Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 5739–5743, July 1991
Biophysics

Parallel inhibition of active force and relaxed fiber stiffness in skeletal muscle by caldesmon: Implications for the pathway to force generation

BERNHARD BRENNER*†, LEEPO C. YU‡, AND JOSEPH M. CHALOVICH§

*Department of General Physiology, University of Ulm, D-7900 Ulm, Federal Republic of Germany; ‡Laboratory of Physical Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892; and §Department of Biochemistry, East Carolina University, Greenville, NC 27858-4354

Communicated by Terrell L. Hill, March 25, 1991 (received for review December 17, 1990)

ABSTRACT **In recent hypotheses on muscle contraction, myosin cross-bridges cycle between two types of actin-bound configuration. These two configurations differ greatly in the stability of their actin–myosin complexes ("weak-binding" vs. "strong-binding"), and force generation or movement is the result of structural changes associated with the transition from the weak-binding (preforce generating) configuration to strong-binding (force producing) configuration [cf. Eisenberg, E. & Hill, T. L. (1985) *Science* 227, 999–1006]. Specifically, in this concept, the main force-generating states are only accessible after initial cross-bridge attachment in a weak-binding configuration. It has been shown that strong and weak cross-bridge attachment can occur in muscle fibers [Brenner, B., Schoenberg, M., Chalovich, J. M., Greene, L. E. & Eisenberg, E. (1982) *Proc. Natl. Acad. Sci. USA* 79, 7288–7291]. However, there has been no evidence that attachment in the weak-binding states represents an essential step leading to force generation. It is shown here that caldesmon can be used to selectively inhibit attachment of weak-binding cross-bridges in skeletal muscle. Such inhibition causes a parallel decrease in active force, while the kinetics of cross-bridge turnover are unchanged by this procedure. This suggests that (*i*) cross-bridge attachment in the weak-binding states is specific and (*ii*) force production can only occur after cross-bridges have first attached to actin in a weakly bound, nonforce-generating configuration.**

It is generally accepted that muscle contraction is the result of myosin cross-bridge interactions with actin. It is a cyclic process that is driven by ATP hydrolysis in the presence of Ca^{2+} (1–3). However, the detailed mechanism of the cross-bridge cycle and its correlation with the various steps of ATP hydrolysis are still unresolved.

Lymn and Taylor (4) made an early attempt to correlate the steps of ATP hydrolysis with the various states postulated for the contractile cycle. In their scheme, ATP irreversibly dissociated myosin from actin. Later studies showed that myosin–ATP complexes did bind to actin but with a relatively low affinity (5, 6). In all recently developed biochemical schemes (6–8), actomyosin alternates between two groups of states known as "weak-binding" and "strong-binding" states. Cross-bridges in weak-binding states, with bound ATP or ADP plus P_i, are characterized by (*i*) low affinity to actin that is only slightly affected by Ca^{2+} (9–13), (*ii*) rapid attachment/detachment kinetics (6, 9, 14), and (*iii*) most importantly, their inability to activate the actin–tropomyosin–troponin complex (15, 16). In contrast, the strong-binding states, with bound ADP or without any bound nucleotide, are characterized by (*i*) a higher actin affinity (17–21), (*ii*) slower attachment/detachment kinetics (20, 22), and (*iii*) their ability to activate the actin–tropomyosin–troponin system (23, 24). Extending the biochemical data to the cross-bridge action in muscle, the force generation is hypothesized to be associated with the transition from an *attached* weak-binding state to a strong-binding state (cf. refs. 8, 25, 26). In this concept, force generation is possible only after a cross-bridge has first attached to actin in a weak-binding state immediately preceding the transition into the strong-binding states (e.g., state "A" in Fig. 1). However, thus far there is no evidence that the preforce-generating attached state, such as state "A" (in Fig. 1), is a weak-binding state.

Evidence for two groups of cross-bridge states, similar to the biochemical weak- and strong-binding states in solution, has been obtained in skinned (demembranated) rabbit psoas fibers (27–29). However, despite the demonstration that weak-binding cross-bridges (e.g., in relaxed fibers) do attach to actin without generating force, the critical question remains whether weak attachment is required for force generation—i.e., whether the state labeled "A" in Fig. 1 is indeed of the low-affinity type.

To characterize this state, an approach is required by which the weak attachment to actin is selectively inhibited. If state A in Fig. 1 is of the low-affinity type, then active force should decrease as the weak cross-bridge attachment is inhibited; otherwise, active force should be unaffected. Diffusion of the smooth muscle actin-binding protein caldesmon (or its actin-binding fragment) into skinned rabbit psoas fibers proved to be an ideal agent for inhibition of weak cross-bridge binding. Caldesmon inhibits the actin-activated ATP hydrolysis of myosin by inhibiting the weak binding of myosin to actin (30–32). In addition, caldesmon inhibits the ATPase activity of fully activated skeletal myofibrils in the presence of the troponin/tropomyosin system (33). Using caldesmon as a competitive inhibitor, the results of the present study support the concept that state A in Fig. 1 is of the weak-binding type—i.e., an *attached* weak-binding state is an essential intermediate for force generation. Preliminary results have been previously presented (34).

MATERIALS AND METHODS

Caldesmon. Caldesmon was prepared from turkey gizzards by a modification of the method of Lynch *et al.* (35). For the preparation of actin-binding fragments, caldesmon was digested for 5 min at 25°C with a 1:1000 molar ratio of chymotrypsin to caldesmon (36). A mixture of three actin-binding fragments originating from a common region of caldesmon was isolated using an Affi-Gel (Bio-Rad) column and HPLC cation-exchange on a Waters SP column. Details of the purification of the fragments and characterization of their properties will be published elsewhere.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†To whom reprint requests should be addressed.

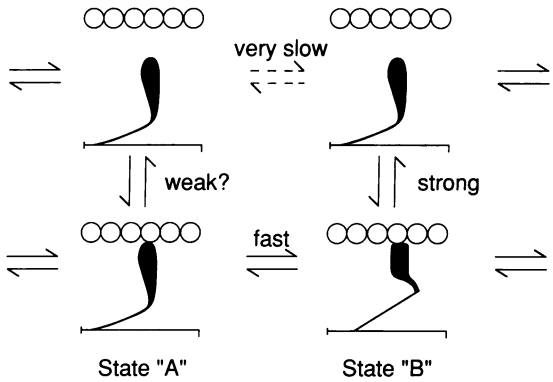

FIG. 1. Schematic representation of part of the cross-bridge cycle where the rate constants for the horizontal reactions are very different for detached and attached cross-bridges. This arrangement accounts for actin activation of actomyosin ATPase since the transition between states A and B is much faster than the corresponding reaction while the cross-bridge is detached. Thus, the cross-bridge can only proceed through the cycle sufficiently fast by undergoing the A to B transition. In the recent biochemical kinetic schemes this reaction step is associated with release of inorganic phosphate and is assumed to represent the transition from the weak- to the strong-binding cross-bridge states. However, it is as yet unclear whether the first pair of states shown here is indeed characterized by low actin affinity (weak-binding) or by high actin affinity (strong-binding). Relative lengths of arrows do not correlate with the equilibrium constants of the corresponding reactions.

Fiber Preparation and Solutions. Single, chemically skinned rabbit psoas fibers were prepared according to Brenner (37, 38). Sarcomere length was 2.3–2.4 μm; temperature was 5°C; ionic strength (μ) was 50 mM or 170 mM as indicated in *Results*. The compositions of solutions (in mM) at pH 7.0 were as follows. Relaxing solution: 10 imidazole, 1 EGTA, 2 $MgCl_2$, 1 MgATP, 1 dithiothreitol; activating solution: 10 imidazole, 1 ATP, 3 $MgCl_2$, 1 CaEGTA, 10 CrP, 250–300 units of creatine phosphokinase (Sigma), 10 caffeine, 1 dithiothreitol; rigor solution: 10 imidazole, 2.5 EGTA, 2.5 EDTA; $MgPP_i$ (pyrophosphate) solution: 10 imidazole, 2 $MgCl_2$, 4 $MgPP_i$, 1 CaEGTA. μ was adjusted by adding potassium propionate.

Mechanical Apparatus. The mechanical setup, including the system for laser light diffraction for recording sarcomere length, was previously described (39, 40).

Fiber Stiffness. Fiber stiffness was measured during rapid ramp-shaped stretches that were imposed on one end of the fiber. Fiber stiffness was defined as the force change when filament sliding has reached 2 nm per half-sarcomere (chord stiffness; ref. 41).

Rate Constant of Force Redevelopment. The rate constant of force redevelopment was determined during the rise in force subsequent to a period of lightly loaded (or unloaded) shortening and restretch of the fiber to the original isometric filament overlap (ref. 42; compare Fig. 4*b Inset*).

Equatorial X-Ray Diffraction. To detect mass shift between myosin and actin, intensities of the two innermost equatorial reflections, [1, 0] and [1, 1], were recorded. The intensity ratio I_{11}/I_{10} was used as a measure of attachment of cross-bridges. Diffraction patterns were recorded from single skinned fibers under the same conditions used for mechanical measurements. Procedures of x-ray experiments follow those of Yu and Brenner (38).

RESULTS

Decrease in Relaxed Fiber Stiffness by Caldesmon. Caldesmon was found to be an effective agent for inhibiting attachment of weak-binding cross-bridges to actin in skinned fibers. Fig. 2*a* shows the stiffness of a segment of a relaxed skinned

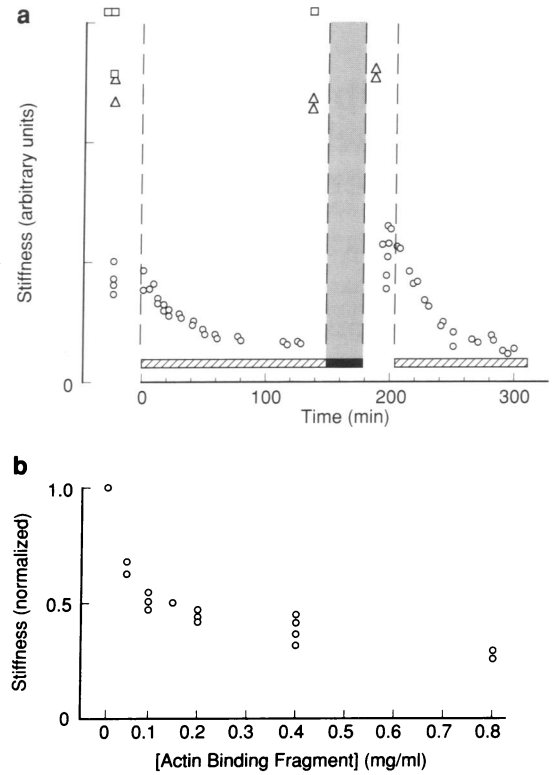

FIG. 2. (*a*) Time course of the effect of intact caldesmon on apparent fiber stiffness under relaxing conditions (○; μ = 50 mM), in rigor (□; μ = 50 mM), and in the presence of $MgPP_i$ (△; μ = 170 mM). The time period when intact caldesmon (0.3 mg/ml) was present is indicated by ▨. The apparent fiber stiffness is the force increase after a stretch of 2 nm per half-sarcomere with a stretch velocity of about 10^4 nm per half-sarcomere·s^{-1}. The caldesmon effect was reversed by washing out the caldesmon in a $MgPP_i$ solution containing 0.1 mg of Ca^{2+}/calmodulin per ml (μ = 170 mM) for 30 min (represented by ■). The effect of intact caldesmon was only partially reversed by washing the fiber in a relaxing solution without caldesmon (μ = 170 mM). The percent inhibition reached in the first and second applications of caldesmon was 61% and 76%, respectively. (*b*) Effect of the actin-binding fragment of caldesmon on fiber stiffness under relaxing conditions (μ = 50 mM). An equilibration period of 1 hr was allowed after each addition of fragment, after which time no further significant change in apparent fiber stiffness was observed. The effect of the actin-binding fragment could be reversed by incubation in $MgPP_i$ solution (pCa 4.5; μ = 170 mM) with 0.1 mg of calmodulin per ml or by raising the ionic strength under relaxing conditions to 170 mM. Slow reversal was also observed by incubation for 2–3 hr in relaxing solution, without the fragment, at μ = 50 mM. The percent inhibition at 0.8 mg of the actin binding fragment per ml was 71%.

fiber as a function of time after addition of intact caldesmon. The large decrease in stiffness suggests that the fraction of cross-bridges attached to actin in the relaxed fiber is decreased through the inhibitive effect of caldesmon. Also, as shown in Fig. 2*a*, the effect of caldesmon is fully reversible.

Caldesmon has an inhibitory, actin-binding region at its C-terminal end (36, 43) and a myosin-binding region at its N-terminal end (32, 44). Under some conditions, caldesmon can link actin to myosin through these binding sites (31). However, the effects on fiber stiffness (Fig. 2*a*) are only due to the binding of caldesmon to actin. The 20-kDa actin-binding fragments isolated from chymotryptically digested caldesmon (32) have the same ability in reducing stiffness of relaxed fibers as intact caldesmon (Fig. 2*b*). At 0.8 mg of the actin-binding fragments per ml, the relaxed stiffness is reduced by 70%. At high concentrations of the fragments (2.8 mg/ml), the relaxed stiffness is further reduced to about 25%

of the unperturbed value (≤5% of the rigor stiffness). Some of this remaining stiffness may well be due to elastic components other than cross-bridges (e.g., cytoskeleton).

Decrease in Equatorial Intensity Ratio I_{11}/I_{10} of the Relaxed Fiber by Caldesmon. The idea that the observed decrease in stiffness of the relaxed fiber is due to decreased cross-bridge attachment is supported by studies of the two innermost equatorial reflections [1, 0] and [1, 1] of the x-ray diffraction pattern. The intensity ratio I_{11}/I_{10} decreases as mass is moved away from the thin filaments (45, 46). Fig. 3 shows that addition of actin-binding fragments of caldesmon increases I_{10} slightly, whereas I_{11} decreases substantially, indicating that the fraction of cross-bridges attached to actin is reduced. Again, this effect is reversed after washing out caldesmon (see the legend to Fig. 2 for conditions).

Attachment in the Strong-Binding States Is Not Inhibited by Caldesmon. In solution, the binding of caldesmon to actin is tighter than the binding of weak-binding cross-bridges to actin but comparable to that of strong-binding cross-bridges (47). Thus, caldesmon readily displaces weak-binding cross-bridges from actin, whereas strong-binding cross-bridges tend to displace caldesmon from actin (31). Thus, the competition between caldesmon and cross-bridge binding to actin, in solution, is determined by the actin affinity of caldesmon relative to that of the various cross-bridge states. Similar effects were found in the fiber: neither the intact caldesmon (Fig. 2*a*) nor its fragments (data not shown) can affect fiber stiffness in rigor or in the presence of $MgPP_i$ (pCa 4.5), whereas the weak interaction, as shown by the decrease in relaxed fiber stiffness, is greatly inhibited.

In the Presence of Caldesmon Active Force Is Reduced in Parallel with Relaxed Fiber Stiffness. Fig. 4*a* shows a parallel decrease in the relaxed fiber stiffness and active force as the caldesmon fragment concentration is increased. The percentage reduction in force is slightly more than that found for relaxed stiffness. At the highest concentration of caldesmon fragment used (2.8 mg/ml), the stiffness was reduced by 75%, and the force was reduced by 80%. This slight discrepancy could mean that a small percentage of the relaxed stiffness originates from some viscoelastic elements of the sarcomere.

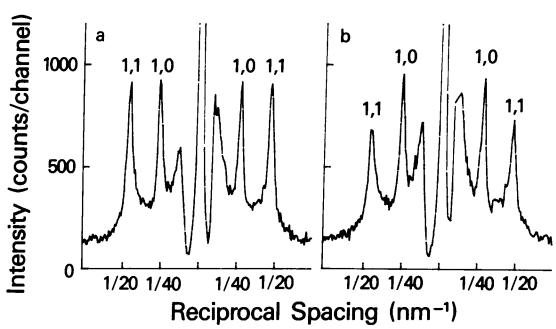

FIG. 3. Effect of the actin-binding fragment of caldesmon on the equatorial x-ray diffraction pattern of a relaxed fiber at μ = 50 mM. Experimental procedures followed those of ref. 25. (*a*) In the absence of caldesmon. (*b*) In the presence of 0.2 mg of actin-binding fragment of caldesmon per ml. Integrated intensities in *b* as compared to *a*: I_{10} increased by 7%; I_{11} decreased by 22%. Hence, I_{11}/I_{10} decreased by 27%. Integrated intensities were obtained by nonlinear curve fitting (38). Quantitative assessment of the change of cross-bridge attachment to actin from the changes in the I_{11}/I_{10} ratio is difficult because of the addition of mass to the actin filament by binding of caldesmon. Adding mass to actin increases the I_{11}/I_{10} ratio, whereas reducing cross-bridge attachment (reduced amount of cross-bridge mass near actin) decreases the I_{11}/I_{10} ratio (45, 46). To assess effects from binding of caldesmon to actin, geometry and extent of caldesmon binding have to be known exactly. In principle, however, since the actin-binding caldesmon fragments are polypeptides of 20 kDa, added mass should lessen the effect of decreasing I_{11}/I_{10} expected from inhibition of cross-bridge binding to actin.

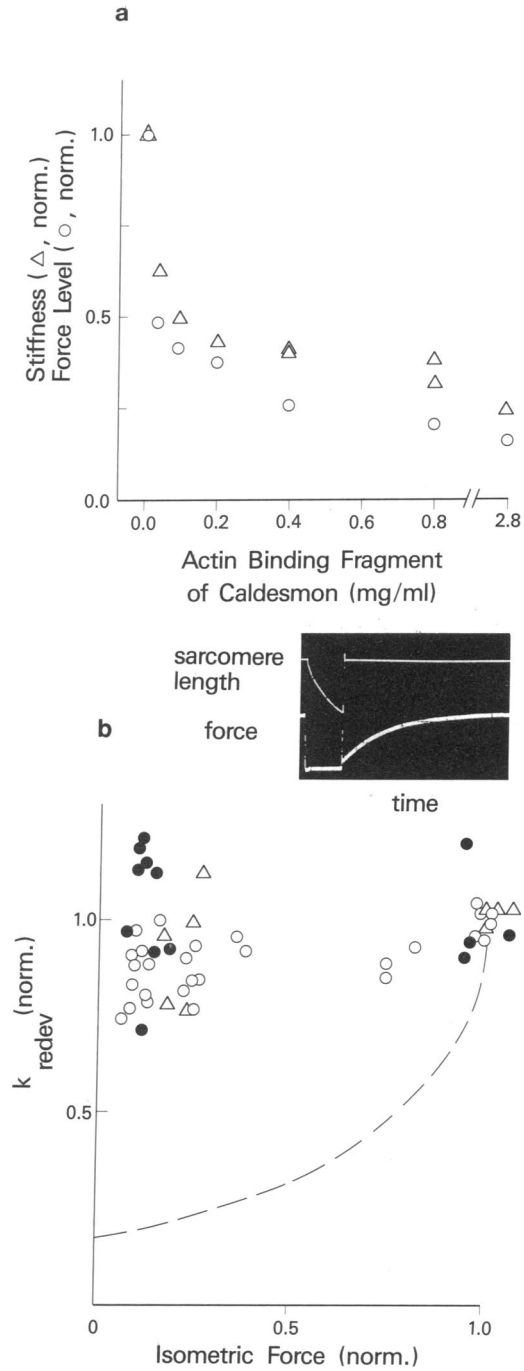

FIG. 4. (*a*) Effect of the actin-binding fragment of caldesmon on stiffness of relaxed fibers (△) and on active force (○) at μ = 50 mM. (*b*) Rate constant of force redevelopment (k_{redev}) vs. active force at increasing concentrations of the actin-binding fragment (○, μ = 170 mM; △, μ = 53 mM) or of intact caldesmon (●, μ = 170 mM). For the experimental procedure of recording force redevelopment subsequent to isotonic shortening, see *Inset*. Further details were previously described (48). Note that increasing concentrations of caldesmon or of its actin-binding fragment reduce isometric force without significantly affecting k_{redev}. This is in contrast to the reduction of active force by troponin–tropomyosin, where k_{redev} decreases to about 20% of its maximum value, as $[Ca^{2+}]$ is lowered (dashed line). norm., Normalized.

The same parallel inhibition of relaxed stiffness and active force was also observed at higher (physiological) μ (120 mM, 170 mM) and at higher temperature (20°C) (49).

Reduction in Force Level Is Not Due to Changes in Turnover Kinetics of Cross-Bridges. The preceding results are consis-

tent with the concept that caldesmon inhibits force generation by blocking actin sites available for weak cross-bridge attachment. However, the results do not rule out effects of caldesmon on the rate constants characterizing the transition between weak- and strong-binding states (f_{app} and g_{app}) (48). Changes in these rate constants could also affect the active force level. The possibility of changing f_{app} and/or g_{app} by caldesmon was investigated by measuring the rate constant k_{redev} of force redevelopment subsequent to a period of isotonic shortening. Previous studies have shown that k_{redev} is a measure of the rate constants of active cross-bridge turnover ($k_{redev} = f_{app} + g_{app}$). Fig. 4*b* shows that the reduction in isometric force caused by caldesmon occurs without a corresponding change in k_{redev}, whereas reduction in force by tropomyosin–troponin (by lowering the Ca^{2+} concentration) is associated with a decrease in k_{redev}. This implies that caldesmon only decreases the number of weak binding cross-bridges attached to actin without affecting the turnover kinetics of the cross-bridge cycle.

DISCUSSION

Our data are consistent with the idea that caldesmon inhibits attachment of weak-binding cross-bridges by blocking sites on actin available for such binding. In parallel, the active force level is reduced without a change in the turnover kinetics.

Binding of Caldesmon to Actin Is Specific. Intact caldesmon (47) and its actin-binding fragment (ref. 50; L. Velaz and J.M.C., unpublished observation) bind to actin with a constant stoichiometry. Both inhibit the binding of one molecule of S-1 for each molecule of actin covered by caldesmon (L. Velaz and J.M.C., unpublished observation; J.M.C. and Y. D. Chen, unpublished observation) and both bind to regions of actin that are known to be involved in myosin binding (51–53). Such high specificity ensures that the effects of caldesmon or its actin-binding fragment on relaxed fiber stiffness and on x-ray diffraction patterns result from its binding to discrete sites on actin. As a consequence, the present data resolve one of the frequently debated questions concerning the attachment of weak-binding cross-bridges in the relaxed states. Although the evidence for cross-bridge attachment in the weak-binding states has been extensive (54), the question remains whether the binding to actin is specific. With the extensive inhibition by the highly specific binding of the filamentous caldesmon molecule (diameter ≈ 20 Å) to actin, it is likely that the attachment of the weak-binding cross-bridges to actin occurs at specific sites.

Attachment in the Force-Producing States Is Not Likely Affected by Caldesmon. The binding constant for $MgPP_i$ cross-bridges in muscle at pCa 4.5 and μ 170 mM is about 3–4 (55). This, however, is about the minimum value necessary for force-generating cross-bridges. Otherwise, the strained cross-bridges required for force generation would preferably be detached and thus the observed active force cannot be accounted for (56). Therefore, the affinity of myosin for actin in $MgPP_i$ under these conditions is thought to be less than or at most equal to that of force-producing cross-bridges. As shown earlier, attachment of $MgPP_i$ cross-bridge is not inhibited by caldesmon (Fig. 2*a*). Consequently, attachment in the force-producing states is highly unlikely to be significantly inhibited by caldesmon.

Actin Attachment of a Weak-Binding State Is Essential for Force Generation. The results presented are consistent with the concept that in the cross-bridge cycle, force-generating states are accessible only from a weakly attached nonforce-generating precursor (25); as shown in Fig. 1, blocking attachment into state A by competitive inhibition of weak cross-bridge binding inhibits the formation of state B—i.e., inhibits transition into the force-generating states. On the other hand, if state A were not of the weak-binding type, caldesmon would not competitively displace it from actin and there would be no effect on force generation. Thus, our data suggest that an attached weak-binding state is an essential intermediate in the pathway to force generation, supporting the hypothesis that at least the first reaction step associated with force generation is the transition from the weak-binding to the strong-binding group of cross-bridge states. This concept of transition from weak to strong configurations is supported by recent x-ray diffraction work that indicates the attachment mode is different for weakly and strongly bound cross-bridges (38, 57). Needless to say, there may be additional structural changes associated with transition between various strong-binding force-generating states. In our view (58), the initial weak cross-bridge attachment could represent the often-postulated attached "preforce-generating state" (e.g., refs. 59 and 60). Several experimental observations (e.g., changes in stiffness and x-ray diffraction patterns preceding force in the rising phase of a tetanus) have led to the proposal of preforce-generating states (59, 60). As we have demonstrated elsewhere (58), the mechanical (27, 29, 61) and structural (28, 38, 57) properties of the weak cross-bridge attachment can account for these observations, and no preforce-generating states other than the weak-binding type of states need to be assumed.

The studies presented here were done at relatively low ionic strength and at low temperature conditions, which facilitate measurement of the weak attachment of cross-bridges to actin. However, using the inhibitory effect of caldesmon to probe for weak cross-bridge attachment to actin, we have recently observed results similar to those shown here, at temperatures as high as 20°C and at μ up to 170 mM (49). Details of these results will be presented elsewhere.

Contrasting Effects of Caldesmon Inhibition vs. Ca^{2+} Regulation by Means of Troponin–Tropomyosin. It was shown in Fig. 4*b* that caldesmon does not affect k_{redev}—i.e., turnover kinetics. In contrast, k_{redev} does change when force generation is modulated by free Ca^{2+} concentration by means of the tropomyosin–troponin system (dashed line). The differences in modulation of active force by caldesmon and tropomyosin–troponin are rather striking. Not only do they have distinct effects on the rate of force redevelopment but also Ca^{2+} regulation by means of tropomyosin–troponin has only a small effect on the fraction of attached weak-binding cross-bridges (9, 61), whereas caldesmon causes a large decrease in weak cross-bridge binding. Thus, tropomyosin–troponin does not prevent the binding of myosin to actin as suggested by the steric blocking model (62–64). Rather, tropomyosin–troponin has its primary effect on the rate of cross-bridge cycling kinetics (48).

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Br849/1-3; B.B.), the National Institutes of Health (AR35216 and AR40540-01A1; J.M.C.), and North Atlantic Treaty Organization (900257; B.B., L.C.Y., and J.M.C.).

1. Huxley, A. F. (1957) *Prog. Biophys. Biophys. Chem.* **7**, 255–318.
2. Pringle, J. W. S. (1967) *Prog. Biophys.* **17**, 1–60.
3. Huxley, H. E. (1969) *Science* **164**, 1136–1366.
4. Lymn, R. W. & Taylor, E. W. (1971) *Biochemistry* **10**, 4617–4624.
5. Sleep, J. A. & Hutton, R. L. (1978) *Biochemistry* **17**, 5423–5430.
6. Stein, L. A., Schwarz, R. P., Chock, P. B. & Eisenberg, E. (1979) *Biochemistry* **18**, 3895–3909.
7. Rosenfeld, S. S. & Taylor, E. W. (1984) *J. Biol. Chem.* **259**, 11908–11919.
8. Hibberd, M. G. & Trentham, D. R. (1986) *Annu. Rev. Biophys. Biophys. Chem.* **15**, 119–161.

9. Chalovich, J. M., Chock, P. B. & Eisenberg, E. (1981) *J. Biol. Chem.* **256,** 575–578.
10. Wagner, P. D. & Giniger, E. (1981) *J. Biol. Chem.* **256,** 12647–12650.
11. Chalovich, J. & Eisenberg, E. (1982) *J. Biol. Chem.* **257,** 2431–2437.
12. Wagner, P. D. (1984) *Biochemistry* **23,** 5950–5956.
13. Chalovich, J. M. & Eisenberg, E. (1986) *J. Biol. Chem.* **261,** 5088–5093.
14. White, H. D. & Taylor, E. (1979) *Biochemistry* **15,** 5818–5826.
15. Chalovich, J. M., Greene, L. E. & Eisenberg, E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4909–4913.
16. Greene, L. E., Williams, D. L. & Eisenberg, E. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3102–3106.
17. Marston, S. B. & Weber, A. (1975) *Biochemistry* **14,** 3868–3873.
18. Highsmith, S. (1977) *Arch. Biochem. Biophys.* **180,** 404–408.
19. Margossian, S. S. & Lowey, S. (1978) *Biochemistry* **17,** 5431–5439.
20. White, H. D. & Taylor, E. (1976) *Biochemistry* **15,** 5818–5826.
21. Greene, L. E. & Eisenberg, E. (1980) *J. Biol. Chem.* **255,** 543–548.
22. Marston, S. B. (1982) *Biochem. J.* **203,** 453–460.
23. Bremel, R. D. & Weber, A. (1972) *Nature (London) New Biol.* **238,** 97–101.
24. Greene, L. E. & Eisenberg, E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2616–2620.
25. Eisenberg, E. & Hill, T. L. (1985) *Science* **227,** 999–1006.
26. Stein, L. A. (1988) *Cell Biophys.* **12,** 29–58.
27. Brenner, B., Schoenberg, M., Chalovich, J. M., Greene, L. E. & Eisenberg, E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7288–7291.
28. Brenner, B., Yu, L. C. & Podolsky, R. J. (1984) *Biophys. J.* **46,** 299–306.
29. Brenner, B., Chalovich, J., Greene, L. E., Eisenberg, E. & Schoenberg, M. (1986) *Biophys. J.* **50,** 685–691.
30. Chalovich, J. M., Cornelius, P. & Benson, C. E. (1987) *J. Biol. Chem.* **262,** 5711–5716.
31. Hemric, M. E. & Chalovich, J. M. (1988) *J. Biol. Chem.* **263,** 1878–1885.
32. Velaz, L., Ingraham, R. & Chalovich, J. M. (1990) *J. Biol. Chem.* **265,** 2929–2934.
33. Chalovich, J. M. (1988) *Cell Biophys.* **12,** 73–85.
34. Brenner, B., Yu, L. C. & Chalovich, J. M. (1990) *Biophys. J.* **57,** 397 (abstr.).
35. Lynch, W. P., Reisman, V. M. & Bretscher, A. (1987) *J. Biol. Chem.* **262,** 7429–7437.
36. Szpacenko, A. & Dabrowska, R. (1986) *FEBS Lett.* **202,** 182–186.
37. Brenner, B. (1983) *Biophys. J.* **41,** 99–102.
38. Yu, L. C. & Brenner, B. (1989) *Biophys. J.* **55,** 441–453.
39. Brenner, B. (1980) *J. Muscle Res. Cell Motil.* **1,** 409–428.
40. Brenner, B. & Eisenberg, E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3542–3546.
41. Simmons, R. M. & Jewell, B. R. (1974) *Recent Adv. Physiol.* **31,** 87–147.
42. Brenner, B. (1985) *J. Muscle Res. Cell Motil.* **6,** 659–664.
43. Bryan, J., Imai, M., Lee, R., Moore, P., Cook, R. & Lin, W. (1989) *J. Biol. Chem.* **264,** 13873–13879.
44. Sutherland, C. & Walsh, M. P. (1989) *J. Biol. Chem.* **264,** 578–583.
45. Huxley, H. E. (1968) *J. Mol. Biol.* **37,** 507–520.
46. Yu, L. C. (1989) *Biophys. J.* **55,** 433–440.
47. Velaz, L., Hemric, M. E., Benson, C. E. & Chalovich, J. M. (1989) *J. Biol. Chem.* **264,** 9602–9610.
48. Brenner, B. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3265–3269.
49. Kraft, T., Chalovich, J. M., Yu, L. C. & Brenner, B. (1991) *Biophys. J.* **59,** 375 (abstr.).
50. Reisman, V. M., Lynch, W. P., Netsky, B. & Bretscher, A. (1989) *J. Biol. Chem.* **264,** 2869–2875.
51. Levine, B. A., Moir, A. J. G., Audemard, E., Mornet, D., Patchell, V. B. & Perry, S. V. (1990) *J. Biochem.* **193,** 687–696.
52. Adams, S., DasGupta, G., Chalovich, J. M. & Reisler, E. (1990) *J. Biol. Chem.* **265,** 19652–19657.
53. Bartegi, A., Fattoum, A. & Kassab, R. (1990) *J. Biol. Chem.* **265,** 2231–2237.
54. Brenner, B. (1987) *Annu. Rev. Physiol.* **49,** 655–672.
55. Brenner, B., Yu, L. C., Green, L. E., Eisenberg, E. & Schoenberg, M. (1986) *Biophys. J.* **50,** 1101–1108.
56. Hill, T. L. (1974) *Prog. Biophys. Mol. Biol.* **28,** 267–340.
57. Yu, L. C., Maeda, Y. & Brenner, B. (1990) *Biophys. J.* **57,** 409 (abstr.).
58. Brenner, B. (1990) in *Molecular Mechanisms in Muscular Contraction*, ed. Squire, J. M. (Macmillan, London), pp. 77–149.
59. Huxley, H. E. & Kress, M. J. (1985) *J. Muscle Res. Cell Motil.* **6,** 153–161.
60. Ford, L. E., Huxley, A. J. & Simmons, R. M. (1986) *J. Physiol. (London)* **372,** 595–609.
61. Kraft, T., Yu, L. C. & Brenner, B. (1990) *Biophys. J.* **57,** 410 (abstr.).
62. Haselgrove, J. C. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **37,** 341–352.
63. Huxley, H. E. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **37,** 361–376.
64. Parry, D. A. D. & Squire, J. M. (1973) *J. Mol. Biol.* **75,** 33–55.