J. Biochem. 128, 1045–1049 (2000)

Proteolysis of Acidic Calponin by μ-Calpain

Ryo Yoshimoto, Masatoshi Hori, Hiroshi Ozaki, and Hideaki Karaki[1]

Department of Veterinary Pharmacology, Graduate school of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113

Received July 24, 2000; accepted October 3, 2000

Acidic calponin is an actin binding protein expressed in smooth muscle and brain. Although the role of smooth muscle calponin (basic calponin) has been well studied, few studies have been performed on acidic calponin. In the present study, we demonstrated that acidic calponin binds to filamentous actin, but not monomeric actin. A co-sedimentation assay indicated that acidic calponin binds to actin with an apparent binding constant of 4×10^5 M^{-1}. In the presence of an excess amount of calmodulin, the binding of acidic calponin to actin was inhibited. The binding of acidic calponin to calmodulin was Ca^{2+}-dependent with K_d of 31 μM. We next investigated whether or not acidic calponin could be a substrate for μ-calpain *in vitro*, since it has been shown that basic calponin is cleaved by μ-calpain. The results showed that acidic calponin was also cleaved by μ-calpain. Neither the proteolytic pattern nor velocity of acidic calponin was different in the absence or presence of calmodulin. When acidic calponin had bound to actin, however, the susceptibility of the acidic calponin to μ-calpain was significantly reduced, which was reversed by the addition of calmodulin. Our results suggest that acidic calponin might be involved in the μ-calpain–regulated actin cytoskeleton.

Key words: acidic calponin, actin, calmodulin, calpain, proteolysis.

Basic calponin is an actin, calmodulin, and tropomyosin binding protein that is exclusively expressed in differentiated smooth muscle cells (*1*). Acidic calponin is one of the genetic variants of basic calponin, and full length cDNA of acidic calponin has been isolated from smooth muscle and cultured neuronal cells (*2*, *3*). Thereafter, an immunocytochemical study involving transfected cells with acidic calponin showed that acidic calponin is colocalized with actin stress fibers (*4*). Another study involving acidic calponin–specific antibodies showed that it is distributed along actin fibers and submembranous cortex in neuronal cells, and glial fibrillary acidic protein and vimentin filaments in glial cells (*5*). These results suggest that acidic calponin plays a critical role as a cytoskeletal or intermediate filamentous component. Although the role of basic calponin has been well elucidated, very few studies have been performed on the biochemical properties of acidic calponin. We attempted the biochemical characterization of acidic calponin and demonstrated that acidic calponin binds to F-actin and Ca^{2+}-calmodulin, and is cleaved by μ-calpain, a Ca^{2+}-dependent cysteine proteinase.

MATERIALS AND METHODS

Preparation of Proteins—The acidic calponin open reading frame was obtained from SD rat brain homogenate using avian reverse transcriptase and amplified by PCR using Taq DNA polymerase (Perkin Elmer, USA). A specific 5′-primer containing a *Nde*I site and corresponding 3′-primers containing a *Bam*HI site were introduced at each end to generate full-length and non-fusion proteins. The PCR products were then ligated into pCR2.1 using a TA cloning kit (Novagen, USA) and sequenced with an ABI cycle sequencer (Perkin Elmer, USA). The pCR2.1 clone encoding the correct sequence of acidic calponin was digested with *Nde*I and *Bam*HI, ligated into pET24a (Novagen, USA), and then transfected in BL21 (DE3) for protein expression. Protein expression was initiated by the addition of IPTG (0.4 mM), and BL 21 (DE3) was harvested after 3 h incubation at 37°C. *Escherichia coli* was homogenized in 50 mM Tris-HCl (pH 7.0), 50 mM KCl, 1 mM EDTA, 1 mM EGTA, and 1 mM DTT, and then centrifuged to remove debris. The supernatant was loaded onto a DEAE-Sepharose CL-6B chromatography column (Amersham Pharmacia Biotech) and eluted with a linear gradient of 50–500 mM KCl. The eluent was collected and the fractions containing acidic calponin were further purified by SP Sephacryl S300 (Amersham Pharmacia Biotech) gel filtration. The purified protein was dialyzed against an appropriate buffer and then stored until use at –80°C.

Skeletal actin and calmodulin were purified from rabbit back muscle and pig testis, respectively, as previously reported (*6*, *7*). Porcine erythrocyte μ-calpain was purchased from Calbiochem (USA). Protein concentrations were determined spectrophotometrically using A_{280} (1%, 1 cm) values of 10.9 for actin, 10.1 for acidic calponin, and 2.0 for calmodulin.

F-Actin Binding Assay—Co-sedimentation assays were performed in a buffer containing 20 mM imidazole (pH 7.0), 50 mM KCl, 1 mM DTT, 5 mM ATP, 2 mM $MgCl_2$, and 5

[1] To whom correspondence should be addressed. Phone: +81-3-5841-5417, Fax: +81-3-5841-8183, E-mail: ahkrki@mail.ecc.u-tokyo.ac.jp

Abbreviations; aCP, acidic calponin; CaM, calmodulin; CBB R-250; Coomassie Brilliant Blue R-250, F-actin, filamentous actin; G-actin, monomeric actin.

© 2000 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

mM $CaCl_2$ at 25°C. Actin (3 μM) and various concentrations of acidic calponin were incubated for 40 min and then centrifuged at 800,000 ×*g* for 40 min at 25°C (Beckman Instruments), the pellet being resuspended in SDS-PAGE sample buffer. The sample was subjected to SDS-PAGE, followed by staining with Coomassie Brilliant Blue R-250 (CBBR-250). The densitometric intensity was quantified with NIH Image. For calibration, 3 μM (180 pmol) actin was polymerized and ultracentrifuged. After the supernatant had been removed, various amounts of acidic calponin were added and mixed in. The mixture was subjected to SDS-PAGE, followed by quantification as the ratio of calponin to actin. The amount of calponin plotted against the ratio of calponin to actin was confirmed to show a linear relation. The pelleted calponin was fitted to the linear equation and the amount of pelletted calponin was estimated.

Electrophoresis—SDS-PAGE was performed as reported previously (*8*). A Western blot of acidic calponin was visualized using monoclonal anti-calponin antibodies (Clone hCP; Sigma, USA).

IAsys—An optical evanescent resonant mirror cuvette system (IAsys; Affinity Sensors, UK) was used to investigate the interaction of acidic calponin with actin or calmodulin. Acidic calponin (0.74 μM) was immobilized on a carboxymethylated dextran–coated cuvette in accordance with the manufacturer's manual. A binding study of acidic calponin to actin was performed with the following buffer systems: G-buffer, containing 2 mM Tris HCl (pH 7.4), 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.5 mM β-mercaptoethanol; and F-buffer, containing G-buffer plus 50 mM KCl, and 2 mM $MgCl_2$. A study with calmodulin was performed in 20 mM imidazole (pH 7.0), 50 mM KCl, 1 mM DTT and 2 mM $CaCl_2$ or EGTA.

Proteolysis with μ-Calpain—Proteolysis with μ-calpain was performed in a buffer containing 20 mM imidazole (pH 7.0), 50 mM KCl, 1 mM DTT, 5 mM $CaCl_2$, and 5 mM cysteine. Acidic calponin (11 μM) was incubated in the above buffer at 25°C, and the reaction was initiated by adding μ-calpain to a final concentration of 4 u/ml. The reaction products were collected at the indicated times and subjected to SDS-PAGE. Acidic calponin remaining undigested was quantified as described under "MATERIALS AND METHODS". In some experiments, 3 μM F-actin or 60 μM calmodulin was added.

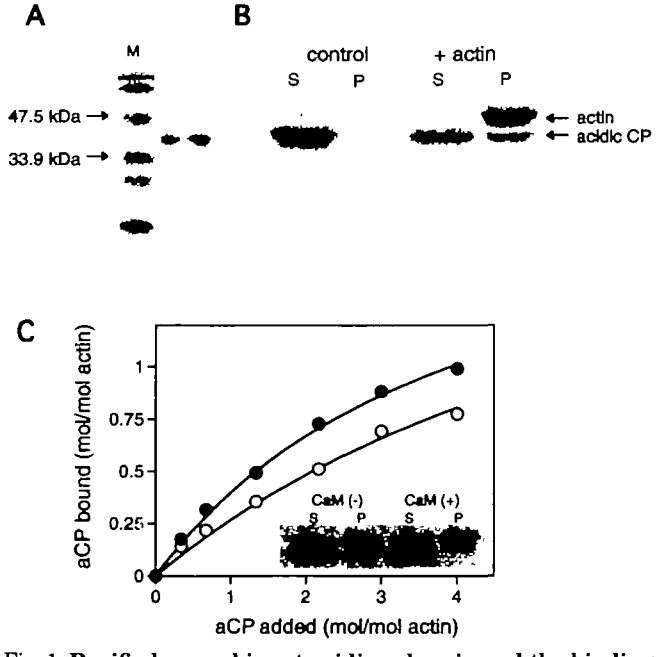

Fig. 1. **Purified recombinant acidic calponin and the binding of acidic calponin to F-actin in a co-sedimentation assay.** Purified acidic calponin was subjected to SDS-PAGE and visualized with CBB R-250 (A, left lane) or anti–smooth muscle calponin (A, right lane). Acidic calponin alone was not precipitated on ultracentrifugation (B, control). In the presence of F-actin (2.9 μM), acidic calponin (6 μM) co-precipitated with F-actin (B, +actin). S, supernatant, P, pellet. C. Various concentrations of acidic calponin were incubated with F-actin (3 μM) in the absence (closed circles) or presence (open circles) of calmodulin (30 μM), and the complex was precipitated by ultracentrifugation. The ratio of bound acidic calponin to actin was plotted against the ratio of added acidic calponin to actin. The inset shows the results of a co-sedimentation assay of acidic calponin (6 μM) and F-actin (2.9 μM) in the absence or presence of calmodulin (30 μM).

RESULTS

Figure 1A shows purified acidic calponin with an apparent molecular weight of 35 kDa observed on 12.5% SDS-PAGE. Acidic calponin cross-reacted with anti-smooth muscle calponin antibodies.

First, we investigated the binding of acidic calponin to actin. Co-sedimentation assays involving acidic calponin and filamentous-actin (F-actin) demonstrated that acidic calponin was pelletted together with F-actin on ultracentrifugation, suggesting that acidic calponin binds to F-actin (Fig. 1B). On the other hand, acidic calponin alone was not pelletted, as shown for the control. We next studied the stoichiometry of the binding of acidic calponin to F-actin. Under our experimental conditions, acidic calponin bound to F-actin in a 2:1 molar ratio with an apparent binding constant of 4×10^5 M^{-1} (Fig. 1C).

We next investigated whether or not acidic calponin binds to monomeric actin (G-actin) using an optical evanescent resonant mirror cuvette system (IAsys). Immobilized

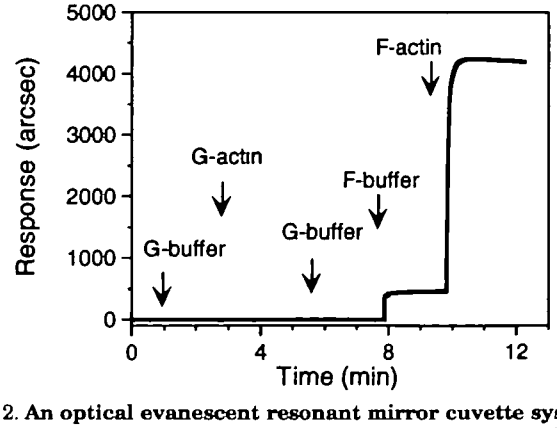

Fig. 2. **An optical evanescent resonant mirror cuvette system study showed acidic calponin binds to F-actin, but not G-actin.** Immobilized acidic calponin was equilibrated in G-buffer and then challenged to G-actin (10 μM). After G-actin had been washed out with G-buffer, acidic calponin was equilibrated in F-buffer and further challenged to F-actin (10 μM). An increase in signal intensity indicates the formation of the acidic calponin and actin complex.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

acidic calponin was equilibrated with G-buffer and then challenged to G-actin (10 μM), which did not increase the signal intensity (Fig. 2). After G-actin had been washed out with G-buffer, acidic calponin was equilibrated with F-buffer, which resulted in a slight increase in the signal intensity, possibly due to the buffer exchange. Further challenge of acidic calponin to F-actin (10 μM) elicited a significant increase in the signal intensity. Therefore, acidic calponin preferentially binds to actin when actin is polymerized.

Since it has been well demonstrated that basic calponin, a genetic variant of acidic calponin, binds to calmodulin (*1*, *9*), we next studied whether or not acidic calponin binds to calmodulin. As shown in Fig. 1C, co-sedimentation of acidic calponin with F-actin was partially inhibited in the presence of calmodulin, suggesting that calmodulin binds to acidic calponin. Since Applegate *et al.* (*2*) previously showed that acidic calponin does not bind calmodulin by means of a co-sedimentation assay, we further employed the IAsys method in order to confirm the binding. When immobilized acidic calponin was incubated in EGTA buffer and further challenged in EGTA buffer containing 10 μM calmodulin, no increase in signal intensity was observed (Fig. 3A). After calmodulin had been washed out with EGTA buffer, acidic calponin was incubated in $CaCl_2$ buffer and further challenged in $CaCl_2$ buffer containing 10 μM calmodulin. The result showed that the addition of calmodulin in the presence of Ca^{2+} significantly increased the binding signal. These results suggest that acidic calponin binds to calmodulin in a Ca^{2+}-dependent manner. We next studied the stoichiometry of the binding of acidic calponin to calmodulin in the presence of Ca^{2+}. The addition of calmodulin, from 1 to 85 μM, increased the binding signal in a concentration-dependent manner. When the concentration of bound calmodulin was plotted against that of free calmodulin, the curvature best fitted non-linear regression with an apparent K_d of 31 μM (Fig. 3B).

Next we investigated whether or not acidic calponin is cleaved by μ-calpain, a Ca^{2+}-dependent cysteine proteinase, since previous studies demonstrated that μ-calpain cleaved basic isoform of calponin (*10*, *11*). Acidic calponin and μ-calpain were incubated in the presence of 5 mM cysteine and 5 mM $CaCl_2$ for the indicated periods, and the reaction was terminated by the addition of SDS-PAGE sample buffer. The reaction products were subjected to SDS-PAGE and stained with CBB-R250. As shown in Fig. 4A, acidic

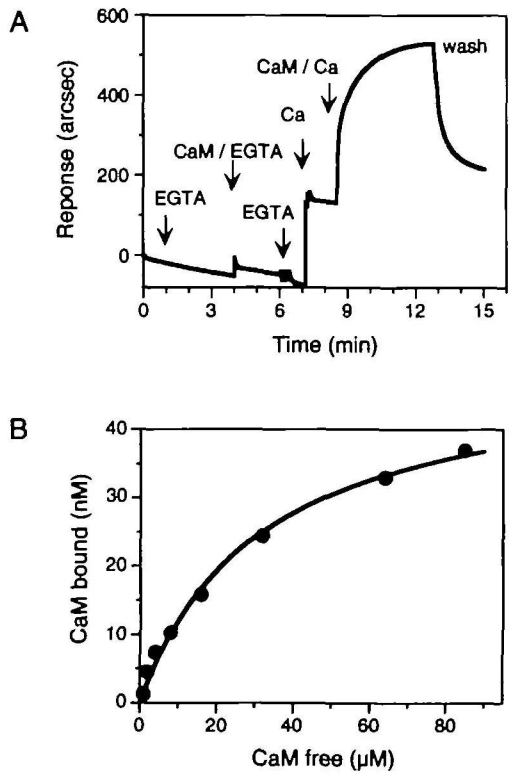

Fig. 3. **An optical evanescent resonant mirror cuvette system study showed acidic calponin binds to calmodulin in a Ca^{2+}-dependent manner.** A: Immobilized acidic calponin was incubated in EGTA (2 mM) buffer and then challenged to calmodulin (CaM, 10 μM) in EGTA buffer. After calmodulin had been washed out with EGTA buffer, the cuvette was refilled with $CaCl_2$ (2 mM) buffer, followed by a further challenge to calmodulin (10 μM) in $CaCl_2$ buffer. B: The concentration of calmodulin bound to acidic calponin was plotted against that of free calmodulin.

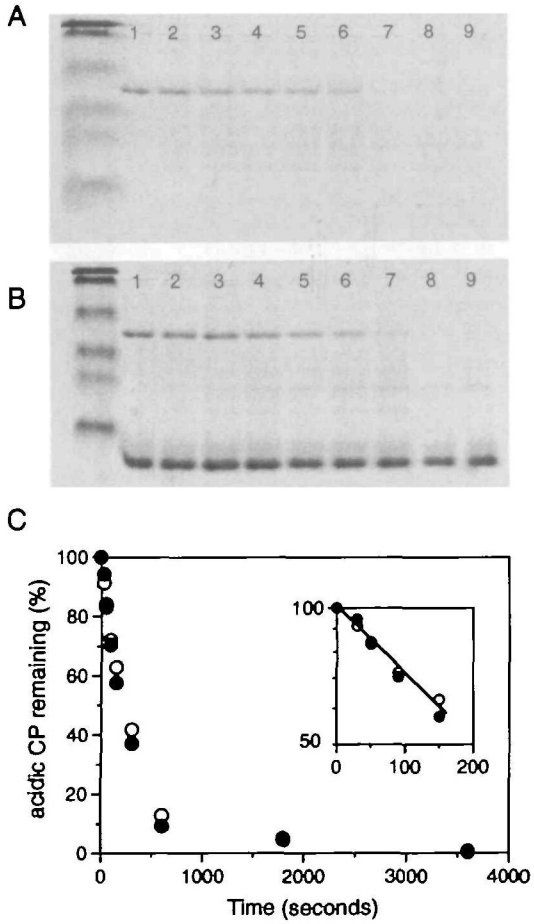

Fig. 4. **Acidic calponin was cleaved by μ-calpain.** Acidic calponin (11 μM) was cleaved with μ-calpain (4 u/ml) in the absence (A) or presence (B) of calmodulin (60 μM) in a time dependent manner (lanes: 1, 0 s; 2, 30 s; 3, 50 s; 4, 90 s; 5, 2.5 min; 6, 5 min; 7, 15 min; 8, 30 min; 9, 60 min), and the amount of acidic calponin remaining undigested was plotted against the incubation period (C: closed circles, control; open circles, + calmodulin). Semilogarithmic plots gave an almost linear relation in the early phase of proteolysis (inset).

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

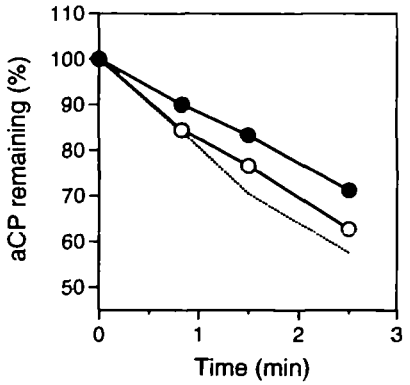

Fig. 5. **F-actin–bound acidic calponin was resistant to μ-calpain, which was reversed by the addition of calmodulin.** Acidic calponin (11 μM) and F-actin (3 μM) were incubated in the absence (closed circles) or presence (open circles) of calmodulin (60 μM). Proteolysis was initiated by the addition of μ-calpain (4 u/ml), followed by reaction for the indicated periods (0 min, 50 s, 90 s, 150 s). The dotted line represents the control, derived from the data in Fig. 4C.

calponin was cleaved in a time-dependent manner and no visible protein band was observed at 30 min. Two major bands corresponding to apparent molecular weights of 26 and 23 kDa, and two minor bands corresponding to 29 and 17 kDa were observed at 60 min. The percentage of the densitometric intensity of the undigested acidic calponin was plotted against the incubation period (Fig. 4C). Semilogarithmic plots gave an almost linear relation in the early phase of protein cleavage, suggesting that the proteolytic reaction is a first-order rate one (Fig. 4C, inset). Previous studies have shown that the proteolytic pattern or velocity of some calmodulin binding proteins differs in the absence or presence of calmodulin. Therefore, we next studied the effect of calmodulin on the proteolysis of acidic calponin by μ-calpain. As shown in Fig. 4, B and C, the presence of calmodulin affected neither the proteolytic pattern nor velocity of acidic calponin with μ-calpain.

It has been shown that acidic calponin is colocalized with actin filaments in living cells (*3*, *5*), hence we next examined the effect of F-actin on the proteolysis of acidic calponin. In the presence of F-actin, the proteolytic velocity of acidic calponin was significantly retarded. The addition of calmodulin increased the proteolysis toward the control level (Fig. 5).

DISCUSSION

In this report, we provided the first evidence that acidic calponin is cleaved by μ-calpain, a Ca^{2+}-activated cysteine proteinase. Evidence is also provided that the proteolysis of acidic calponin was significantly retarded in the presence of F-actin, which was reversed by the addition of calmodulin.

A co-sedimentation assay showed that acidic calponin binds to F-actin (Fig. 1, B and C) with an apparent binding constant of 4×10^5 M^{-1}. We further demonstrated that the binding of acidic calponin to actin is restricted when actin is filamentous, but not monomeric, using the IAsys system (Fig. 2). Previous studies have demonstrated that acidic calponin is colocalized along actin fibers in living cells (*4*, *5*), and furthermore that acidic calponin is enriched in the growth cones of cerebellar and cortical cells in the brain (*5*). Since growth cone motility has been demonstrated to be dependent on actin filament assembly (*12*), acidic calponin might be involved in the remodeling of filamentous actin *in vivo*.

Binding of acidic calponin to F-actin was observed to be inhibited in the presence of calmodulin in a co-sedimentation assay (Fig. 1C), suggesting that acidic calponin binds to calmodulin. Previous studies have shown that basic calponin, an actin and calmodulin binding protein, was co-sedimented with F-actin and that the binding was inhibited in the presence of calmodulin (*1*, *13*, *14*). The amino acid residues which are responsible for the binding of basic calponin to calmodulin and actin are located within the N-terminus 22 kDa region (*14*), which shows high homology to that of acidic calponin. We further employed the IAsys system to investigate the binding of acidic calponin to calmodulin and its Ca^{2+} dependency. The results showed that acidic calponin directly binds to calmodulin in a Ca^{2+}-dependent manner (Fig. 3A). Applegate *et al.* (*2*) demonstrated that calmodulin did not inhibit the binding of acidic calponin to F-actin in a co-sedimentation assay, and they suggested a functional distinction between acidic and basic isoforms. In this study, we demonstrated the binding of acidic calponin to calmodulin using two different experimental procedures. One possible reason for the conflicting results would be that the acidic calponin used in their study was purified partially and we used the more than 95% purified protein. Alternatively, since the binding affinity of acidic calponin to calmodulin is not high (K_d = 31 μM), they might have failed to detect the binding under their experimental conditions.

Figure 4 shows that acidic calponin was cleaved by μ-calpain, a Ca^{2+}-dependent cysteine proteinase. The results of a kinetic study in the early phase of proteolysis indicated that the proteolysis is a first-order rate reaction. It has been reported that the proteolytic velocity of some calmodulin binding proteins such as brain spectrin and basic calponin is accelerated in the presence of calmodulin (*10*, *11*, *15*), and that that of others such as caldesmon and erythroid spectrin is not (*15*, *16*). Furthermore, the cleaved patterns of myosin light chain kinase, Ca^{2+}-ATPase and calcineurin differ in the absence and presence of calmodulin (*16–18*). Therefore, we next investigated the effect of calmodulin on the proteolysis of acidic calponin. The results indicated that neither the velocity nor the cleaved pattern was affected in the presence of calmodulin, indicating different regulation of acidic calponin by calmodulin from that of the basic isoform (*10*). Since acidic calponin binds to F-actin *in vitro* (*2*, *4*) and *in vivo* (*3–5*), the F-actin–bound form of acidic calponin was incubated with μ-calpain. The results showed that the proteolysis of acidic calponin was significantly inhibited, like that of basic calponin (*10*), suggesting that the F-actin–bound form of acidic calponin in living cells is resistant to μ-calpain. Furthermore, the addition of calmodulin restored the proteolysis of acidic calponin in the presence of F-actin. μ-Calpain is a proteinase that is activated at micromolar cytosolic Ca^{2+} concentrations, when calmodulin has bound to Ca^{2+} and is fully activated. Therefore, it is likely that under certain conditions when the cytosolic Ca^{2+} concentration is sufficiently elevated to activate μ-calpain, the Ca^{2+}-calmodulin complex binds to acidic calponin and dissociates it from the actin cytoskeleton, which contributes

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

to the proteolysis of acidic calponin by μ-calpain. Although the precise role of μ-calpain has not been elucidated, previous reports have suggested that μ-calpain is activated in cell migration, and spreading and apoptosis in living cells, and cleaves some actin-associated proteins such as integrin, spectrin and focal adhesion kinase (*19–23*). Furthermore, Ferhat *et al.* have reported that acidic calponin is abundant in migrating or proliferating neuronal cells in the developing rat hippocampus (*3*), suggesting that the proteolysis of acidic calponin might be involved in cell migration.

In this report, we demonstrated that acidic calponin, a calmodulin- and F-actin binding protein, is a substrate for the proteolysis of μ-calpain. The role of acidic calponin in living cells remains unclear, however, our results suggest that the proteolysis of acidic calponin might play a role in actin-linked cytoskeletal regulation, which might be modulated by Ca^{2+}-calmodulin.

REFERENCES

1. Winder, S.J. and Walsh, M.P. (1996) Calponin. *Curr. Top. Cell Regul.* **34**, 33–61
2. Applegate, D., Feng, W., Green, R.S., and Taubman, M.B. (1994) Cloning and expression of a novel acidic calponin isoform from rat aortic vascular smooth muscle. *J. Biol. Chem.* **269**. 10683–10690
3. Ferhat, L., Charton, G., Represa, A., Ben-Ari, Y., der Terrossian, E., and Khrestchatisky, M. (1996) Acidic calponin cloned from neural cells is differentially expressed during rat brain development. *Eur. J. Neurosci.* **8**, 1501–1509
4. Gimona, M. and Mital, R. (1998) The single CH domain of calponin is neither sufficient nor necessary for F-actin binding. *J. Cell Sci.* **111**, 1813–1821
5. Plantier, M., Fattoum, A., Menn, B., Ben-Ari, Y., Der Terrossian, E., and Represa, A. (1999) Acidic calponin immunoreactivity in postnatal rat brain and cultures: subcellular localization in growth cones, under the plasma membrane and along actin and glial filaments. *Eur. J. Neurosci.* **11**, 2801–2812
6. Spurdich, J.A. and Watt, S. (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* **246**, 4866–4871
7. Gopalakrishna, R. and Anderson, W.B. (1982) Ca^{2+}-induced hydrophobic site on calmodulin: application for purification of calmodulin by phenyl-Sepharose affinity chromatography. *Biochem. Biophys. Res. Commun.* **104**, 830–836
8. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
9. Takahashi, K., Hiwada, K., and Kokubu, T. (1986) Isolation and characterization of a 34,000-dalton calmodulin- and F-actin-binding protein from chicken gizzard smooth muscle. *Biochem. Biophys. Res. Commun.* **141**, 20–26
10. Tsunekawa, S., Takahashi, K., Abe, M., Hiwada, K., Ozawa, K., and Murachi, T. (1989) Calpain proteolysis of free and bound forms of calponin, a troponin T-like protein in smooth muscle. *FEBS Lett.* **250**, 493–496
11. Croall, D.E., Chacko, S., and Wang, Z. (1996) Cleavage of caldesmon and calponin by calpain: substrate recognition is not dependent on calmodulin binding domains. *Biochim. Biophys. Acta* **1298**, 276–284
12. Yamada, K.M. and Wessells, N.K. (1973) Cytochalacin B: effects on membrane ruffling, growth cone and microspike activity, and microfilament structure not due to altered glucose transport. *Dev. Biol.* **31**, 413–420
13. Winder, S.J., Walsh, M.P., Vasulka, C., and Johnson, J.D. (1993) Calponin-calmodulin interaction: properties and effects on smooth and skeletal muscle actin binding and actomyosin ATPases. *Biochemistry* **32**, 13327–13333
14. Mezgueldi, M., Fattoum, A., Derancourt, J., and Kassab, R. (1992) Mapping of the functional domains in the amino-terminal region of calponin. *J. Biol. Chem.* **267**, 15943–15951
15. Seubert, P., Baudry, M., Dudek, S., and Lynch, G. (1987) Calmodulin stimulates the degradation of brain spectrin by calpain. *Synapse* **1**, 20–24
16. Kosaki, G., Tsujinaka, T., Kambayashi, J., Morimoto, K., Yamamoto, K., Yamagami, K., Sobue, K., and Kakiuchi, S. (1983) Specific cleavage of calmodulin-binding proteins by low Ca^{2+}-requiring form of Ca^{2+}-activated neutral protease in human platelets. *Biochem. Int.* **6**, 767–775
17. Wang, K.K., Roufogalis, B.D., and Villalobo, A. (1988) Further characterization of calpain-mediated proteolysis of the human erythrocyte plasma membrane Ca^{2+}-ATPase. *Arch. Biochem. Biophys.* **267**, 317–327
18. Tallant, E.A., Brumley, L.M., and Wallace, R.W. (1988) Activation of a calmodulin-dependent phosphatase by a Ca^{2+}-dependent protease. *Biochemistry* **27**, 2205–2211
19. Croall, D.E. and DeMaritino, G.N. (1991) Calcium-activated protease (calpain) system, structure, function and regulation. *Physiol. Rev.* **71**, 813–847
20. Sorimachi, H., Ishiura, S., and Suzuki, K. (1997) Structure and physiological function of calpains. *Biochem. J.* **328**, 721–732
21. Cooray, P., Yuan, Y., Schoenwaelder, S.M., Mitchell, C.A., Salem, H.H., and Jackson, S.P. (1996) Focal adhesion kinase (pp-125FAK) cleavage and regulation by calpain. *Biochem. J.* **318**, 41–47
22. Huttenlocher, A., Palecek, S.P., Lu, Q., Zhang, W., Mellgren, R.L., Lauffenburger, D.A., Ginsberg, M.H., and Horwitz, A.F. (1997) Regulation of cell migration by the calcium-dependent protease calpain. *J. Biol. Chem.* **272**, 32719–35722
23. Potter, D.A., Tirnauer, J.S., Janssen, R., Croall, D.E., Hughes, C.N., Fiacco, K.A., Mier, J.W., Maki, M., and Herman, I.M. (1998) Calpain regulates actin remodeling during cell spreading. *J. Cell Biol.* **141**, 647–662

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012