Characterization of a Caldesmon Fragment That Competes with Myosin-ATP Binding to Actin

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ABSTRACT The protein caldesmon inhibits actin-activated ATP hydrolysis of myosin and inhibits the binding of myosin·ATP to actin. A fragment isolated from a chymotryptic digest of caldesmon contains features of the intact molecule that make it useful as a selective inhibitor of the binding of myosin·ATP complexes to actin without having the complexity of binding to myosin. The COOH-terminal 20 kDa region of caldesmon binds to actin with one-sixth the affinity of caldesmon with a stoichiometry of binding of one fragment per two actin monomers. This contrasts with the 1:6–9 stoichiometry of intact caldesmon. The binding of the 20 kDa fragments to actin is totally reversed by Ca^{2+}-calmodulin and, like intact caldesmon, the 20 kDa fragments are competitive with the binding of myosin subfragments to actin. This competition with myosin binding is largely responsible for the inhibition of ATP hydrolysis, although both the fragments and intact caldesmon also reverse the potentiation of ATPase activity caused by tropomyosin. These polypeptides are useful both in defining the function of caldesmon and in studying the role of weakly bound cross-bridges in muscle.

INTRODUCTION

Caldesmon is associated with actin filaments in smooth muscle and nonmuscle cells and has been implicated in the regulation of motility (1–8). The inhibition of actin-activated myosin ATPase activity by caldesmon appears to occur by a mechanism different from that of the tropomyosin-troponin complex of striated muscle. Studies in solution (9, 10) and in single skeletal muscle fibers (6) have shown that tropomyosin-troponin alters the rate of transition from non-force-producing cross-bridge states (weak binding states) to force producing states (strong binding states). In contrast, the results of several studies indicate that caldesmon inhibits the binding of myosin to actin. Thus, under conditions where caldesmon does not bind to myosin, caldesmon inhibits both the ATPase activity and binding to actin of skeletal myosin subfragment 1 (S-1) (11) and smooth heavy meromyosin (HMM) (12–14). Caldesmon has also been shown to compete with the binding of various myosin subfragments to actin in the presence of pyrophosphate (11) and adenyl-5′-yl imidodiphosphate (AMP-PNP) (15–17) and in the absence of nucleotide (18, 19). The binding of caldesmon to actin is reversed by antibodies that reverse the binding of S-1·ATP to actin (20). Furthermore, the inhibition of ATPase activity by caldesmon occurs with a large change in the apparent K_m (13, 21).

We have shown that caldesmon may diffuse into single-skinned striated muscle fibers with subsequent inhibition of binding of weakly bound cross-bridges and the production of force (6, 10). However, intact caldesmon has two properties that detract from its usefulness as a probe of cross-bridge function. First, the diffusion of caldesmon into single fibers is slow, requiring more than an hour to obtain full equilibration. Second, caldesmon binds to myosin, as well as to actin (15), and this could cross-link actin to myosin and complicate the analysis of mechanical measurements of fibers. These problems can be avoided by using smaller proteolytic fragments of caldesmon that do not contain the myosin-binding region.

We have found that the most useful of the inhibitory actin-binding fragments originate at either Lys^{579} or Leu^{597} in the primary structure of caldesmon and terminate at or near the COOH terminus (19). These fragments diffuse rapidly into fibers and inhibit the active force production of both skeletal (6) and smooth (7) muscle fibers. We now report the details of the interactions of these fragments that provide the basis for their use as probes of weak cross-bridge binding. The results presented here also help to define further the mechanism by which caldesmon inhibits actin-myosin interactions.

EXPERIMENTAL PROCEDURES

Skeletal actin was isolated from rabbit back and leg muscles by the method of Spudich and Watt (22) as modified by Eisenberg and Kielley (23). Smooth muscle tropomyosin was isolated from turkey gizzards (24). Calmodulin was purified from porcine brains (25). Skeletal muscle myosin was isolated from the back and leg muscles of rabbits (26), and chymotryptic S-1 was prepared by the method of Weeds and Taylor (27). Caldesmon was isolated from turkey gizzards by the method of Bretscher (24) as modified by Velaz et al. (28). Chymotryptic fragments were prepared according to our published procedure (19). The 20 kDa fragments used correspond to combined peaks 1 through 3 of Fig. 2 of Chalovich et al. (19). In one experiment a single 20 kDa fragment (equivalent to peak 2 of Fig. 2, Ref. 19) was used. Protein concentrations were determined by absorbance at 280 nm except for caldesmon, which was determined by the Lowry assay, using bovine serum

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Abbreviations used: S-1, myosin subfragment 1; HMM, heavy meromyosin; AMP-PNP, adenyl-5′-yl imidodiphosphate; SDS, sodium dodecyl sulfate.

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albumin as a standard. The molecular weights used for calculation of protein concentrations were 120,000 (S-1), 42,000 (actin), 68,000 (tropomyosin), 16,500 (calmodulin), and 87,000 (caldesmon). The average molecular weight of the three actin-binding fragments of caldesmon was estimated to be 20,000 (19).

Binding studies

Prior to binding, caldesmon or the 20 kDa fragments were reacted with a twofold molar excess of [^{14}C]iodoacetamide in a buffer containing 100 mM NaCl and 10 mM Tris-HCl (pH 8.0) for 5 h at 17°C. The extent of labeling was about 0.4 mol of sulfhydryl groups modified per mole of caldesmon. In the case of the 20 kDa fragments, only those polypeptides beginning with Lys^{579} contain the radioactive label on Cys^{580}. The binding of ^{14}C-labeled caldesmon to actin was determined by the centrifugation method described previously (19, 28) under conditions given in the figure legends. Binding data were corrected for (*a*) the fraction of caldesmon that did not form a sediment even at saturating actin concentrations, (*b*) the amount of caldesmon that was present in the sediment in the absence of actin, and (*c*) the fraction of actin (5%) that did not form a sediment (28). S-1 binding and caldesmon binding to caldesmon were sometimes measured simultaneously. In these cases, S-1 binding was measured using the NH_4^+/EDTA ATPase assay as described by Chalovich and Eisenberg (29).

ATPase assays

The ATPase activity of myosin skeletal S-1 was measured by the liberation of $^{32}P_i$ from [$^{32}P_i$]ATP as described earlier (29).

Affinity chromatography

Smooth muscle tropomyosin was cross-linked to CNBr-activated Sepharose 4B according to the manufacturer's protocol (Pharmacia LKB Biotechnology, Inc.). The conditions of chromatography are described in the text. Bound proteins were identified by electrophoresis using sodium dodecyl sulfate (SDS) gels (30), with a 7–20% gradient of polyacrylamide, and stained with Coomassie blue.

RESULTS

Several actin-binding fragments of caldesmon are formed during the digestion of caldesmon with chymotrypsin. Four closely related polypeptides of molecular weight of 18,000–23,000 are easily separated from the caldesmon digest by high-performance liquid cation exchange chromatography (Fig. 2 of Ref. 19). These fragments, collectively called 20 kDa fragments, differ by several residues at either the NH_2- or COOH-terminal regions as shown in Fig. 1. While these four fragments can be resolved by high-performance liquid ion exchange chromatography, most experiments were performed using this mixture of fragments, since we have found no significant differences among these fragments. The binding of the 20 kDa fragments to actin was measured by cosedimentation of the ^{14}C-labeled fragments with F-actin. Fig. 2 shows the results of binding to both pure actin (open symbols) and to actin-tropomyosin (closed symbols). Each symbol represents a different caldesmon fragment preparation and a single binding experiment. The model of McGhee and von Hippel (31) was fitted to the data to estimate the binding constant K for the binding of a single fragment to an isolated lattice site composed of n actin monomers and the cooperativity parameter ω, which is involved in the binding of tandem caldesmon molecules (28).

FIGURE 1 Location of COOH-terminal polypeptide fragments of caldesmon. The region of caldesmon from residue 550 through the carboxyl terminus, residue 756, is shown (see Ref. 43). The 20 kDa fragment is a mixture of polypeptides differing slightly in the NH_2- and COOH-terminal regions (▨, ▧). The composition of these fragments is, typically: 42% fragment Lys^{579} (22.9 kDa), 47% fragments Lys^{579} (20.1 kDa) + Leu^{597} (20.1 kDa), and Leu^{597} (18.2 kDa) was 11% of the total mass (the fragments are described by their NH_2-terminal residue, and the apparent molecular weight is given in parentheses). The 7.3 kDa (19) and 10 kDa (40) fragments, derived from the 20 kDa fragments, contain a common region (■).

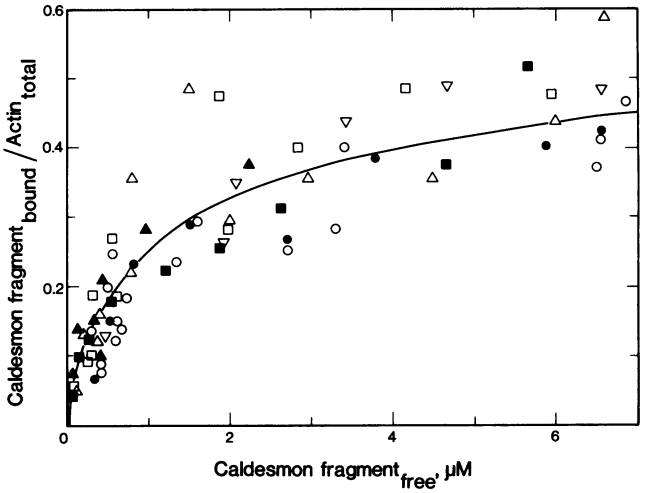

FIGURE 2 Binding of the 20 kDa ^{14}C-labeled fragments of caldesmon to actin. Binding is shown in the absence (△, □, ○) and in the presence (▲, ■, ●) of smooth muscle tropomyosin in a solution containing 10 μM skeletal muscle actin, 3.5 μM smooth muscle tropomyosin, 0.3 μM skeletal muscle myosin S-1, 2 mM ATP, 42 mM NaCl, 4.8 mM $MgCl_2$, 0.25 mM EGTA, 0.5 mM dithiothreitol, and 9.6 mM imidazole (pH 7.2). Each symbol represents a single binding experiment with different protein preparations. The theoretical curve was fitted by using $K = 0.63 \times 10^6$ M^{-1}, $\omega = 1$, and $n = 1.7$. For the best fit of binding parameters to each data set see Table 1.

Table 1 summarizes the data for the binding of the COOH-terminal actin-binding fragment of caldesmon to actin in the presence and absence of smooth muscle tropomyosin. The value of n ranges from 1.7 to 2.6 (with an average of all experiments of 2.0) mol actin/mol of the caldesmon fragment. The value of ω is between 1 and 2. The binding constant K varies from 0.2 to 0.7 $\times$ 10^6 M^{-1}. The lack of effect of tropomyosin on the binding was not due to incomplete saturation of actin with tropomyosin since similar results were obtained at 0, 2.5, 3.5, and 6.0 μM tropomyosin. Furthermore, for comparison, an experiment has been included showing that tropomyosin increases the affinity of native caldesmon to actin from 0.9 to 1.9 $\times$ 10^6 M^{-1}. In this case,

TABLE 1 Analysis of the binding of the COOH-terminal, 20 kDa fragments of caldesmon to actin

Tropomyosin (μM)	ω	n	K_1 ($\times 10^{-6}$ M^{-1})	No.*
0‡	1.0	1.7	0.66	11
0	2.0	2.0	0.20	8
	1.8	2.5	0.61	14
	1.8	2.0	0.68	7
	2.0	1.7	0.40	6
	1.7	2.1	0.63	16
	2.0	2.0	0.35	18
	1.0	2.6	0.55	11
	1.0	1.7	0.66	11
0§	5.0	6.0	0.90	7
2.5‡	1.0	1.7	0.66	9
3.5	2.0	2.0	0.20	10
	1.8	2.5	0.61	7
	1.8	2.0	0.68	7
	1.7	2.1	0.63	12
	2.0	2.0	0.35	8
	1.0	2.0	0.55	9
	1.0	1.7	0.66	9
6.0	1.0	2.5	0.55	9
2.5§	5.0	6.0	1.94	6

* Number of experimental points.

‡ This experiment contained 7 μM actin, and different free concentrations (0.1–10 μM) of the 20 kDa fragments of caldesmon. All other experiments contained 10 μM actin.

§ Intact caldesmon, 7.0 μM actin.

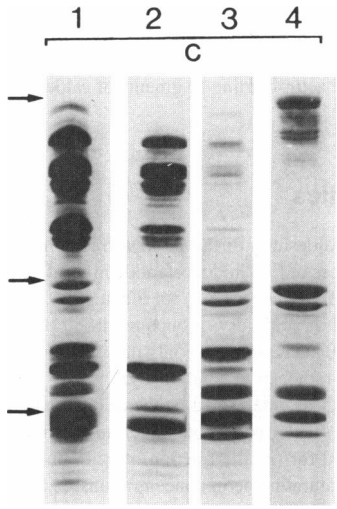

FIGURE 3 SDS gel of the elution profile of chymotryptically cleaved caldesmon on a smooth muscle tropomyosin-Sepharose affinity column. The arrows indicate the position of standards (not shown): caldesmon (M_r 120,000 on SDS gels), actin (M_r 42,000), and soybean trypsin inhibitor (M_r 21,500). (*Lane 1*) Digested caldesmon applied to the column in a buffer containing 30 mM NaCl, 3 mM $MgCl_2$, 0.5 mM EGTA, and 7 mM imidazole-HCl (pH 7.2). (*Lane 2*) Caldesmon fragments not retained by the column. (*Lanes 3* and *4*) Fractions eluted at 0.16 and 0.2 M NaCl, respectively.

the binding of native caldesmon to actin is 2.1 times tighter in the presence of tropomyosin as compared with the 2.7-fold increase reported earlier (28).

Caldesmon is known to bind directly to tropomyosin (32), and it is often assumed that this direct interaction results in the enhanced binding of caldesmon to actin in the presence of tropomyosin. However, while there is little effect of tropomyosin on the binding of the 20 kDa fragments to actin, the 20 kDa fragments do bind to tropomyosin. Fig. 3 is an SDS polyacrylamide gel showing the chromatographic profile of digested caldesmon applied to a tropomyosin-Sepharose affinity column. The polypeptides bound include undigested caldesmon, a fragment of apparent molecular weight 71,000, the large M_r 35,000–40,000 actin-binding fragment, the myosin-binding fragment (apparent molecular weight about 28,000), and the three small actin-binding polypeptides (M_r 22,000, 20,000, and 18,000). Thus, polypeptides from both the NH_2- and COOH-terminal regions of caldesmon appear to bind to tropomyosin. In a separate experiment, purified ^{14}C-labeled M_r 20,000 fragments were found to bind to the same column and elute in a NaCl gradient between 0.14 and 0.16 M (not shown).

Fig. 4 shows the rate of ATP hydrolysis as a function of the amount of the caldesmon 20 kDa polypeptides bound to actin (open circles) and to actin-tropomyosin (closed circles). In the absence of tropomyosin, the rate of ATP hydrolysis decreases from 1.0 to 0.15 s^{-1} in nearly a linear fashion. However, maximal inhibition of ATPase activity actually occurs at less than stoichiometric binding of fragments to actin. In this experiment, maximum binding occurred with a stoichiometry of 1 fragment to 2–2.5 actin monomers, while maximum inhibition occurred at about a 1:3 stoichiometry.

Although tropomyosin does not alter the binding of the 20 kDa fragments of caldesmon to actin, it does substantially enhance the degree of inhibition of actin-activated ATP hydrolysis. In the presence of tropomyosin, the deviation between full saturation of actin with the 20 kDa fragments and total inhibition of ATPase activity is large, as with intact caldesmon (33, 28), with maximum inhibition occurring at about 50% saturation. This could occur if tropomyosin made caldesmon more effective in displacing bound S-1 or if there were additional reductions in the k_{cat}. Tropomyosin, by itself, increases the actin activated ATPase activity of S-1 by about twofold under the conditions of this experiment, and the 20 kDa fragments apparently reverse this potentiating effect. A similar limiting ATPase rate is obtained in both the presence and absence of tropomyosin, which is 0.15 s^{-1} in excess of the rate of S-1 in the absence of actin. This feature was also observed with intact caldesmon (33, 28). The inset to Fig. 4 shows the ATPase rates expressed relative to the rates in the absence of caldesmon to show the difference in caldesmon sensitivity caused by tropomyosin.

We had reported previously that intact caldesmon functions largely by competitively inhibiting the binding of S-1·ATP to actin (11, 14, 15, 18, 28). Fig. 5 shows normalized values of the ATPase rate and S-1·ATP bound to actin as a function of the amount of the caldesmon fragment bound to actin. In this experiment, only a single 20 kDa fragment was used, which is the 20.1 kDa fragment (equivalent to peak 2 of Fig. 2, Ref. 19). Furthermore, the A1 isozyme of myosin S-1 was used, and the measurements were made at low ionic strength to increase the accuracy of measurement of S-1 binding. The inhibition of ATPase activity, shown here in the

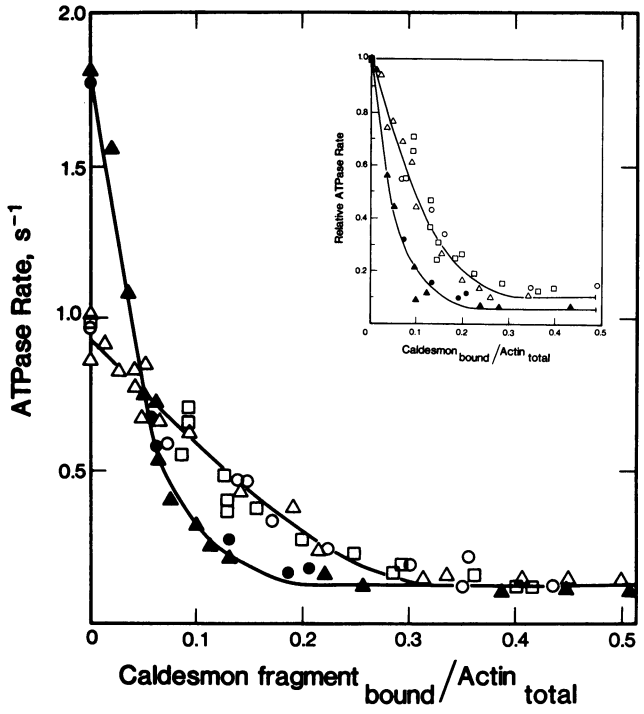

FIGURE 4 Correlation between binding of the 20 kDa peptides of caldesmon to actin and inhibition of ATPase activity. The actin-activated myosin skeletal S-1 ATPase rate and the binding of [^{14}C]caldesmon peptides to actin were measured simultaneously in mixtures containing 10 μM actin, 0.3 μM S-1, variable caldesmon fragments concentrations, and either 0 μM (○, △, □), 3.5 μM (▲) or 6.0 μM (●) smooth muscle tropomyosin. The other components of the solution were identical to those in Fig. 2. In the absence of caldesmon, the ATPase rates were 1.8 and 1.0 s^{-1}, in the presence and absence of tropomyosin, respectively. The limiting ATPase rates at very high caldesmon concentrations were 0.12 and 0.15 s^{-1}, respectively, in the presence and absence of tropomyosin. (*Inset*) A replot of the same data in which the ATPase rates, relative to the maximum value in each series, is shown as a function of the fraction actin containing bound caldesmon fragment.

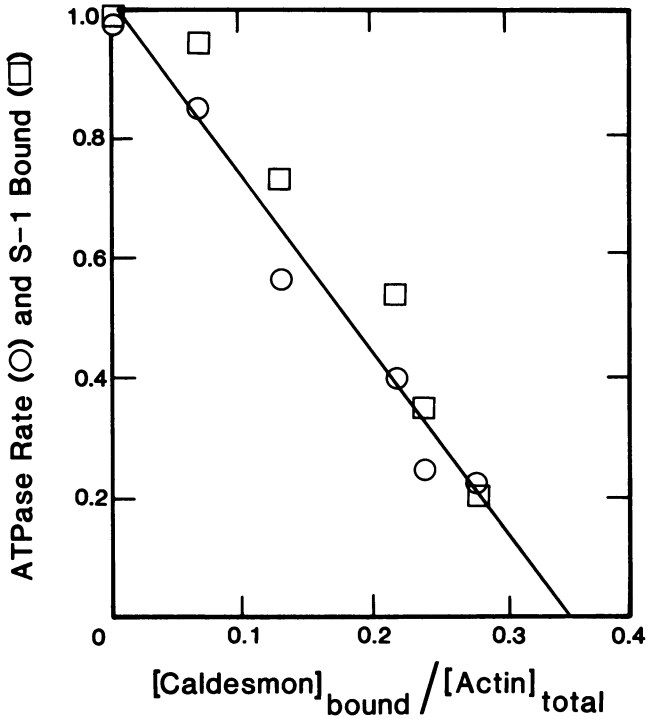

FIGURE 5 ATPase activity and (A1)S-1-ATP binding to actin as a function of the amount of 20.1 kDa caldesmon fragment bound to actin. The pure 20.1 kDa fragment of caldesmon (Ref. 19, Fig. 2, peak 2) was used rather than the mixture of 20 kDa fragments. Both ATPase activity (○) and S-1·ATP binding (□) were measured at 25°C in solutions containing 1 mM ATP, 3 mM $MgCl_2$, 11 mM imidazole (pH 7.0), 0.1 μM (A1)S-1, and 10 μM actin. In the absence of the caldesmon fragment 84% of the (A1)S-1 was bound and the ATPase activity was 8.8 s^{-1}.

absence of tropomyosin, closely parallels the inhibition of binding of S-1·ATP to actin. The ATPase rate data, for the single 20.1 kDa fragment, closely resemble those from the mixture of 20 kDa fragments shown in Fig. 4. This is further justification of the use of a mixture of these closely related polypeptides.

The 20 kDa fragments of caldesmon, like intact caldesmon, compete with the equilibrium binding of myosin S-1 to actin in non-ATP-bound states. Fig. 6 demonstrates the competition between S-1·AMP-PNP (a strong binding state) and 20 kDa caldesmon fragments for binding to actin. In this experiment, the total caldesmon concentration was constant while increasing amounts of S-1·AMP-PNP were added. The fraction of actin containing bound caldesmon fragments (Theta II) decreased as the fraction of actin containing bound S-1·AMP-PNP (Theta I) increased. Stoichiometric binding of the strong binding S-1·AMP-PNP totally displaced the caldesmon fragments from actin. This shows that the 20 kDa fragments, like intact caldesmon, are mutually exclusive with S-1 binding to actin. Both the pure competitive model (dashed line) and the mosaic multiple-binding model (solid line) of binding were fit to these data (see Ref. 17). The two models give similar fits at small values of n as in the present case.

Fig. 7 shows that Ca^{2+}-calmodulin displaces bound 20 kDa fragments from actin in a manner similar to that observed earlier with intact caldesmon (11) and the 7.3 kDa actin-binding fragment of caldesmon (19). Fifty percent inhibition of binding is achieved at a calmodulin concentration of 25 and 12.5 μM for intact caldesmon (11) and the 20 kDa fragments, respectively.

DISCUSSION

The 20 kDa fragments of caldesmon are similar to intact caldesmon in their inhibition of acto-S-1 ATPase activity, their response to tropomyosin in this inhibition, and their competition with S-1 for binding to actin. Also of importance is the observation that the effects of the 20 kDa fragments are reversed by Ca^{2+}-calmodulin since this provides a convenient way of neutralizing the effects of these probes of cross-bridge action. Two advantages of the 20 kDa fragments are their more rapid diffusion into single muscle fibers (6) and their lack of interaction with myosin (14). In contrast, intact caldesmon binds to skeletal HMM and even to some extent to skeletal S-1 at low ionic strength (15, 34). The simultaneous binding to myosin and actin can be misleading.

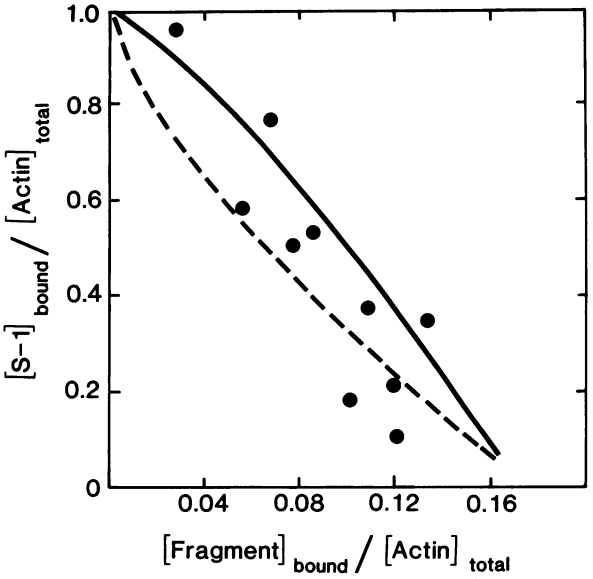

FIGURE 6 Competition between the 20 kDa caldesmon fragments and myosin skeletal S-1 for binding to actin. The conditions used were: 4 mM AMP-PNP, 42 mM NaCl, 4.2 mM $MgCl_2$, 0.25 mM EGTA, 10 mM imidazole (pH 7.0), and 0.5 mM dithiothreitol with 10 μM actin, and 7.5 μM ^{14}C-labeled caldesmon fragments. The skeletal S-1 concentration was varied from 3.5 to 46.8 μM. Both the pure competitive (– – – –) and mosaic multiple (——) models were fit to these data. The association constants of both S-1·AMP-PNP and the 20 kDa fragments to actin were 2×10^6 M^{-1}, and the cooperativity parameter, y, was 2. See Chen and Chalovich (17) for details of the models.

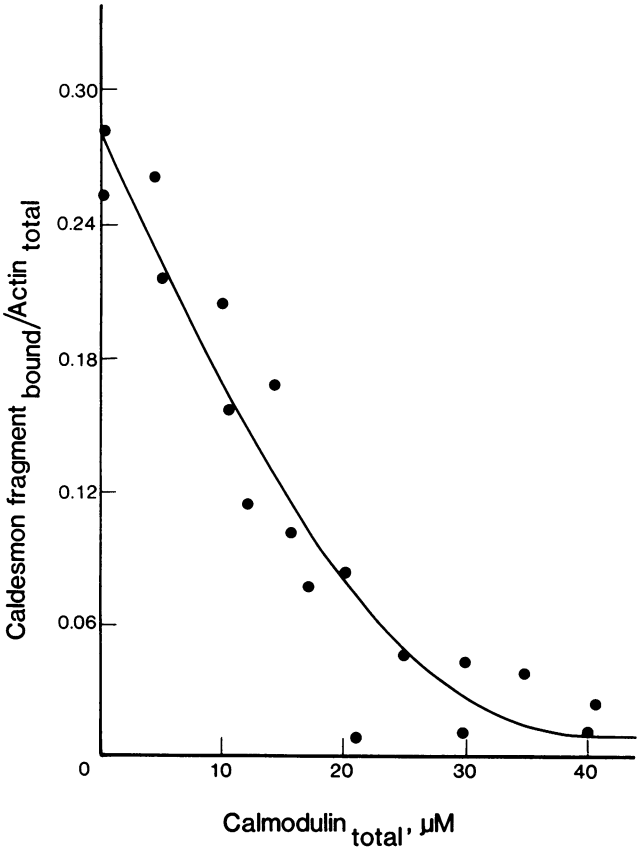

FIGURE 7 Reversal of binding of the 20 kDa, COOH-terminal caldesmon peptides to actin by Ca^{2+}-calmodulin. Binding was measured at 25°C in a solution containing 42 mM NaCl, 4.8 mM $MgCl_2$, 10.5 mM imidazole (pH 7.0), 0.25 mM EGTA, 0.5 mM dithiothreitol, 1.0 mM $CaCl_2$, 10 μM actin, and 8 μM ^{14}C-labeled caldesmon fragments, and calmodulin was varied from 5 to 80 μM.

The affinity of the 20 kDa fragments for actin ($K \times \omega = 8 \times 10^5$ M^{-1}) is one-sixth of that for intact caldesmon (28) and 18 times that of the 7.3 kDa fragment (19). The 20 kDa fragments must contain the strongest actin binding site(s) of the caldesmon molecule. The binding of the 20 kDa fragments to actin is considerably stronger than the binding of S-1·ATP to actin ($K < 10^4$ M^{-1} at 50 mM ionic strength). This means that the 20 kDa fragments are good inhibitors of the weak binding interaction. It is also important that the 20 kDa fragments bind less tightly than the strong binding cross-bridges. For example, $K > 2 \times 10^6$ M^{-1} at 60 mM ionic strength for HMM·AMP-PNP (35). The S-1·ADP complex binds to actin with an even higher affinity (35). Thus the 20 kDa fragments are selective inhibitors of the population of states known as weak binding states. This selectivity was demonstrated in single rabbit psoas fibers, where it was shown that caldesmon inhibits the attachment of cross-bridges in the presence of ATP but not in the presence of pyrophosphate (6).

A continuing question is whether caldesmon regulates ATPase activity solely by blocking the binding of myosin·ATP to actin or whether there is an additional component of regulation. The case for inhibition of myosin·ATP binding is based on direct binding measurements in solution (11, 14–16) on polarized fluorescence changes in ghost fibers (36) and by the effect of caldesmon on the kinetics of ATP hydrolysis (13, 21, 37). Recent studies have also shown that diffusion of caldesmon into rabbit psoas fibers results in a reduction in stiffness measured in the presence of ATP, a reduction in the equatorial intensity ratio I_{11}/I_{10}, and no change in the rate constant of force redevelopment (6). Therefore, in the skeletal fiber, caldesmon clearly causes cross-bridge detachment, and there is no indication of regulation of a transition between two attached states. In our present results, from experiments done in the absence of tropomyosin, we observe slightly more inhibition of ATPase activity than expected from the amount of bound caldesmon (Fig. 4, open symbols). Since this effect is small, it is difficult to rule out errors in measuring caldesmon binding. It is also possible that while the 20 kDa fragment binds tightly to two actin monomers, it could inhibit the binding of S-1·ATP to adjacent sites. Thus, in Fig. 5, there is a strong correlation between the inhibition of ATPase activity and inhibition of S-1·ATP binding to actin.

While there is now agreement that caldesmon competes with myosin binding, there is an active debate over the importance of this inhibition of binding in the presence of tropomyosin (38, 39). It is clear from our past (19, 28, 33) and present work that caldesmon is a more effective inhibitor of ATPase activity in the presence of tropomyosin. Fig. 4 shows that the rate of ATPase activity is enhanced by tropomyosin

and that this rapid ATPase activity is strongly inhibited by caldesmon. One-third as much bound caldesmon is required for 50% inhibition as in the absence of tropomyosin. There is cooperativity in the inhibition of ATPase activity that is seen only in the presence of tropomyosin. However, while caldesmon inhibits the cooperative activation of ATPase activity by smooth tropomyosin (12) there is considerable evidence that competition of binding remains a dominant feature in regulation (6, 11, 13, 14, 17, 21, 37). For example, we have observed that the ability of caldesmon to compete with S-1 binding is enhanced by tropomyosin (17). It is particularly noteworthy, as already discussed, that the 20 kDa fragments inhibit cross-bridge binding in skinned striated muscle fibers (6). This fiber preparation contains tropomyosin, albeit skeletal muscle tropomyosin, and in addition maintains the geometry and relative abundance of proteins expected in a physiological system.

The results of our studies suggest that the stoichiometry of binding of caldesmon fragments to actin is related to the size of the caldesmon fragment. This implies that different regions of caldesmon are binding to different actin monomers (see Ref. 17). Thus, in our hands, intact caldesmon binds to about seven actin monomers (28), the 20 kDa fragment binds to about two actin monomers (present study), and the 7.3 kDa fragment, which is the NH_2 terminal region of the 20 kDa fragment, binds to one actin monomer (19). We would have predicted, from these results, that the COOH-terminal half of the 20 kDa fragment would also bind to a single actin monomer. However, a CNBr fragment of caldesmon, which is essentially the COOH-terminal region of the 20 kDa fragment, is reported to bind to actin with a 1:7 stoichiometry (40), much like intact caldesmon. We cannot explain this result at present.

Caldesmon has been shown earlier to bind to tropomyosin (32), and this has been localized to the 38 kDa COOH-terminal region (41). We have observed that fragments from both the NH_2- and COOH-terminal regions of caldesmon bind to tropomyosin, although it is not clear if these are both physiologically significant interactions. Likely regions for the tropomyosin-binding sites are the two tropomyosin-binding consensus sequences at residues 508–565 and 592–623 (42–44). Our 20 kDa fragments contain this second region. Watson et al. (45) have suggested that residues 142–227 of tropomyosin are involved in the binding of intact caldesmon but the site to which this region of tropomyosin binds has not been identified. Although the 20 kda caldesmon fragments bind to tropomyosin, there is little effect of tropomyosin on the binding of caldesmon to actin. This is not totally unexpected since earlier studies have shown that tropomyosin has a relatively small (about threefold) effect on the binding of intact caldesmon to actin (28), even though caldesmon binds as tightly to tropomyosin in solution (46) as caldesmon binds to actin (28). These results indicate that the caldesmon-binding site on tropomyosin may be partially inaccessible in the presence of actin.

In conclusion, the 20 kDa actin-binding fragments of caldesmon have the inhibitory properties normally associated with caldesmon but lack the complication of binding to myosin. These fragments are well suited for perturbing the "weak" binding of myosin to actin that occurs during ATP hydrolysis both in solution and in muscle.

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