Limited digestion of α-actinin in the presence of F-actin

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N-terminal actin-binding domain of α-actinin is connected to central rod domain through flexible neck region that is susceptible to proteolysis. It is suggested that the neck region assumes variable orientations by actin binding. In order to examine the effect of actin binding to α-actinin, we carried out limited digestion of α-actinin by chymotrypsin in the presence and absence of F-actin. Although the cleavage process was retarded when bound to F-actin, digestion to 32 kDa-head and 55 kDa-rod domains occurred through the same intermediate products as the digestion in the absence of F-actin. N-terminal sequencing of 55 kDa-fragment showed the neck region was cleaved at 276-Leu. The cleavage site was not affected by binding to F-actin nor ionic strength of the solvent. It was also indicated that α-actinin was cleaved at 15-Tyr by chymotrypsin. Quantitation of the cleavage products by densitometry of the SDS-gels suggested the conformational change of α-actinin at domain-connecting regions by F-actin binding.

Key words: actin binding domain, chymotrypsin digestion

α-Actinin is an actin binding protein that plays a critical role for the integration of actin cytoskeleton. α-Actinin crosslinks actin filaments and causes bundling and gelation of actin filaments. In muscle cells α-actinin localizes at actin bundling structure as Z-disks of striated muscles and dense bodies in smooth muscle. Non-muscle isoforms of α-actinins distribute periodically along actin filament bundles like stress fibers and then participate in anchoring structure of these actin bundles to membrane, adhesion plaques or focal contacts. Moreover, recent works have shown that α-actinins offer binding sites to multiple structural and regulatory proteins as multi-talent platform.

α-Actinin is a rod shaped homodimer of polypeptides of ~100 kD each that are oriented anti-parallel. Based on its domain structure, α-actinin is classified as a member of spectrin superfamily. Each subunit consists of three functional domains; N-terminal containing actin binding domain (ABD) with tandem calponin homology motifs, rod domain with four spectrin-repeats, and regulatory tail domain with EF-hand like motifs. The binding activity of non-muscle α-actinins was shown to be Ca²⁺-sensitive while that of muscle isoforms was regulated by phosphoinositides.

Structural analysis on α-actinin molecules indicates that the central rod domain of the dimer registered, thus suggesting the interaction of ABD and regulatory domain between the opposite subunits regulates the binding activity of α-actinin to actin filaments. The linker regions between the domains are assumed to be flexible and are susceptible to limited digestion by proteases. Taylor and his colleagues have proposed that flexible joint connecting ABD and rod domain assume variable orientations by actin binding. Although crystallography has revealed detailed structure of isolated ABDs and rod domains, the conformational change of this region by actin binding is not well known.

In this paper, we carried out the limited digestion of α-actinin that bound to F-actin. In the presence of F-actin, the cleavage process became retarded when compared with that in the absence. However, N-terminal sequence study showed that cleavage site between actin binding domain and rod-domain was not affected by F-actin binding.
Materials and Methods

Protein purification

**Purification of α-actinin**: α-Actinin was prepared from glycerinated chicken breast muscle as described previously. Briefly, myofibrils were extracted with high salt solution containing 0.6 M KCl, 5 mM Mg-pyrophosphate, and 50 mM Tris-maleate pH 6.5, and resulting I-Z-I brush was extracted with 5 mM Tris-KCl (pH 8.0) and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). The extract was fractionated with ammonium sulfate fractionation and precipitates obtained between 40–60% ammonium sulfate saturation were applied to DEAE-cellulose column equilibrated with 5 mM Tris-HCl (pH 8.0), 0.2 mM DTT and 0.5 mM PMSF. α-Actinin was eluted with linear KCl gradient and fractions eluted between 0.15–0.20 M KCl.

**Purification of actin**: Actin was extracted from an acetone-dried powder of chicken skeletal muscle by the method of Spudich and Watt and purified by Sephadex G-150 column chromatography equilibrated with 0.2 mM ATP, 0.01% sodium azide and 5 mM Tris-HCl, pH 8.0.

**SDS-polyacrylamide gel electrophoresis and densitometry**

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was carried out according to Laemmli with a 10% separating gel. Protein samples were treated for three minutes over boiling water with one-third volumes of SDS-solution containing 5% SDS, 5% 2-mercaptoethanol, 0.1 mM PMSF, and 50% glycerol. Samples for protein sequencing studies were treated with the SDS-solution for 10 min at 37°C. After electrophoresis, the gels were fixed for 10 min with 50% ethanol and 12% acetic acid and stained with 0.2% Coomassie Brilliant Blue (CBB). Molecular weights of protein bands were determined using molecular weight standard purchased from Sigma (Broad Range).

CBB-stained gels were photographed with a digital camera (Canon Power Shot G-40) at the resolution of 300 dpi. Amount of proteins detected on a SDS-gel was quantitated from the digitized files in tif-format using Image J (ver. 1.4).

**Protein Sequencing**

Protein bands resolved on a SDS-gel were electrically transferred to polyvinylidene fluoride (PVDF) membranes for 3 h at a constant current of 80 mA using 25 mM Tris-glycine (pH 8.3), 20% ethanol and 0.1 mM ethylene dithio- glycol as an electrolyte. Protein bands on transferred polyvinylidene fluoride (PVDF) membranes were visualized by brief staining with 0.1% CBB, and 32 kDa (head domain) and 55 kDa (rod domain) bands were excised from PVDF membranes. Five amino acid residues of N-terminal sequences of those bands were identified by Edman degradations using Shimazu PSQ-1 gas phase protein sequencer.

Other methods

Protein concentrations were determined by DC-Protein assay (BioRad) standardized with bovine serum albumin (Sigma). Concentration of purified α-actinin and actin were determined spectrophotometrically using extinction coefficient at 278 nm of 0.97 mg\(^{-1}\) ml cm\(^{-1}\) for α-actinin and at 290 nm of 0.62 mg\(^{-1}\) ml cm\(^{-1}\) for actin respectively.

Results and Discussion

**Time course of α-actinin digestion in the presence of F-actin**

Limited digestion with a protease is a useful method to access conformation of native proteins. α-Actinin is cleaved by chymotrypsin into protease resistant domains; namely N-terminal containing actin binding and rod-domain. In a previous paper, we have shown that ionic strength of the solvents affects the conformation of skeletal muscle α-actinin molecule. In the presence of 0.1 M KCl, the conformation of α-actinin is more compact than that in the absence of added salts. Reflecting the conformation, α-actinin becomes less susceptible to limited digestion although cleavage sites between domains were not affected (refer cleavage map shown in Fig. 4).

In this report, we examined the cleavage pattern of α-actinin that bound to F-actin. α-Actinin (0.82 mg/ml) was mixed with 0.93 mg/ml of F-actin and centrifuged for 1 hr with a Hitachi 55P-72 ultracentrigue with a 40P rotor at 300,000 rpm. Pelleted α-actinin-F-actin complex was dispersed in 0.1 M KCl and 20 mM Tris-HCl, pH 7.5. Densitometry of the SDS-gel of the mixture indicated that the molar ratio of α-actinin to actin was 0.13, showing saturated binding of α-actinin to F-actin. As F-actin was fairly resistant...
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to chymotryptic digestion (refer Fig. 1B), concentration of α-actinin in the complex was estimated from the molar ratio and 1/100 (w/w) chymotrypsin (Sigma, type I-S) was added to chymotryptic digestion after clarification of α-actinin for 1 hr at 30,000 rpm. (A) α-Actinin (0.42 mg/ml) was subjected to chymotryptic digestion at 25°C. (A) α-Actinin (0.42 mg/ml) was subjected to chymotryptic digestion after clarification of α-actinin for 1 hr at 30,000 rpm, (B) α-Actinin-F-actin complex was pelleted by the ultracentrifugation and dispersed in 0.1 M KCl and 20 mM Tris-HCl (pH 7.5) at a protein concentration of 0.80 mg/ml. α-Actinin in the complex was digested with chymotrypsin. Numbers on the top of gel images indicate incubation time in hour. Numbers on the side of the image show molecular weight of marker proteins (MW) and those of cleavage products shown in k (×10^3). The 98 kDa-fragment that appeared only early period of digestion in small amount was marked by asterisk. The last lane in Plate B (-Fa5) showed the 5h-cleavage product without actin just shown for comparison of products. Polyacrylamide concentration, 10%. See the text for detail.

**Figure 2** Time course of α-actinin digestion. α-Actinin was digested with 1/100 (w/w) chymotrypsin in 0.1 M KCl and 20 mM Tris-HCl (pH 7.5) at 25°C. (A) α-Actinin (0.42 mg/ml) was subjected to chymotryptic digestion after clarification of α-actinin for 1 hr at 30,000 rpm, (B) α-Actinin-F-actin complex was pelleted by the ultracentrifugation and dispersed in 0.1 M KCl and 20 mM Tris-HCl (pH 7.5) at a protein concentration of 0.80 mg/ml. α-Actinin in the complex was digested with chymotrypsin. Numbers on the top of gel images indicate incubation time in hour. Numbers on the side of the image show molecular weight of marker proteins (MW) and those of cleavage products shown in k (×10^3). The 98 kDa-fragment that appeared only early period of digestion in small amount was marked by asterisk. The last lane in Plate B (-Fa5) showed the 5h-cleavage product without actin just shown for comparison of products. Polyacrylamide concentration, 10%. See the text for detail.

**Figure 3** Change in the amount of cleavage products detected during digestion. The amount of cleavage products were measured from digitized images of the SDS-gel with NIH ImageJ in the presence (A) and the absence (B) of F-actin. Ordinate, the relative amount of each product to the amount of 105 kDa-band in zero-time was expressed in percentage.
The actin-binding domain and the rod domain of α-actinin are linked by about 30 residues protease-sensitive linker. The neck region is flexible and actin-binding domain can assume variable conformations over rod domain. Therefore it is suggested that binding of α-actinin to F-actin induced conformational changes on this flexible neck region. Corgan et al. showed that binding of phosphoinositides to smooth muscle α-actinin affected the cleavage site on the neck region. Then, the question arises whether the binding of α-actinin to F-actin or ionic strength of the solvent influences the cleavage site on the neck region.

We examined N-terminal sequence of 32 kDa head and 55 kDa rod by Edman reaction and assigned the cleavage site on the reported primary sequence of chicken skeletal α-actinin. As shown in Table 1, N-terminal sequence of 55 kDa-rod domains was Ala-Val-Asn-Gln-Glu– indicating chymotrypsin hydrolyzed carbonyl side of 276-Leu. This cleavage site was not affected by actin binding nor by ionic strength of the solvent. It is interesting that this cleavage site was identical to the cleavage site reported to smooth muscle α-actinin. In addition, it was shown that chymotrypsin also cleavaged at 15-Tyr. N-terminal sequence of the 32 kDa-actin-binding domain was Asn-Tyr-Glu–Glu- irrespective to the experimental conditions examined. Cleavage of N-terminal fragment of actin-binding domain with thermolysin at 24-Leu was reported for smooth muscle α-actinin. Figure 4A illustrates the cleavage map of chicken skeletal α-actinin. Site Ia located at 15-Tyr and Site I at 276-Leu. Judged by the primary sequence of skeletal muscle α-actinin, Site II and Site III was assigned around 750th and 850th residue, respectively. Regulatory domain with EF-hand-like motifs exits between Site II and near C-terminal end.

Determination of N-terminal peptide sequences of digested products

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32 k-ABD and C-terminal containing 72 kDa-fragments are formed by cleavage of native α-actinin at Site I. Cleavage of native α-actinin at Site II and Site III result in the formation of ABD-containing 87 kDa- and 98 kDa-fragment respectively. Formation of 98 kDa-fragment was limited only at early periods of the digestion and the amount of the 98 kDa was very small (refer Fig. 2). Flow chart of the degradation of α-actinin into actin-binding and rod-domain through subfragments are summarized in Figure 4B. There are three unique pathways of the degradation of native α-actinin. As the amount of 98 kDa-fragment was very small and limited, contribution of Path C for accumulation of other subfragments would be negligible for the present experiment.

As shown in Figure 3, the ratios of subfragments detected during digestion were affected by binding of α-actinin to F-actin. In the absence of F-actin, 87 kDa-fragment was a major product throughout the digestion process. 72 k- and 68 k-fragment were also detected continuously during the cleavage. Therefore, it is suggested that both Path A and B were available for the digestion of α-actinin into 32 k- and 55 k-domain in the absence of F-actin. On the other hand, in the presence of F-actin, 72 kDa-fragment was detected as a major product and its amount increased with time of digestion. These results suggest that binding to F-actin caused some conformational change at the neck region of α-actinin.

Table 1 Amino acid sequences of 32 k and 55 k fragments

| 32 k fragment | 55 k fragment |
|--------------|--------------|
| −KCl Asn-Tyr-Glu–Glu-Asp | Ala-Val-Asn-Glu-Glu |
| 0.1 M KCl Asn-Tyr-Glu–Glu-Asp | Ala-Val-Asn-Glu-Glu |
| +FA Asn-Tyr-Glu–Glu-Asp | Ala-Val-Asn-Glu-Glu |

Limited digestion with chymotrypsin was carried out in the absence of added salt (−KCl), in the presence of 0.1 M KCl, and in the presence of F-actin.

Figure 4 Cleavage map of skeletal muscle α-actinin. Cleavage sites of α-actinin by chymotrypsin were mapped on the primary sequence of skeletal muscle α-actinin (A). Flow chart for the degradation of 105 k- subunit into 55 k- and 32 k-domain (B). See the text for details.
and enhanced the cleavage at Site I. In addition, the formation of 87 kDa-fragment was limited at early period of digestion in the presence of F-actin. As shown in Figure 1, the central rod domain of the dimer registered. This model indicates that Site I of one subunit locates close vicinity to Site II of another subunit. Therefore, it is quite probable that conformational change on neck region of one subunit affect the conformation of Site II of counterpart subunit, then reduce the formation of 87 kDa-fragment.

References
1. Blanchard, A., Ohanian, V. & Critchley, D. The structure and function of α-actinin. J. Muscle Res. Cell Motil. 10, 280–289 (1989).
2. Sjöblom, B., Salmazo, A. & Djönović-Carugo, K. α-Actinin structure and regulation. Cell Mol Life Sci. 65, 2688–2701 (2008).
3. Masaki, T., Endo, M. & Ebashi, S. Localization of 6S component of α-actinin at Z-band. J. Biochem. 62, 630–632 (1967).
4. Luther, P. K. The vertebrate muscle Z-disc: sarcomere anchor for structure and signaling. J. Muscle Res. Cell Motil. 30, 171–185 (2009).
5. Geiger, B., Dutton, A. H., Tokuyasu, K. T. & Singer, S. J. Immunoelectron microscope studies of membrane-microfilament interactions, distributions of α-actinin, tropomyosin, and vinculin in intestinal epithelial brush border and chicken gizzard smooth muscle cells. J. Cell Biol. 91, 614–628 (1981).
6. Draeger, A., Amos, W. B., Ikebe, M. & Small, J. V. The cytoskeletal and contractile apparatus of smooth muscle: contraction bands and segmentation of the contractile elements. J. Cell Biol. 111, 2463–2473 (1990).
7. Burridge, K., Nuckolls, G., Otey, C., Pavalko, F., Simon, K. & Turner, C. Actin-membrane interaction in focal adhesions. Cell Diff. Dev. 32, 337–342 (1990).
8. Naumenko, P., Lappalainen, P. & Hotulainen, P. Mechanisms of actin stress fibre assembly. J. Microsc. 231, 446–454 (2008).
9. Otey, C. A. & Carpen, O. α-Actinin revisited: A fresh look at an old player. Cell Motil. Cytoskeleton 58, 104–111 (2004).
10. Djönović-Carugo, K., Gautel, M., Ylänne, J. & Young, P. The spectrin repeat: A structural platform for cytoskeletal protein assemblies. FEBS Lett. 513, 119–123 (2002).
11. Young, P. & Gautel, M. The interaction of titin and α-actinin is controlled by a phospholipid-regulated intramolecular pseudodigand mechanism. EMBO J. 19, 6331–6340 (2000).
12. Sanger, J. W., Kang, S., Siebrands, C. C., Freeman, N., Du, A., Wang, J., Staehelin, T. & Sanger, J. M. How to build a myofibril. J. Muscle Res. Cell Motil. 26, 343–354 (2005).
13. Burridge, K. & Féraudis M. J. R. Non-muscle α-actinins are calcium-sensitive actin-binding proteins. Nature 294, 565–567 (1981).
14. Noegel, A., Witke, W. & Schleicher, M. Calcium-sensitive non-muscle α-actinin contains EF-hand structures and highly conserved regions. FEBS Lett. 221, 391–396 (1987).
15. Fukami, K., Furuhashi, K., Inagaki, M., Endo, T., Hatano, S. & Takenawa, T. Requirement of phosphatidylinositol 4,5-bisphosphate for α-actinin function. Nature 359, 150–152 (1992).
16. Fraley, T. S., Pereira, C. B., Tran, T. C., Singleton, C. & Greenwood, J. A. Phosphoinositide binding regulates α-actinin dynamics: mechanism for modulating cytoskeletal remodeling. J. Biol. Chem. 280, 15479–15482 (2005).
17. Corgan, A. M., Singleton, C., Santoso, C. B. & Greenwood, J. A. Phosphoinositides differentially regulate α-actinin flexibility and function. Biochem. J. 378, 1067–1072 (2004).
18. Flood, G., Kahana, E., Gilmore, A. P., Rowe, A. J., Gratzer, W. B. & Critchley, D. R. Association of structural repeats in the α-actinin rod domain. Alignment of inter-subunit interactions. J. Mol. Biol. 252, 227–234 (1995).
19. Taylor, K. A. & Taylor, D. W. Projection image of smooth muscle α-actinin from two-dimensional crystals formed on positively charged lipid layers. J. Mol. Biol. 230, 196–205 (1993).
20. Djönović-Carugo, K., Young, P., Gautel, M. & Saraste, M. Structure of the α-actinin rod: molecular basis for cross-linking of actin filaments. Cell 98, 537–546 (1999).
21. Hampton, C. M., Taylor, D. W. & Taylor, K. A. Novel structures for α-actinin: F-actin interactions and their implications for actin-membrane attachment and tension sensing in the cytoskeleton. J. Mol. Biol. 368, 92–104 (2007).
22. Liu, J., Taylor D. W. & Taylor, K. A. A 3-D reconstruction of smooth muscle α-actinin by cryoEm reveals two different conformations at the actin-binding region. J. Mol. Biol. 338, 115–125 (2004).
23. Borrego-Diaz, E., Kerff, F., Lee, S. H., Ferron, F. L. & Domínguez, R. Crystal structure of the actin-binding domain of α-actinin I: evaluating two competing actin-binding models. J. Struct. Biol. 155, 230–238 (2006).
24. Lee, S. H., Weins, A., Hayes, D. B., Pollak, M. R. & Domínguez, R. Crystal structure of the actin-binding domain of α-actinin-4 Ly525Glu mutant implicated in focal segmental Glomerulosclerosis. J. Mol. Biol. 376, 317–324 (2008).
25. Galkin, V. E., Orlova, A., Salmazo, A., Djönović-Carugo, K. & Egelman, E. H. Opening of tandem calponin homology domains regulates their affinity for F-actin. Nat. Struct. Mol. Biol. 17, 614–616 (2010).
26. Ylänne, J., Schefzik, K., Young, P. & Saraste, M. Crystal structure of the α-actinin rod reveals an extensive torsional twist. Structure 9, 597–604 (2001).
27. Golgi, J., Collins, R. & Mofrad, M. R. Molecular mechanics of the α-actinin rod domain: bending, torsional, and extensional behavior. PLoS Comput. Biol. 5, e1000389 (2009).
28. Kuroda, M., Kohira, Y. & Sasaki, M. Conformational change of skeletal muscle α-actinin induced by salt. Biochim. Biophys. Acta 1205, 97–104 (1994).
29. Spudich, J. A. & Watt, S. The regulation of rabbit skeletal muscle contraction. J. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. J. Biol. Chem. 246, 4866–4871 (1971).
30. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685 (1970).
31. Towbin, H., Staehelin, T. & Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350–4354 (1979).
32. Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M. & Stromer, M. H. Some properties of purified skeletal muscle α-actinin. J. Biol. Chem. 251, 6870–6870 (1976).
33. Gordon, D. J., Yang, Y. Z. & Korn, E. D. Polymerization of gizzard smooth muscle α-actinin-4 Lys255Glu mutant implicated in focal segmental Glomerulosclerosis. J. Mol. Biol. 376, 317–324 (2008).
34. Golgi, D. E., Suzuki, A., Temple, J. & Holmes, G. R. Studies on purified α-actinin: I. Effect of temperature and tropomyosin
on the α-actinin/F-actin interaction. *J. Mol. Biol.* 67, 469–472 (1972).

35. Meyer, R. K. & Aebi, U. Bundling of actin filaments by alpha-actinin depends on its molecular length. *J. Cell Biol.* 110, 2013–2024 (1990).

36. Winkler, J., Lärnsdorf, H. & Jockusch, B. M. Flexibility and fine structure of smooth-muscle alpha-actinin. *Eur. J. Biochem.* 248, 193–199 (1997).

37. Arimura, C., Suzuki, T., Yanagisawa, M., Imamura, M., Hamada, Y. & Masaki, T. Primary structure of chicken skeletal muscle and fibroblast alpha-actinins deduced from cDNA sequences. *Eur. J. Biochem.* 177, 649–655 (1988).

38. Parr, T., Waite, G. T., Patel, B., Millake, D. B. & Critchley, D. R. A chick skeletal-muscle alpha-actinin gene gives rise to two alternatively spliced isoforms which differ in the EF-hand Ca(2+)-binding domain. *Eur. J. Biochem.* 210, 801–809 (1992).

39. Imamura, M., Endo, T., Kuroda, M., Tanaka, T. & Masaki, T. Substructure and higher structure of chicken smooth muscle alpha-actinin molecule. *J. Biol. Chem.* 263, 7800–7805 (1988).

40. Davison, M. D., Baron, M. D., Wootton, J. C. & Critchley, D. R. Structural analysis of homologous repeated domains in α-actinin and spectrin. *Int. J. Biol. Macromol.* 11, 81–90 (1989).

41. Xu, J., Wirtz, D. & Pollard, T. D. Dynamic cross-linking by alpha-actinin determines the mechanical properties of actin filament networks. *J. Biol. Chem.* 273, 9570–9576 (1998).