Mapping of the Microvillar 110K–Calmodulin Complex: Calmodulin-associated or -free Fragments of the 110-kD Polypeptide Bind F-Actin and Retain ATPase Activity

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Abstract. The 110K–calmodulin complex isolated from intestinal microvilli is an ATPase consisting of one polypeptide chain of 110 kD in association with three to four calmodulin molecules. This complex is presumably the link between the actin filaments in the microvillar core and the surrounding cell membrane. To study its structural regions, we have partially cleaved the 110K–calmodulin complex with α-chymotrypsin; calmodulin remains essentially intact under the conditions used. As determined by ^{125}I-calmodulin overlays, ion exchange chromatography, and actin-binding assays, a 90-kD digest fragment generated in EGTA remains associated with calmodulin. The 90K–calmodulin complex binds actin in an ATP-reversible manner and decorates actin filaments with an arrowhead appearance similar to that found after incubation of F-actin with the parent complex; binding occurs in either calcium- or EGTA-containing buffers. ATPase activity of the 90-kD digest closely resembles the parent complex. In calcium a digest mixture containing fragments of 78 kD, a group of three at ∼40 kD, and a 32-kD fragment (78-kD digest mixture) is generated

with α-chymotrypsin at a longer incubation time; no association of these fragments with calmodulin is observed. Time courses of digestions and cyanogen bromide cleavage indicate that the 78-kD fragment derives from the 90-kD peptide. The 78-kD mixture can also hydrolyze ATP. Furthermore, removal of the calmodulin by ion exchange chromatography from this 78-kD mixture had no effect on the ATPase activity of the digest, indicating that the ATPase activity resides on the 110-kD polypeptide. The 78 kD, two of the three fragments at ∼40 kD, and the 32-kD fragments associate with F-actin in an ATP-reversible manner. Electron microscopy of actin filaments after incubation with the 78-kD digest mixture reveals coated filaments, although the prominent arrowhead appearance characteristic of the parent complex is not observed. These data indicate that calmodulin is not required either for the ATPase activity or the ATP-reversible binding of the 110K–calmodulin complex to F-actin. In addition, since all the fragments that bind F-actin do so in an ATP-reversible manner, the sites required for F-actin binding and ATP reversibility likely reside nearby.

IN intestine, the 110-kD polypeptide is presumed to be a component of the lateral link between the microvillar membrane and the bundle of cross-linked filaments composing the microvillar core (Matsudaira and Burgess, 1979; Glenney et al., 1981). Each 110-kD polypeptide is associated with three to four 17-kD calmodulin molecules to form the 110K–calmodulin complex. Previous studies have shown that binding of the 110K–calmodulin complex to F-actin in vitro is ATP, calcium, and protein concentration regulated (Howe and Mooseker, 1983; Verner and Bretscher, 1985; Coluccio and Bretscher, 1987; Conzelman and Mooseker, 1987). Incubation of 110K–calmodulin with F-actin results in the formation of decorated filaments similar to those seen after incubation of actin filaments with the myosin fragment, S_1. In some areas 33-nm cross-links project from actin filaments that often associate laterally in the presence of 110K–calmodulin (Coluccio and Bretscher, 1987). Other

similarities to myosin include ATP sensitivity of this binding to actin and the ability of the complex to split ATP (Collins and Borysenko, 1984; Verner and Bretscher, 1985; Coluccio and Bretscher, 1987; Conzelman and Mooseker, 1987). This raises the exciting possibility that the 110K–calmodulin complex is a motor molecule similar to myosin, although only a limited actin-activatable Mg^{2+}-ATPase activity has been detected (Conzelman and Mooseker, 1987; Krizek et al., 1987) and no evidence of motility has yet been described. In any event, by virtue of this molecule's role as a presumptive link between the cell membrane and the underlying cytoskeleton, its study is of special importance.

In an effort to investigate the interaction of the 110K–calmodulin complex with F-actin, we have used limited proteolysis of the complex with α-chymotrypsin to identify actin-binding peptides. Whether or not these peptides are associated with calmodulin has been investigated. The frag-
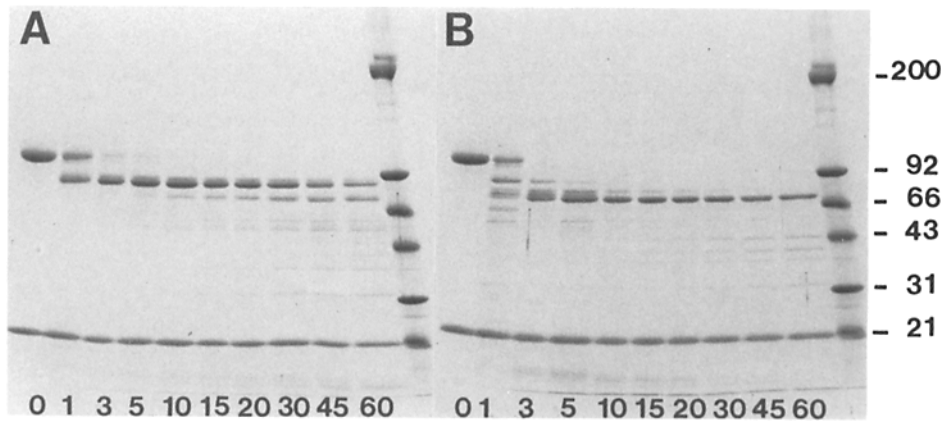
Figure 1. Digestion of the 110K-calmodulin complex analyzed by SDS-PAGE. (*A*) Digestion of 110K-calmodulin at 0.3 mg/ml in 1 mM EGTA with 0.006 mg/ml α-chymotrypsin. The digestion times in minutes are indicated below the corresponding lanes; (*B*) a similar digestion, but in the presence of 0.2 mM CaCl₂. Molecular masses (× 10⁻³) of standards, present in the last lane of each panel, are indicated.

ments have been characterized according to their calcium and/or ATP sensitivity of binding to F-actin. The abilities of the digest mixtures to hydrolyze ATP have been assessed. Our results demonstrate that the ATPase activity and binding of the 110-kD polypeptide to F-actin is independent of calmodulin.

Materials and Methods

Protein Purification

Chicken intestinal 110K-calmodulin complex was isolated from brush borders as recently described by Coluccio and Bretscher (1987). Actin was prepared from rabbit skeletal muscle according to Spudich and Watt (1971) and further purified by gel filtration as described by MacLean-Fletcher and Pollard (1980). Calmodulin was isolated from bovine brain by the method of Lin et al. (1974).

Protease Digests

110K-calmodulin complex at 0.3 mg/ml was digested at 25°C with 0.006 mg/ml α-chymotrypsin (Sigma Chemical Co., St. Louis, MO) in buffer containing 10 mM Tris, pH 8.0, 0.2 mM CaCl₂ or 1 mM EGTA, 100 mM KCl, 1 mM MgCl₂, and 1 mM dithiothreitol (DTT). The reaction was stopped by addition of phenylmethylsulfonyl fluoride (PMSF) and benzamidine to a final concentration of 0.3 and 0.5 mM, respectively.

Generation of the 90K-calmodulin complex was routinely achieved by digestion in EGTA for 3 min. The 78-kD digest mixture was generated in CaCl₂ after 45 min, at which time the reaction was terminated. After each digestion, the resulting digest patterns were examined by SDS gel electrophoresis to determine if any parent molecule remained.

Anion Chromatography

Digest fragments were chromatographed on a 3-ml column packed with Fast Flow Q-Sepharose, an anion exchange resin (Pharmacia AB, Uppsala, Sweden), in buffer containing 20 mM triethanolamine, pH 7.4, 1 mM EGTA, 1 mM DTT, 0.2 M NaCl. After washing the column with the same buffer, a 16-ml salt gradient ranging from 0.2-1 M NaCl was developed. Fractions were analyzed for protein composition by SDS gel electrophoresis. Alternatively, in a 1.5-ml microfuge tube, 35 μl of Q-Sepharose was mixed directly with 300 μl digest mixture and incubated for 10 min on ice with mixing. The Q-Sepharose was then removed by centrifugation in the microfuge.

Actin-binding Assays

The interaction of F-actin with 110K-calmodulin or its cleavage fragments was investigated by cosedimentation assays. Mixtures were incubated in buffer containing 10 mM Tris, pH 8.0, 1 mM DTT, 100 mM KCl, 1 mM MgCl₂ and either 0.2 mM CaCl₂ or 1 mM EGTA at room temperature for 30 min and then centrifuged in an airfuge (Beckman Instruments, Inc., Palo Alto, CA) at 30 psi for 20 min to pellet the F-actin and any associated mate-

rial. Supernatants were separated from pellets and equivalent aliquots of each were prepared for SDS-PAGE.

Gel Electrophoresis

SDS-PAGE of proteins was done according to Laemmli (1970) using 7.5/15% or 7.5/17.5% acrylamide split mini gels. Sample buffer contained final concentrations of 80 mM Tris, pH 6.8, 1% β-mercaptoethanol, 5 mM EGTA, and 10% glycerol. Gels were stained in 0.1% Coomassie Brilliant Blue in 50% methanol, 10% acetic acid, then destained in 50% methanol, 10% acetic acid, or, when indicated, by silver stain according to Oakley et al. (1980).

Protein Concentration

Protein concentrations were determined according to the method of Bradford (1976) using BSA as a standard.

ATPase Assays

A colorimetric assay to detect ATPase activity was performed as described by Pollard (1982) in 10 mM Tris, pH 8.0, 50 mM KCl, 1 mM DTT containing
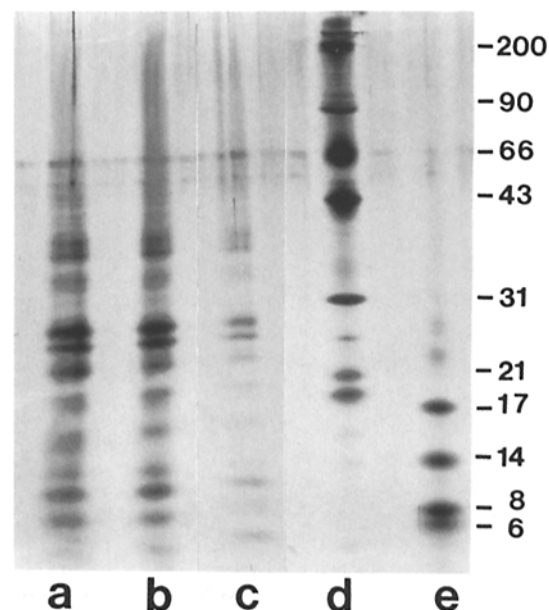

Figure 2. Cyanogen bromide digestion of the 110-kD (lane *a*), 90-kD (lane *b*), and 78-kD (lane *c*) polypeptides as identified by SDS-PAGE. Molecular masses (× 10⁻³) of markers in lanes *d* and *e* are indicated at the right. Proteins are visualized on a 5-25% gradient gel by silver stain.
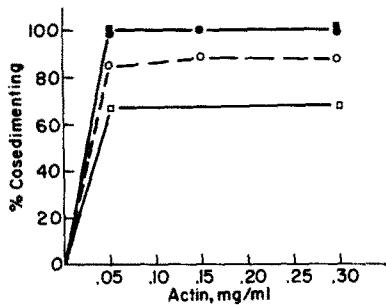
Figure 3. Cosedimentation of 90K–calmodulin complex with F-actin. 90-kD digest mixture at 0.18 mg/ml was incubated for 30 min with F-actin, then centrifuged, and the supernatants and pellets analyzed by SDS-PAGE. The percentage of 90 kD (●) and calmodulin (○) cosedimenting in 1 mM EGTA or 0.2 mM CaCl₂ ([■] 90 kD; [□] calmodulin) as determined by densitometry is plotted vs. the actin concentration.

either 5 mM CaCl₂, 5 mM MgCl₂, or 2 mM EDTA and 2 mM EGTA, as indicated. Absorbance at 720 nm was measured with a spectrophotometer (DU-50; Beckman Instruments, Inc., Wakefield, MA).

Activities of the intact complex were compared with that of the 90-kD digest and the 78-kD digest ± calmodulin. For samples in which calmodulin had been removed by addition of Fast Flow Q, a dilution factor resulted. To compare activities of the 78-kD digest with that of the same digest minus calmodulin, gel samples were subjected to electrophoresis and then scanned with a densitometer (Quick-Scan R + D; Helena Laboratories, Beaumont, TX). The areas under the curve were excised and weighed with a Mettler analytical balance. Specific activities of the mixtures were then determined, taking into account the average difference in intensities of the five major bands.

Electron Microscopy

Samples were applied to collodion-coated copper grids stabilized by a thin film of carbon and rendered hydrophilic by glow discharge before use. After staining for 30 s with 1% aqueous uranyl acetate, excess stain was removed by touching the side of the grid with a filter paper strip. The grid was then allowed to air-dry. Specimen examination was with a Philips 301 electron microscope at an accelerating voltage of 100 kV.

Other

Calmodulin was radiolabeled with iodine using Bolten-Hunter reagent (Bolton and Hunter, 1973). ¹²⁵I-calmodulin overlays were performed according to the method of Glenney and Glenney (1984). Cyanogen bromide cleavage was done according to the method of Pepinsky (1983).

Results

Protease Cleavage

Digestion of the 110K–calmodulin complex by α-chymotrypsin was strongly influenced by the presence of calcium. Limited digestion of 110K–calmodulin in 1 mM EGTA with α-chymotrypsin at a 1:50 wt/wt ratio resulted within the first few minutes in the generation of a digest mixture consisting predominantly of a 90-kD digest fragment and calmodulin that remained essentially uncleaved (Fig. 1 *A*). No corresponding complimentary 20-kD fragment to the 90-kD polypeptide could be identified. In 0.2 mM CaCl₂ at 45 min, a major fragment of 78 kD and minor products of 45, 40, 38, and 32 kD resulted; still, little change in intensity of the 17-kD band was observed, indicating that calmodulin remained virtually intact (Fig. 1 *B*).

Inspection of the digest pattern indicated that the 78-kD fragment results from the 90-kD polypeptide. Moreover, excision and treatment of the 78- and 90-kD fragments with cyanogen bromide that cleaves at methionine residues gave very similar digest patterns, indicating that the 78-kD portion of the molecule is most likely entirely contained within the larger 90-kD fragment (Fig. 2).

Identification of F-Actin–binding Fragments

In EGTA-containing buffer, 100% of the α-chymotryptic 90-kD fragment together with 85% of the calmodulin, as determined by densitometry, cosedimented after incubation with F-actin, ranging in concentration from 0.3–0.05 mg/ml (Fig. 3). In calcium-containing buffer, all of the 90-kD fragment also associated with F-actin; however, only 65% of the
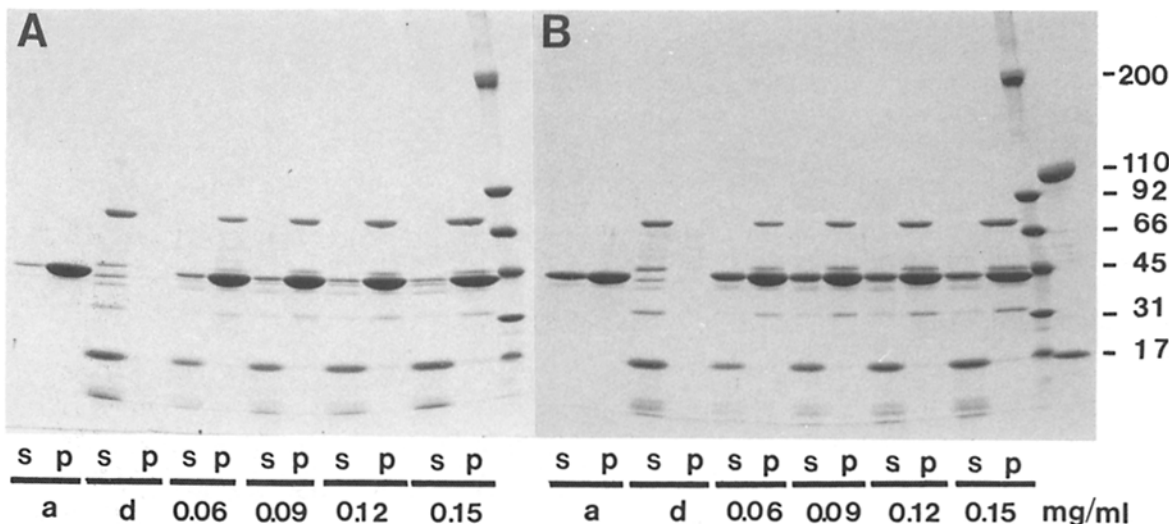

Figure 4. Cosedimentation with F-actin of the 78-kD digest fragments. F-actin at 0.3 mg/ml was incubated with increasing amounts of 78-kD digest mixture in either 0.2 mM CaCl₂ (*A*) or 1 mM EGTA (*B*) for 30 min, then centrifuged, and the supernatants (lanes *s*) and pellets (lanes *p*) analyzed by SDS-PAGE. The concentrations indicated represent the total amount of digest used. Control samples contained either actin only at 0.3 mg/ml (lanes *1* and *2*) or digest only (lanes *3* and *4*) at 0.15 mg/ml.
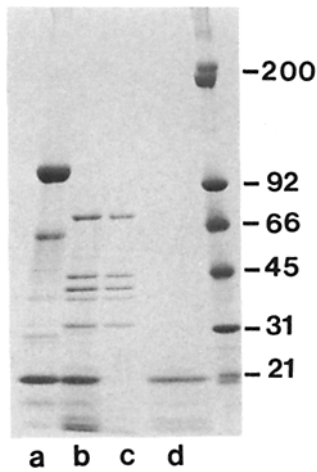
Figure 5. Removal of calmodulin from the 78-kD digest by Q-Sepharose. After digestion in calcium of the 110K–calmodulin (lane *a*) with α-chymotrypsin, the resulting 78-kD digest mixture (lane *b*) was incubated with Q-Sepharose. Flow-through fractions (lane *c*) contained the 78-, 45-, 40-, 38-, and 32-kD fragments, but no calmodulin. Calmodulin (lane *d*) eluted with a 1-M NaCl wash.

Figure 6. Identification of calmodulin-binding fragments by ^{125}I-calmodulin gel overlays. (*A*) Coomassie-stained samples of the 110K–calmodulin parent (lane *1*), 90-kD digest mixture (lane *2*), 78-kD digest mixture (lane *3*) in equivalent loads of ∼2.5 μg, and standard molecular mass markers (lane *4*). After transfer to nitrocellulose the proteins were overlayed with ^{125}I-calmodulin in the presence of 0.2 mM CaCl₂ and then autoradiographed (*B*).

calmodulin cosedimented with the F-actin (Fig. 3). In a similar experiment, in either the presence (Fig. 4 *A*) or absence (Fig. 4 *B*) of calcium, the addition of increasing amounts of 78-kD digest resulted in cosedimentation of the 78-, 45-, and 32-kD fragments with actin filaments, although even at the highest concentration of digest used, no fragments pelleted in the absence of F-actin. None of the calmodulin in the 78-kD digest mixture cosedimented with the actin filaments.

78-kD and Smaller Digest Fragments Are Not Associated with Calmodulin

Chromatography of the 78-kD digest mixture on the anion exchange resin Q-Sepharose resulted in removal of calmodulin; calmodulin could be eluted with high salt (Fig. 5). This indicated that calmodulin was not associated with these digest fragments. Chromatography of the 1-min EGTA digest containing the 90-kD fragment on Q-Sepharose resulted in coelution of the 90-kD peptide and calmodulin by high salt (data not shown).

[125]I-calmodulin overlays of the digest fragments confirmed that the 78-kD or smaller digest fragments bind no calmodulin (Fig. 6, lane *3*). ^{125}I-calmodulin overlays of the 90-kD digest verified, however, that the 90-kD fragment, like the 110-kD parent chain, shows an affinity for calmodulin in calcium (Fig. 6, lanes *1* and *2*) as well as in 1 mM EGTA (data not shown).

Association of 78-kD Digest Fragments with F-Actin Is Independent of Calmodulin

Calmodulin-depleted 78-kD digest incubated with F-actin in either calcium or EGTA resulted in association of the 78-, 45-, and 32-kD fragments with the F-actin (Fig. 7); neither the 40- nor the 38-kD polypeptide cosedimented with the actin filaments.

ATP Sensitivity of F-Actin-Binding Fragments

After incubation of either the 90- or 78-kD digest mixture with F-actin, ATP was added to a final concentration of 5 mM. Binding of the 90-kD fragment to F-actin was reversible by the addition of ATP (Fig. 8 *A*). Dissociation of the 78-, 45- and 32-kD fragments from F-actin also occurred in the presence of ATP (Fig. 8 *B*). Presumably, binding of
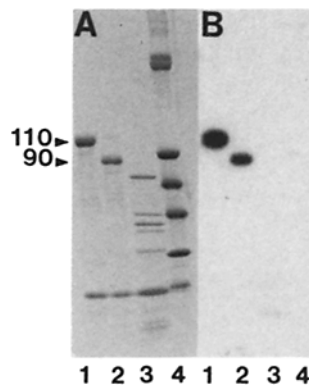
ATP to the fragments precludes their association with actin filaments.

To explore further the reversible binding of the 90K–calmodulin complex to F-actin and to compare it with the parent 110K–calmodulin complex, parent complex was added to 90K–calmodulin in a 1:1 ratio in EGTA buffer containing ATP concentrations ranging from 0 to 5 mM. Although binding of either complex to F-actin was sensitive to the presence of ATP, the 90-kD polypeptide showed less association with F-actin than the 110-kD polypeptide did (Fig. 9). As pointed out by one reviewer, one possible explanation is that two populations of 110K–calmodulin differing in their ability to bind F-actin may exist.

ATPase Activities

The activity of the 90-kD digest mixture in 5 mM CaCl₂ or K⁺, EDTA-EGTA was very similar to the parent molecule, yet the activity in MgCl₂ was substantially enhanced (Table I). The 78-kD digest also retained all the ATPase activity in CaCl₂ and was enhanced in 5 mM MgCl₂, but was substantially lower in K⁺, EDTA-EGTA. All the ATPase activity
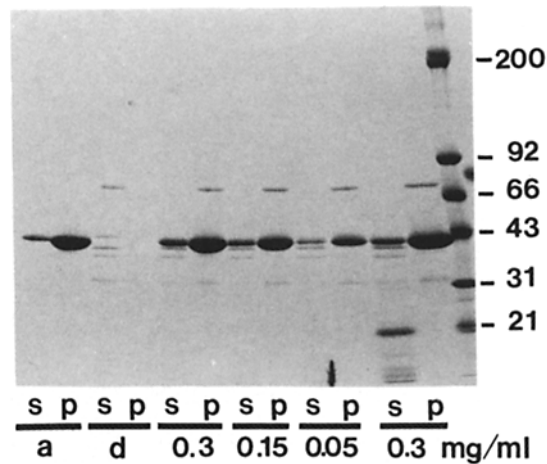

Figure 7. Cosedimentation of actin with calmodulin-depleted 78-kD digest. Calmodulin-depleted 78-kD digest mixture at ∼0.18 mg/ml was incubated in calcium with actin ranging from 0.3 to 0.05 mg/ml. Association with 0.3 mg/ml F-actin of the original calmodulin-containing digest also at ∼0.18 mg/ml is illustrated at right.
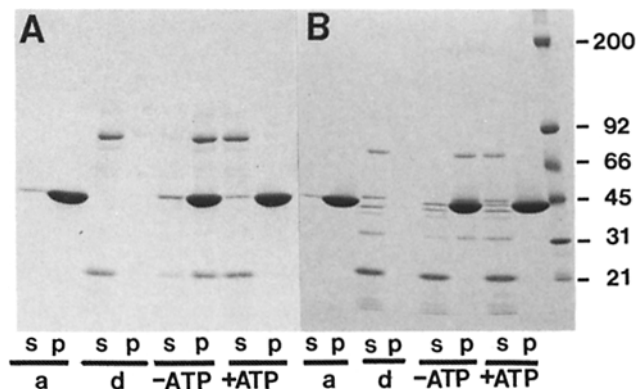
Figure 8. Reversibility of actin binding by the addition of ATP. F-actin at 0.3 mg/ml was incubated with 0.18 mg/ml 90-kD digest (A) or the 78-kD digest (B). To some aliquots, ATP was added to a final concentration of 5 mM for 20 min (+ATP) before the samples were centrifuged. Supernatants (lanes s) and pellets (lanes p) were analyzed by SDS gel electrophoresis. Samples containing either actin only (a) or digest only (d) are shown.
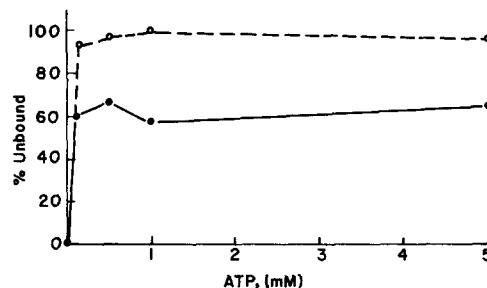

Figure 9. Effect of ATP on binding of 90K–calmodulin vs. the parent complex. At ATP concentrations ranging from 0–5 mM, F-actin at 0.2 mg/ml was incubated for 30 min in EGTA-containing buffer with 0.3 mg/ml 110K–calmodulin and an equal amount of the 90-kD digest mixture. Samples were centrifuged and the amount of 90-kD (O) or 110-kD (●) remaining in the supernatant was determined by densitometry after SDS-PAGE and plotted vs. the initial ATP concentration.

was retained in 78-kD digest mixture from which calmodulin had been removed by incubation with Q-Sepharose.

The different activities for the 110K–calmodulin preparations indicate the typical variability in the ATPase activities found.

Decoration of F-Actin with Digest Fragments

Titration of the 78-kD digest mixture with various amounts of F-actin resulted in coated filaments at high ratios of digest to actin; however, no arrowhead appearance characteristic of F-actin after incubation with the 110K–calmodulin parent complex was observed. Filament–filament associations were also visible at high ratios of 78-kD digest mixture to actin (Fig. 10 A). In some areas, cross-bridges could be seen; however, the extent of filament interaction observed never reached that found in samples with the parent complex.

Incubation of F-actin with the 90-kD digest mixture also resulted in association of the actin filaments (Fig. 10 B); moreover, individual filaments displayed a strikingly obvious arrowhead configuration (Fig. 10 C) indistinguishable from that previously observed with the 110K–calmodulin parent complex.

Discussion

We have recently demonstrated that the isolated microvillous 110K–calmodulin complex binds F-actin in a cooperative and calcium-regulated manner and also decorates actin filaments

with a polarized arrowhead appearance. These, together with the reports that the complex has an intrinsic ATPase activity characteristic of myosin (Collins and Borysenko, 1984), provide enticing evidence that the 110K–calmodulin complex, like myosin, may also be a mechanochemical motor molecule. We have now used limited proteolysis of the native complex to explore its functional organization and to identify and characterize fragments with the ability to bind actin and/or calmodulin and those that retain the ability to split ATP.

The digestion time course of 110K–calmodulin with α-chymotrypsin in the presence of calcium is more extensive than in excess EGTA. The rate of digestion by α-chymotrypsin is considered to be sensitive to calcium (Wilcox, 1970); however, our finding may be partially attributed to a calcium-induced conformational change in the 110K–calmodulin complex because the pattern of fragments generated differs depending on whether or not calcium is present. Although the initial product under either condition is a 90-kD fragment of the 110-kD parent molecule, this 90-kD fragment is relatively stable in EGTA, whereas it is rapidly cleaved to a 78-kD polypeptide in the presence of calcium. One attractive possibility is that this difference in sensitivity to proteolysis is related to our previous finding that in calcium one calmodulin molecule can be released from the 110K–calmodulin complex. This change could easily make a site previously protected by calmodulin now accessible to digestion.

Removal of the associated calmodulin molecules from the purified 110K–calmodulin complex renders the 110K–calmodulin complex insoluble (Coluccio and Bretscher, 1987); partial removal of the calmodulin also leads to partial insolu-

Table I. ATPase Activities of the 90- and 78-kD Digest Mixtures*

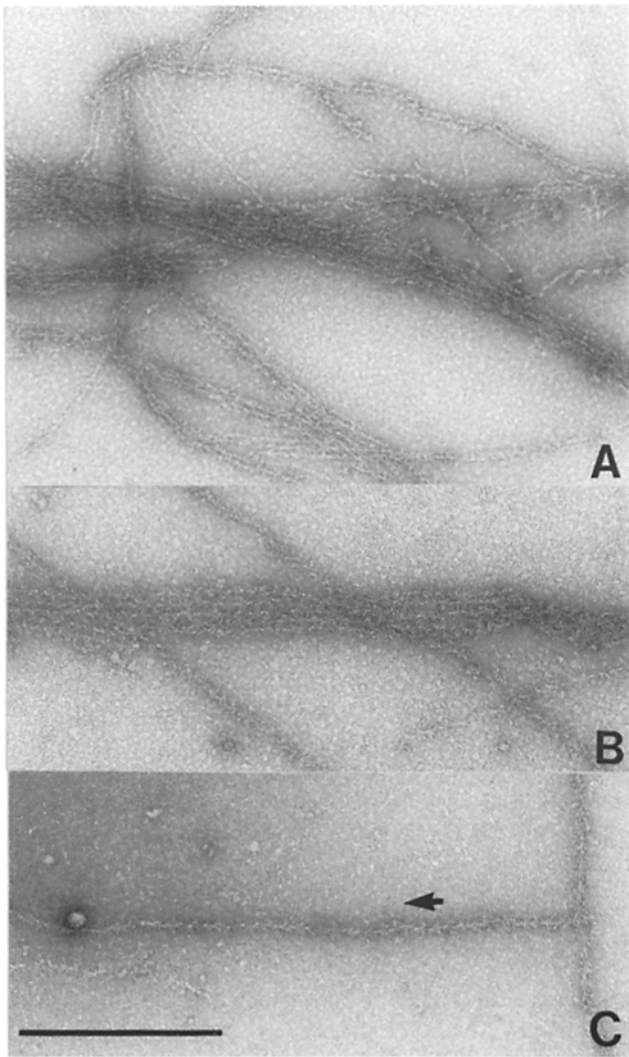|	5 mM Ca++	5 mM Mg++	K+ EDTA-EGTA
Parent molecule	341.5 ± 68	27.2 ± 6.9	394.1 ± 66
90-kD Digest	488.9 ± 55	74.3 ± 6.6	424.6 ± 30
Parent molecule	424.0 ± 44.5	7.1 ± 5.0	263.7 ± 45
78-kD Digest	379.7 ± 32.7	63.7 ± 11.7	72 ± 19.8

* nmol P_i/mg protein per min.

Figure 10. Negatively stained actin filaments incubated with the 78-kD digest mixture in 0.2 mM CaCl₂ (actin, 0.05 mg/ml; digest, 0.3 mg/ml) (*A*) or the 90-kD digest mixture in 1 mM EGTA (actin, 0.05 mg/ml; digest, 0.3 mg/ml) (*B*) as viewed by electron microscopy. An individual filament decorated with the 90-kD–calmodulin complex is shown in *C*. Bar, 0.5 μm.

bility of the resulting complex (Howe and Mooseker, 1983). We note here that several of the digest fragments <78 kD are not associated with calmodulin, yet do not precipitate from solution. It is possible that a 20–30-kD portion of the parent chain usually associated with the calmodulin molecules is particularly hydrophobic. Once exposed by loss of the calmodulin molecules, the 110-kD polypeptide chain is rendered insoluble.

Isolation of the individual 78-kD digest fragments by ion exchange chromatography has been particularly frustrating, since it appears that the digest products are similarly charged. The use of anion exchangers is a main step in the isolation of the parent complex. Binding of the complex to the resin may require the calmodulin molecules associated with the 110-kD polypeptide since the fragments that have no associated calmodulin chains are presumably basic and pass through this resin unimpeded. The 90-kD fragment together

with calmodulin elute from Q-Sepharose with increasing salt.

Each 90-kD polypeptide, like the 110-kD parent chain, has calmodulin associated with it. This conclusion is based on three observations: (*a*) in EGTA, 85% of the calmodulin associates with the 90-kD polypeptide in actin-binding assays, (*b*) calmodulin elutes with the 90-kD polypeptide on anionic exchange columns (data not shown) and (*c*), the 90-kD polypeptide binds radiolabeled calmodulin in overlays. We have so far not detected any major differences in F-actin–binding properties between the 90K–calmodulin complex and the parent complex. Both bind F-actin in an ATP-reversible manner and have a single calcium-dependent dissociable calmodulin chain. Moreover, each decorates F-actin to give a characteristic arrowhead appearance. However, although all the Ca²⁺ and K⁺, EDTA-EGTA ATPase activity is retained in the 90K–calmodulin complex, the Mg²⁺-ATPase activity of the digest exceeds that of the parent. Whether this is related to the lower concentration of ATP needed to dissociate 90K–calmodulin from F-actin remains to be investigated.

Loss of a 12-kD segment from the 90-kD polypeptide is responsible for generation of the 78-kD fragment and consequently loss of the calmodulin chains associated with the heavy chain. Removal of the calmodulin from the digest mixture by ion exchange has no effect on the ability of the fragments to bind actin in an ATP-reversible manner or on ATPase activity. This indicates that the calmodulin molecules in association with the 110-kD chain are not necessary for either F-actin binding or ATPase activity. Electron microscopy of F-actin saturated with the 78-kD digest mixture does not reveal polarly decorated filaments, as seen after incubation of actin filaments with either the 110K–calmodulin or 90K–calmodulin complex. The arrowhead decoration must therefore be a result of the additional 12-kD sequence with its associated calmodulins. Interestingly, the arrowhead appearance on thin filaments obtained with scallop S-1 complex from which the regulatory light chain has been removed is less dramatic than that seen with S-1 complexes in the presence of regulatory chain (Craig et al., 1980). It is likely that the ATP regulatory site resides in relatively close proximity to the sites required for actin binding since fragments exhibiting the ability to bind actin release actin when ATP is added. This provides further support for a functional relationship between F-actin binding and ATP hydrolysis. The Ca²⁺ and Mg²⁺ ATPase activities characteristic of the 90K–calmodulin complex are fully retained by the calmodulin-depleted 78-kD digest mixture; however, at this point it is not definitive as to which fragments retain the ATPase activities. The reason for the low level of K⁺, EDTA-EGTA ATPase activity in this digest is also unclear. Like 110K–calmodulin and 90K–calmodulin, the calmodulin-depleted 78-kD digest mixture reduces the apparent critical concentration of actin (see Figs. 4 and 7); this is probably an intrinsic property of the F-actin–binding domain on the heavy chain.

The finding that we have so far not identified any fragments that bind actin both in the presence and absence of ATP contrasts with the studies of Lynch et al. (1986) on *Acanthamoeba* myosin 1A. Chymotryptic digestion of myosin 1A yields two F-actin–binding fragments, one associated with the ATPase activity and another that binds F-actin in an ATP-independent manner. Another difference from the *Acanthamoeba* single-headed myosins is that in the fragments so

far isolated, the F-actin–binding fragments that retain ATPase activity always have associated light chains; our 78-kD digest retains these properties in the absence of the calmodulin light chains. The smallest fragment so far identified that binds actin reversibly is 32 kD.

A case is rapidly building in favor of classifying the 110K–calmodulin complex as a myosin-like molecule. In addition to its ability to split ATP, it binds actin in a calcium-sensitive manner, and decoration of actin filaments with 110K–calmodulin complex resembles by electron microscopy that which occurs after incubation of actin with myosin fragments. We now observe that the enzymatic activity resides on the 110-kD heavy chain; the calmodulin chains, as has been found for the light chains of skeletal muscle myosin (Wagner and Giniger, 1981; Sivaramakrishnan and Burke, 1982) and *Acanthamoeba* myosin (Maruta et al., 1978), are not essential.

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