observed by biochemical (5–8) and electron microscopic analysis (9). In support of this hypothesis, a polyclonal anti-S2 antibody was shown to depress tension or isometric force generation in maximally activated glycerinated muscle fibers (10, 11) and to reduce the extent of shortening in isolated myofibrils (11).

We investigated the influence of S2 on active shortening and motion using an antibody directed against a peptide 20 amino acids long that is totally within the hinge region. Control of crossbridge cycling by myosin occurs physiologically in the heart, and so we selected cardiac myosin and myocytes to test the relevance of the hinge in contractility.

We show that the hinge exerts a pronounced long-range effect that impairs contractile motion without preventing crossbridge attachment under conditions where little tension transmission by S2 occurs. This finding raises a larger question, as to how information about the status of the hinge is physically communicated along S2 and how it is transduced by S1 to affect its interaction with actin. A preliminary report of these observations was presented earlier (12).

MATERIALS AND METHODS

Preparation of Myosin and the Hinge Peptide. Human or dog cardiac and turkey gizzard myosins were prepared according to procedures described elsewhere (13, 14). A 20-amino acid peptide within the hinge was selected from published sequences based on cDNA data identical for α- and β-myosin heavy chains (15). The sequence of amino acids in the peptide (synthesized by Applied Biosystems) was Glu-Arg-Leu-Glu-Glu-Ala-Gly-Gly-Ala-Thr-Ser-Val-Gln-Ile-Glu-Met-Asn-Lys-Lys-Arg. This sequence spans residues starting at Glu-711 and terminating at Arg-730 (15) or Glu-1150 to Arg-1169 (16) and is completely within the hinge region.

Preparation of Anti-Hinge Peptide Antibody. The hinge peptide was crosslinked to bovine serum albumin according to Walter *et al.* (17), and the resulting bovine serum albumin–peptide antigen complex was used to immunize rabbits as described (18). The antibody produced was first purified on a DEAE column followed by chromatography on a Sepharose-hinge peptide affinity column (19). Affinity- and DEAE-purified antibodies were used as indicated in the experiments described below.

Radioimmunoassay (RIA) and Inhibition Experiments. Pure hinge peptide and six different myosins (normal and myopathic human cardiac, turkey gizzard, rabbit skeletal, and dog and rat cardiac) were tested for reactivity versus a double-antibody RIA using rabbit polyclonal affinity-purified anti-hinge IgG, competing with ^{125}I-labeled hinge peptide (18).

Preparation of Samples for Electron Microscopic Mapping. Myosin was dialyzed into 1 M NH_4OAc (pH 8.0). A P-300 column of V_b = 1.2 ml in a Pasteur pipette was poured and rinsed in the same buffer. The myosin and α-hinge IgG were mixed (100 μg of myosin plus 45 μg of IgG; 100 μg of myosin plus 150 μg of IgG) and allowed to react for 15 min at 4°C; the sample was loaded on the P-300 column and eluted at about 5 ml/hr. Samples for electron microscopy were prepared for electron microscopy as described (19, 20).

Preparation of Cardiac Myocytes and Evaluation of Contractile Response to Antibodies. Calcium-resistant, electrically excitable rat ventriculocytes were enzymatically isolated with 0.1–0.2% collagenase and 0.001% trypsin, washed, and resuspended in a physiological salt solution (21). Within 30 min after isolation, the cells were permeabilized by resuspending the cells in 3–4 ml of nonionic detergent (0.5–1.0% Triton X-100) on ice for >30 min in a relaxing solution

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of 125 mM potassium proprionate, 2.0 mM $Mg(OAc)_2$, 5.0 mM Na_2ATP, 5.0 mM K_2EGTA, and 5.0 mM imidazole (pH 7.4). An aliquot of permeabilized cells in 2 ml of fresh relaxing solution was deposited in a small test chamber mounted on the stage of an inverted microscope. Antibody was added to achieve final concentrations of (*i*) 100–300 μg of DEAE-purified anti-hinge antibody per ml or (*ii*) 25–90 μg of affinity-purified anti-hinge antibody per ml. Contraction of individually selected myocytes was determined by measurement of their sarcomere shortening in response to the transient iontophoretic deposition of calcium from a small pipette containing 0.1 M $CaCl_2$ placed ≈10 μm from the cell. Sarcomere length was measured 530 times·s^{-1} by frequency modulation detection of the striated pattern using an extension of the phase-locked loop method (22, 23). All observations were conducted at 16–17°C.

Effect of the Anti-Hinge upon Shortening in Response to Steady Calcium Activation. Aliquots of ≈5000 permeabilized cells were exposed overnight to 0.1–3 mg of anti-hinge antibody or anti-LC1 antibody per ml or to nonimmune IgG at molar ratios of >25:1 (protein to computed cellular myosin). A 50-μl sample of the cells was placed in a chamber containing 200 μl of fresh experimental solution in which $[Ca^{2+}]$ was adjusted by varying the ratio of CaEGTA/K_2EGTA keeping total EGTA at 5 mM and retrospectively adjusted for the K_2EGTA originally present in the added aliquot of cells.

***In Vitro* Motility Assays of Anti-Hinge Antibody-Coupled Myosin and ATPase Measurements.** The sliding actin *in vitro* motility assay was performed essentially as described by Umemoto and Sellers (24) with modifications described below to reduce the influence of rigor-like heads. To prepare for the sliding actin motility assay, cardiac myosin (4.7 mg/ml) in 0.5 M KCl, 0.01 M imidazole (pH 7.0), and 1 mM dithiothreitol was made 3 mM in $MgCl_2$/2 mM ATP and 10 μM in phalloidin. To this, 0.4 mg of F-actin per ml was added immediately before centrifugation at 30 psi (1 psi = 6.89 kPa) in a Beckman Airfuge for 15 min. The supernatant was removed and used directly in the motility assay.

Following the above procedure, myosin was diluted to 0.2 mg/ml with 0.5 M NaCl, 10 mM Mops (pH 7.0), 0.1 mM EGTA, and 1 mM dithiothreitol and applied to the flow cell. Under these conditions the myosin binds as monomers to the nitrocellulose surface. After washing with a buffer containing 0.5 mg of bovine serum albumin per ml, the flow cell was washed with motility buffer [20 mM KCl, 10 mM Mops (pH 7.2), 5 mM $MgCl_2$, 0.1 mM EGTA, and 2 mM dithiothreitol] containing 0.2 mg of the appropriate IgG per ml and incubated for 30 min at room temperature or 3 hr at 4°C. The antibody solution was then washed out with motility buffer and this was replaced with fresh motility buffer containing 20 μM F-actin labeled with phalloidin that had been incubated in a sonicating water bath for 10 min (this treatment also tends to reduce the effect of rigor-like myosin heads). After 1–2 min of incubation this solution was replaced with 10 nM rhodamine phalloidin-labeled F-actin in motility buffer and then washed with motility buffer containing 1 mM ATP to start the reaction. Fields of actin filaments were videotaped for a duration of 3–5 min. ATPase assays were performed as described (13, 25) in a 5-ml assay mixture containing 2.5 mM ATP, 3.25 mM $MgCl_2$, 20 mM KCl, and either 0.1 mM $CaCl_2$ or 2 mM EGTA at 25°C or 30°C and pH 7.5. Control assays were performed using S1 and actin. S1 concentration in the reaction mixture was 0.05 mg/ml and that of actin was 0.27 mg/ml. The molar ratio of antibody to S1 was varied from 1:3 to 1:1.

RESULTS

Specificity of the Antibody to the Cardiac Myosin Hinge Region. The effective crossreactivity of the various myosins with the antibody against hinge peptide was evaluated by competitive inhibition RIAs. Cardiac myosin from normal and dilated cardiomyopathic human hearts as well as from dog and rat heart inhibited the binding of purified hinge peptide to surface-bound anti-hinge IgG (Fig. 1). Turkey gizzard smooth and rabbit skeletal muscle myosins were relatively inefficient as inhibitors. Quantitative dot blot analysis confirmed the specificity of the anti-hinge antibody for cardiac myosin (data not shown). The antibody had no crossreactivity with bovine serum albumin, which was used to couple the hinge peptide for preparation of antibody. SDS/PAGE (Fig. 2*D*, lanes 1–3) and Western blot analysis also showed no reaction with either cardiac S1 (Fig. 2*D*, lane 5) or bovine aortic smooth muscle myosin (Fig. 2*D*, lane 6), but the antibody did react with the whole cardiac myosin (Fig. 2*D*, lane 4).

Macromolecular Electron Microscopy Localizing the Hinge Peptide Antigenic Site on Human Cardiac Myosin. Platinum-shadowed particles clearly indicate an extra globular structure on the myosin tail (Fig. 2*A*) corresponding to the anti-hinge IgG. The last frame in this series (on the right) shows a pair of myosin molecules crosslinked by the antibody. Parameters A and B (Fig. 2*B*) are the distances from the S1/S2 junction or from the distal tip of the myosin head to the center of the globular IgG, respectively. The distance from the S1/S2 junction (parameter A) was 55 nm (Fig. 2*C*), and that from the tip of the myosin head to the IgG (parameter B) was 72 nm, which agrees well with expected distance of the hinge from the S1/S2 junction, after allowing for the dimensions of the IgG marker.

Effect of Antibody on *in Vitro* Motility Measurements and ATPase Activity. The myofibrillar ATPase activity performed in the presence and absence of anti-hinge peptide antibody as a function of calcium did not change significantly. In control

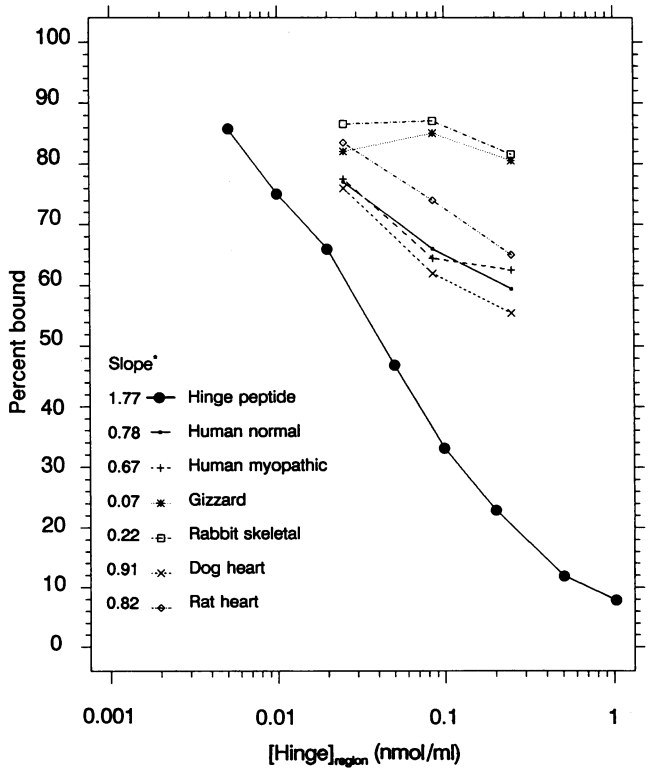

FIG. 1. Inhibitory effect in the RIA. The hinge peptide was compared with that of various myosin preparations in inhibitory capacity versus rabbit polyclonal anti-hinge antibody precipitation of ^{125}I-labeled hinge peptide. Symbols for the various competing myosins are shown. Slope*, slopes of the various inhibition curves are compared in the range 0.025–0.25 nmol/ml.

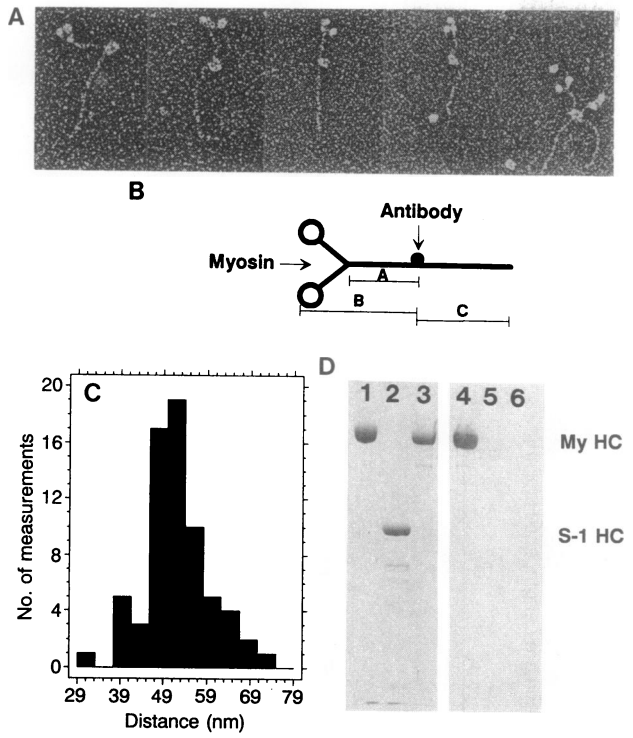

FIG. 2. Electron micrographs of rotary-shadowed complexes of myosin with anti-hinge antibody. (*A*) The first four frames (from the left) show the attachment of the antibody at the hinge and the last frame (on the right) shows two myosin molecules linked through the antibody at the hinge. (*B*) Model of the myosin–antibody complex based on the electron micrograph and the location of the antibody measured from several reference points—i.e., from the S1/S2 junction (parameter A) and from the end tip of the myosin tail (parameter C). (*C*) Histogram showing the distribution of measurements for parameter A—i.e., distance from the S1/S2 junction to the center of the antibody; this value corresponds to 55 nm. (*D*) Lanes 1–3, SDS/PAGE of dog cardiac myosin, dog cardiac S1, and bovine aortic myosin, respectively, stained with Coomassie brilliant blue; lanes 4–6, autoradiograms of Western blots (parallel with lanes 1–3), revealed with rabbit anti-hinge peptide antibody, showing no reactivity with dog cardiac S1 or with bovine aortic myosin. My HC, myosin heavy chain position; S-1 HC, S-1 heavy chain position.

assays, myofibrils responded to calcium as expected: the activity in the presence of calcium was 0.12 μmol of $P_i \cdot min^{-1} \cdot mg^{-1}$, and in its absence it was reduced to 0.034 μmol of $P_i \cdot min^{-1} \cdot mg^{-1}$ (Table 1). The corresponding activities in the presence of the antibody were 0.135 and 0.037 μmol of $P_i \cdot min^{-1} \cdot mg^{-1}$ (Table 1). Also, the antibody had no effect on the actin-activated ATPase of S1 in the presence of a 15-fold molar excess of actin. The specific activity in the absence of antibody was 0.27 μmol of $P_i \cdot min^{-1} \cdot mg^{-1}$ and 0.32 and 0.34 μmol of $P_i \cdot min^{-1} mg^{-1}$ in the presence of affinity-purified antibody at antibody-to-S1 molar ratios of 1:2 and 1:1, respectively. When DEAE-purified antibody was used at molar ratios of 1:3 and 1:1 the corresponding activities were 0.20 and 0.23 μmol of $P_i \cdot min^{-1} \cdot mg^{-1}$. Thus, the actin-activated ATPase activity remained essentially constant at all concentrations of the antibody used, indicating that it did not interfere with the active site of myosin.

The results obtained from motility measurements were drastically different. The measured rate of movement of control myosin was 0.510 ± 0.042 $\mu m \cdot s^{-1}$. This velocity was reduced to 0.247 ± 0.021 $\mu m \cdot s^{-1}$ in the presence of DEAE-purified antibody and it was zero in the presence of the affinity-purified antibody. The rate did not significantly change when nonimmune IgG was included in the assay mixture (Table 1). The rate of movement of actin filaments on

Table 1. Effect of anti-hinge peptide antibody on the motility of F-actin filaments on myosin-coated surfaces and on ATPase activity

Reaction mixture	Rate of movement, $\mu m \cdot s^{-1}$	ATPase activity, μmol of $P_i \cdot min^{-1} \cdot mg^{-1}$	
		Ca^{2+}	EGTA
Myosin	0.510 ± 0.042		
+ DEAE anti-hinge*	0.247 ± 0.021†		
+ affinity anti-hinge‡	0.00†		
+ DEAE anti-LC1	0.180 ± 0.022†		
+ affinity anti-LC1	0.00†		
+ nonimmune IgG	0.470 ± 0.023†		
Subfragment 1 (S1)	0.375 ± 0.04		
+ affinity anti-hinge‡	0.391 ± 0.01		
Myofibrils		0.12	0.034
+ anti-hinge§		0.135	0.037

LC1, light chain 1.
*Assayed in the presence of antibody purified on a Whatman DE-52 column alone.
†Assayed for motility after 30 min of incubation with the antibody.
‡Assayed in the presence of antibody further purified on an affinity column.
§Affinity-purified antibody was mixed with myofibrils (26) at a molar ratio of either 1:1 or 1.5:1 with respect to myosin, considering a 50% myosin content in myofibrils (27). The mixture was incubated overnight on ice to allow equilibration and binding of antibody to myosin. The KCl concentration during incubation was adjusted to 0.6 M to allow interaction of the antibody with myofibrillar myosin. Either nonimmune IgG or phosphate-buffered saline alone was included in separate assays to determine the specificity of the antibody for the observed effect.

S1 used as control was also not influenced in the presence of the antibody: the rates were 0.375 ± 0.04 and 0.391 ± 0.01 $\mu m \cdot s^{-1}$ (Table 1) in the presence and absence of the antibody, respectively, suggesting that the observed effect was specifically due to the binding of the antibody in the hinge region of the monomeric myosin used in these assays.

Measurements of Sarcomere Shortening. The response of the isolated myocytes to calcium iontophoresis was blocked within 30 min after addition of anti-hinge antibody (15 mg/ml, DEAE-purified) and 0.65 mg/ml (affinity-purified) to achieve a final concentration of 0.1 mg/ml. For example, all 13 control cells tested responded to iontophoresis, whereas only 1 in 14 and 0 in 4 responded after addition of DEAE- or affinity-purified antibody, respectively. However, shortening could still be induced by direct addition of large amounts of calcium (≈50 μl of 0.1 M $CaCl_2$) to the cells suppressed with either anti-hinge antibody (righthand panels in Fig. 3 *B* and *C*). The effect of antibody was reproduced in another heart where the cells were uniformly preexposed to 0.1 mg of antibody per ml overnight, at a calculated concentration of 25:1 antibody to total cellular myosin by weight in each aliquot. All 11 control cells responded by an average shortening of 0.39 ± 0.12 μm (mean ± SD); all 9 cells tested that were incubated in DEAE-purified antibody responded but shortened less, 0.18 ± 0.12 μm, whereas only 2 in 6 cells tested with affinity-purified antibody shortened (0.07 ± 0.16 μm), despite use of currents twice as long as used in the control cells. Observations in three additional hearts indicated that the peak velocity of shortening was reduced after 2–3 hr of exposure to antibody. For control the peak velocity was 1.86 ± 0.78 $\mu m \cdot s^{-1}$ (mean ± SD, 13 cells); peak velocity was 1.44 ± 1.20 $\mu m \cdot s^{-1}$ (18 cells), 0.62 ± 0.58 $\mu m \cdot s^{-1}$ (16 cells), and 0.48 ± 0.23 $\mu m \cdot s^{-1}$ (7 cells) for cells treated with 1.3 mg of DEAE-purified antibody per ml and 25 and 50 μg of affinity-purified antibody per ml, respectively. Thus, the antibody influenced the dynamics of shortening in the calcium-regulated contractile lattice.

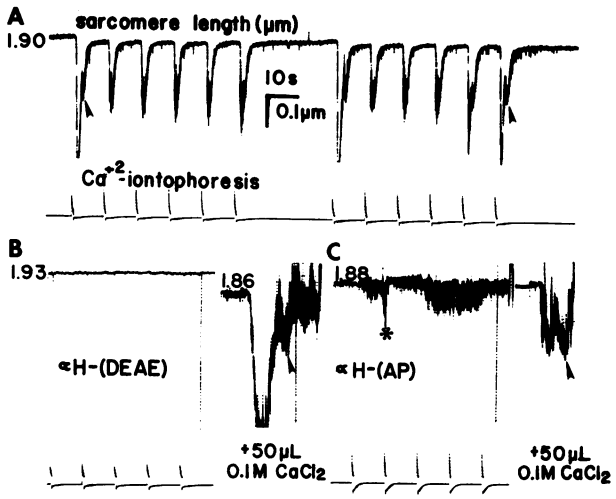

FIG. 3. Effect of anti-hinge antibody on shortening in unattached, isolated cardiac myocytes. Sarcomere length (top trace) and iontophoretic calcium current (lower trace). (*A*) Control cells reproducibly shorten and relengthen in response to 700-ms iontophoretic calcium pulses. (*B*) After addition of 100 μg of DEAE-purified antibody [αH-(DEAE)]. At right, addition of 50 μl of 0.1 M $CaCl_2$ shows that antibody-suppressed cells retain ability to shorten in response to unphysiologically high calcium concentrations. (*C*) After addition of 100 μg of affinity-purified antibody [αH-(AP)]. At right, the same cell shortens in response to 50 μl of 0.1 M $CaCl_2$. The asterisk denotes a focusing artifact. A biphasic relengthening trace, denoted by the arrowhead, may represent an artifact in the phase-locked loop method due to the difference in the formation of contraction bands in shortening and lengthening sarcomeres.

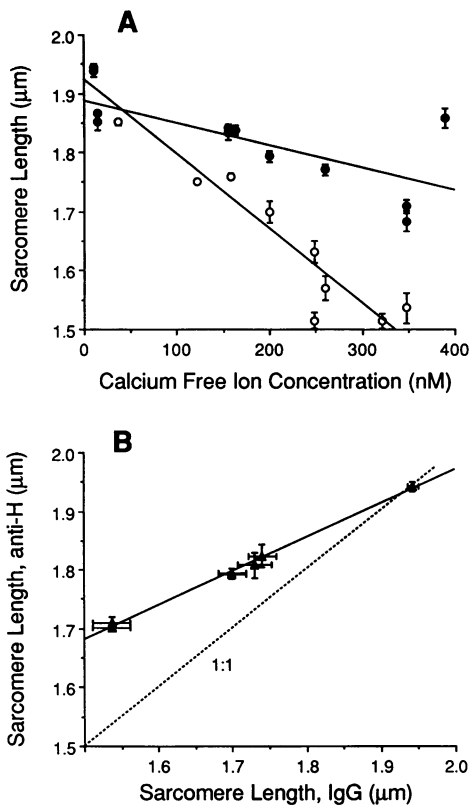

FIG. 4. (*A*) Demonstration of an effect of anti-hinge antibody upon shortening in response to controlled activation by steady calcium. Open symbols represent the mean ± SEM for 233 cells from three hearts that were preexposed to nonimmune IgG to test for nonspecific effects. Filled symbols represent data from 216 cells (four hearts) that were exposed to affinity-purified anti-hinge antibody. Lines represent the respective linear regression using mean data points: $Y = 1.89 - 0.38[Ca\ (\mu M)]$; $R^2 = 0.507$ (anti-hinge); $Y = 1.93 - 1.26[Ca\ (\mu M)]$; $R^2 = 0.92$. Individual data points represent the mean of the sarcomere length measured at given conditions. (*B*) Parametric display of the effects of anti-hinge (anti-H) with respect to a matched concentration of nonimmune IgG. Data represent mean ± SEM. In all cases, sarcomere length is graded by varying $[Ca^{2+}]$, which was also similar for any set of paired data. Points lying above a 1:1 correspondence indicate retarded shortening. The equation represents a linear regression of the means. Its slope, 0.58, indicates that shortening was reduced by 42% by the anti-hinge peptide antibody.

Contraction was studied when activation was steady, uniform, and controlled to rule out an unspecified viscous hindrance to motion. Fig. 4 illustrates the effect of overnight incubation with antibody upon the relationship between free calcium concentration and sarcomere length. To eliminate any potential effect of nonspecific protein binding and of added protein on the calcium ion equilibria (28, 29), control measurements were made in the presence of nonimmune IgG. Linear regression of the means indicated that calcium-activated steady shortening was reduced ≈50% by the anti-hinge antibody (Fig. 4*A*), although in one instance (see Fig. 4*A*, $[Ca^{2+}] = 400$ nM) the affinity-purified anti-hinge antibody almost completely repressed shortening. The antibody appeared to depress systematically steady sarcomere shortening when paired to controls in IgG under matched conditions of study (Fig. 4*B*). These parametric relations indicated that the slope of the dependence of length of sarcomeres exposed to anti-hinge antibody relative to those exposed to IgG (0.58, $R^2 = 0.99$) was equivalent to that of anti-hinge antibody relative to control cells (0.60, $R^2 = 0.77$).

A paired comparison of the effect of 0.15 mg of antibody per ml on cells isolated from the same hearts indicated that depression of shortening by affinity-purified antibody was always greater than that by DEAE-purified antibody. The greater impairment of steady shortening by the affinity-purified anti-hinge antibody parallels the corresponding differences observed in *in vitro* motility assays.

DISCUSSION

The actomyosin interaction constitutes the molecular mechanism of motility, regulation of contractility, and power generation in muscle. Since the S1 head of myosin contains all of the actin binding and enzymatic activities, it is likely to be involved in such a power stroke. At the same time, involvement of the S2 region in contraction has also been proposed, based on physical-chemical (5, 30) and immunochemical (10, 11) studies. Huxley (31) had earlier postulated a two-way coupling between performance of mechanical work and the splitting of ATP, where the hinge at the heavy meromyosin/light meromyosin junction provides an inherent mobility of myosin that allows cycling of crossbridges.

A recent molecular genetic study also suggested that the importance of S2 may extend beyond serving as a mechanical linkage between the thick filament backbone and S1. Alternative splicing of the myosin heavy chain (MHC) gene coding for the rod demonstrated the existence of MHC isoforms based on the variability of the hinge region. Expression of alternate hinges was selective and specific according to muscle function. When the expression of the hinge region was altered in flightless *Drosophila* mutants, the result was a disorganized sarcomere lattice and contractile dysfunction (32).

In vitro motility assays, however, have shown that the S2 region is not obligatory for movement. The myosin head alone, S1, is capable either of moving along actin filaments in the *Nitella*-based assay, of inducing actin filaments to move on S1-coated surfaces (1–3), or of generating force (4). However, the rate of movement induced by S1 in motility

assays is less than that induced by myosin or heavy meromyosin. It is not known whether this is due to improper binding of the S1 head to the surface or to a lack of an S2-hinge region. An explanation of shortening in terms of an obligatory role of simple, vectorial change in the length of S2 appears to be ruled out by the observation that the direction of myofilament sliding is determined by actin polarity rather than the orientation of myosin in native thick filaments (33). However, the slower velocity observed when actin filaments move away from the center of the thick filaments suggests that a properly oriented S2 region may be required for a maximal effect.

The biochemical and physiological data reported here provide a direct demonstration of the effect of the hinge on contraction by using a 20-amino acid residue peptide as antigen (15) rather than the whole S2 (10). In general, these observations parallel those made using anti-S2, which induced a marked decrease in force generation (10) and shortening (11) in ATP-activated skeletal muscle. The 50% reduction in the extent of shortening of cardiac myocytes as opposed to the total inhibition of *in vitro* motility may be due to limits to diffusion of the antibody or heterogeneity of myosin-antibody binding in the myocytes.

The results of our motility assays allow a more direct evaluation of the effect of antibody on cardiac myosin hinge. Affinity-purified anti-hinge peptide antibody stopped movement completely while not inhibiting the MgATPase activity. Anti-LC1 antibody, on the other hand, repressed movement, but this could be due to inhibition of ATP hydrolysis, which was suppressed by the antibody in cardiac myofibrils (S.S.M., unpublished data). Thus, the possibility of direct steric interference by the anti-hinge antibody is not likely because the binding site of the antibody is too far from the head/tail junction (55 nm) to obstruct S1 function directly. The fact that this occurs with myosin bound to the nitrocellulose-coated surface as monomers eliminates the possibility that the antibody acted by disrupting the normal packing order of myosin in filaments.

Mobility conferred by the S2 has been previously thought to be necessary to overcome the geometrical constraints to crossbridge attachment (31). However, the antibody to the hinge region used in these studies suppresses motility and contractility without impairing the ability of myosin to hydrolyze ATP. Thus, under conditions where geometric constraints and tension generation are minimal, the influence of the hinge extends also to the power stroke and/or subsequent steps in the crossbridge cycle.

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1. Toyoshima, Y. Y., Kron, S. J., McNally, E. M., Niebling, K. R., Toyoshima, C. & Spudich, J. A. (1987) *Nature (London)* **328,** 536–539.
2. Manstein, D. J., Ruppel, K. M. & Spudich, J. A. (1989) *Science* **246,** 656–658.
3. Hynes, T. R., Block, S. M., White, B. T. & Spudich, J. A. (1987) *Cell* **48,** 953–963.
4. Kishino, A. & Yanagida, T. (1988) *Nature (London)* **334,** 74–76.
5. Ueno, H. & Harrington, W. F. (1986) *J. Mol. Biol.* **190,** 69–82.
6. Ueno, H. & Harrington, W. F. (1986) *Biochemistry* **26,** 3589–3596.
7. Applegate, D. & Reisler, E. (1983) *J. Mol. Biol.* **169,** 455–468.
8. Tsong, T. Y., Karr, T. & Harrington, W. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
9. Walker, M. & Trinick, J. (1986) *J. Mol. Biol.* **192,** 661–667.
10. Lovell, S., Karr, T. & Harrington, W. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1849–1853.
11. Harrington, W. F., Karr, T., Busa, W. B. & Lovell, S. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7453–7456.
12. Margossian, S. S., Krueger, J. W., Sellers, J. R., Cuda, G. & Slayter, H. S. (1991) *Biophys. J.* **59,** 431a (abstr.).
13. Margossian, S. S. (1985) *J. Biol. Chem.* **260,** 13747–13754.
14. Margossian, S. S., Chantler, P. D., Sellers, J. R., Malhotra, A., Stafford, W. F. & Slayter, H. S. (1984) *J. Biol. Chem.* **259,** 13534–13540.
15. Kavinsky, C. J., Umeda, P. K., Levin, J. E., Sinha, A. M., Nigro, J. M., Jakovcic, S. & Rabinowitz, M. (1984) *J. Biol. Chem.* **259,** 2775–2781.
16. McNally, E. M., Kraft, R., Bravo-Zehnder, M., Taylor, D. A. & Leinwand, L. A. (1989) *J. Mol. Biol.* **210,** 665–671.
17. Walter, G., Scheidtmann, K.-H., Carbone, A., Laudano, A. P. & Doolittle, R. F. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5197–5200.
18. Saglio, S. D. & Slayter, H. S. (1982) *Blood* **59,** 162–166.
19. Slayter, H. S. (1989) *Methods Enzymol.* **169,** 251–269.
20. Slayter, H. S. (1976) *Ultramicroscopy* **1,** 341–357.
21. Krueger, J. W., Forletti, D. & Wittenberg, B. A. (1980) *J. Gen. Physiol.* **88,** 475–607.
22. Myers, J., Tirosh, R., Jacobson, R. C. & Pollack, G. H. (1982) *IEEE Trans. Biomed. Eng.* **29,** 463–466.
23. Krueger, J. W., Denton, A., Siciliano, G. & Tiberio, R. C. (1989) *Biophys. J.* **55,** 268a (abstr.).
24. Umemoto, S. & Sellers, J. R. (1990) *J. Biol. Chem.* **265,** 14864–14869.
25. Margossian, S. S. & Lowey, S. (1973) *J. Mol. Biol.* **74,** 313–330.
26. Solaro, R. J., Pan, D. C. & Briggs, F. N. (1971) *Biochim. Biophys. Acta* **245,** 259–262.
27. Dreizen, P. & Gershman, L. C. (1970) *Trans. N.Y. Acad. Sci.* Ser. 2 **32,** 170–203.
28. Fabiato, A. & Fabiato, F. (1979) *J. Physiol. (Paris)* **75,** 413–463.
29. Fabiato, A. (1981) *J. Gen. Physiol.* **78,** 457–497.
30. Harrington, W. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5066–5070.
31. Huxley, H. E. (1969) *Science* **164,** 1356–1366.
32. Collier, V. L., Kronert, W. A., O'Donnell, P. T., Edwards, K. A. & Bernstein, S. I. (1990) *Genes Dev.* **4,** 885–895.
33. Sellers, J. R. & Kachar, B. (1990) *Science* **249,** 406–408.