The binding of skeletal muscle C-protein to regulated actin

Katsuhiro Yamamoto

Department of Dairy Science, Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan*

Received 5 August 1986

The binding of C-protein, a component of thick filament of myofibrils, to regulated actin filaments in the presence or absence of Ca^{2+} was studied. The amount of C-protein bound to regulated actin filaments in the presence of Ca^{2+} was higher than those in the absence of Ca^{2+}. The addition of C-protein to regulated actin caused an increase in turbidity, especially in the presence of Ca^{2+}, and this was found to result from side-by-side association of actin filaments into bundles. In the absence of Ca^{2+}, no actin filament bundles were formed.

C-protein *Actin filament* *Tropomyosin-troponin* *(Skeletal muscle)*

1. INTRODUCTION

C-protein is a component of the thick filaments of skeletal [1] and cardiac [2] muscle myofibrils. It binds to the rod portion of the myosin molecule [3,4] and affects the configuration of myosin filaments [3,5]. C-protein also binds to F-actin [6] and to the I-band of myofibrils [7]. The latter binding is Ca^{2+}-dependent, i.e. C-protein binds to the I-band in the presence of Ca^{2+} but not in the absence of Ca^{2+}. The actin-activated myosin ATPase is altered by C-protein [8], although C-protein itself has no ATPase activity [1]. Its effect on ATPase depends on ionic strength; it inhibits actomyosin ATPase at low ionic strength, but it slightly activates ATPase at physiological ionic strength [8]. Recent electron-microscopic studies on C-protein from rabbit [9], chicken [10] skeletal and cardiac [11] muscles indicate that the C-protein molecules are V, L, C or U-shaped and their contour lengths range from 15 to 40 nm. Thus, the size of C-protein is enough to span the gap between the thick and thin filaments. From these properties of C-protein, a regulatory function of C-protein in muscular contraction has been suggested [7,8,12].

Here, I demonstrate that the binding of C-protein to regulated actin is Ca^{2+}-sensitive and that C-protein causes side-by-side association of regulated actin filaments in the presence of Ca^{2+}. These results are consistent with the observations of Moos [7] on C-protein binding to myofibrils, and suggest a regulatory role of C-protein in actin-myosin interactions.

2. MATERIALS AND METHODS

Rabbit skeletal muscle was used for protein preparations. Actin was extracted with water from an acetone powder of the residue after myosin extraction, and actin was further purified by the method of Spudich and Watt [13]. The tropomyosin-troponin (TT) complex was prepared from ether-dried actomyosin powder by the method of Hartshorne and Mueller [14]. Hydroxyapatite column purified C-protein was prepared as described [15].

Protein concentrations were determined spectrophotometrically using extinction coefficients at 280 nm of 1.09 $mg^{-1} \cdot ml^{-1} \cdot cm^{-1}$ for C-protein

* Formerly The College of Dairying

Abbreviations: Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Mops, 3-(*N*-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

[1], 1.15 for actin [16] and 0.38 for TT complex [14]. The M_r values used for actin and C-protein are 42000 and 135000, respectively.

Binding of C-protein to regulated actin was calculated from the protein concentration of the supernatant after ultracentrifugation. Actin and TT complex were mixed at 1:1 weight ratio in 50 mM KCl, 10 mM Mops, 1 mM $MgCl_2$, 0.2 mM ATP (pH 7.0), and 1 mM $CaCl_2$ or 1 mM EGTA. C-protein was added at varying ratios and the mixture was incubated for about 1 h at 4°C, and 175 μl samples were then centrifuged in a Beckman airfuge at 30 psig (135000 × g) for 10 min at room temperature. After centrifugation, 100 μl of the supernatant was transferred to a microcuvette and its absorbance measured at 280 and 320 nm. The A_{320} was subtracted from A_{280} as a correction for turbidity.

The precipitates after centrifugation were dissolved in SDS-PAGE sample buffer (10 mM Tris-Bicine, 1% SDS, 2% 2-mercaptoethanol, pH 8.3) and electrophoresed in 7.5% acrylamide gel on a minislab gel apparatus as in [2].

For electron microscopy, the protein samples were applied to carbon-coated grids, rinsed with several drops of 50 mM KCl, and negatively stained by 2% uranyl acetate. The specimens were observed in a Hitachi H-800 electron microscope at an accelerating voltage of 75 kV.

3. RESULTS

It was important to determine the regulatory activity of the TT complex used here. The regulatory activity of TT complex was tested by measuring regulated actin-activated myosin ATPase in a reaction mixture containing 0.13 mg/ml each of myosin, actin and TT, 50 mM KCl, 2 mM $MgCl_2$, 1 mM ATP and 1 mM $CaCl_2$ or EGTA at pH 7.0 and 25°C on a pH stat. Ca^{2+} sensitivity, (Ca^{2+} – ATPase) – (EGTA – ATPase)/(Ca^{2+} – ATPase) × 100(%), was over 80%, indicating that TT complex had enough regulatory activity.

The effect of C-protein on the turbidity of regulated actin is shown in fig.1. The addition of C-protein to regulated actin resulted in an increase in turbidity of the filament suspension. The turbidity of the C-protein itself, which was less than 0.015 irrespective of whether Ca^{2+} was present or absent, was subtracted at each data point. Even in the absence of Ca^{2+}, the turbidity increased with C-protein concentration; however, the turbidities in the presence of Ca^{2+} were always higher than those in its absence. The changes in turbidity caused by adding C-protein suggested that Ca^{2+}-dependent interactions between actin filaments were occurring.

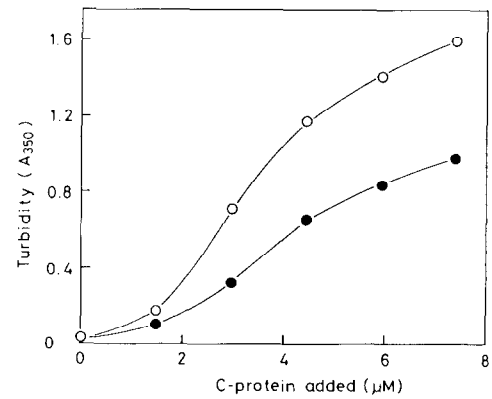

Fig.1. Effect of C-protein on turbidity of regulated actin. All samples contained 50 mM KCl, 10 mM Mops, 1 mM $MgCl_2$, 0.2 mM ATP (pH 7.0), and 1 mM $CaCl_2$ (○) or EGTA (●). The protein concentrations of actin and TT-complex were 0.2 mg/ml each, and the C-protein concentration was varied. Turbidity was measured as the absorbance at 350 nm with 10 mm path-length cuvettes.

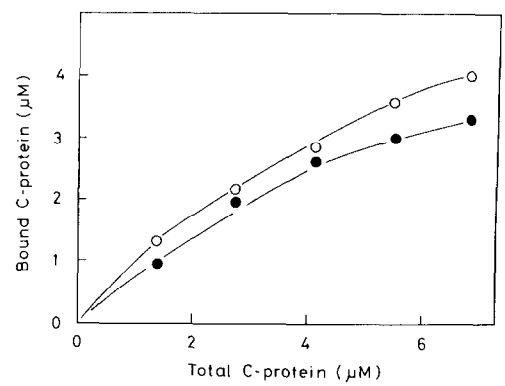

Fig.2. Binding of C-protein to regulated actin. Conditions were as in fig.1. C-protein was added to regulated actin in the presence or absence of Ca^{2+}, incubated for about 1 h at 4°C, and then ultracentrifuged. Binding was calculated from the protein concentration of the supernatant. (○) Ca^{2+}, (●) EGTA.

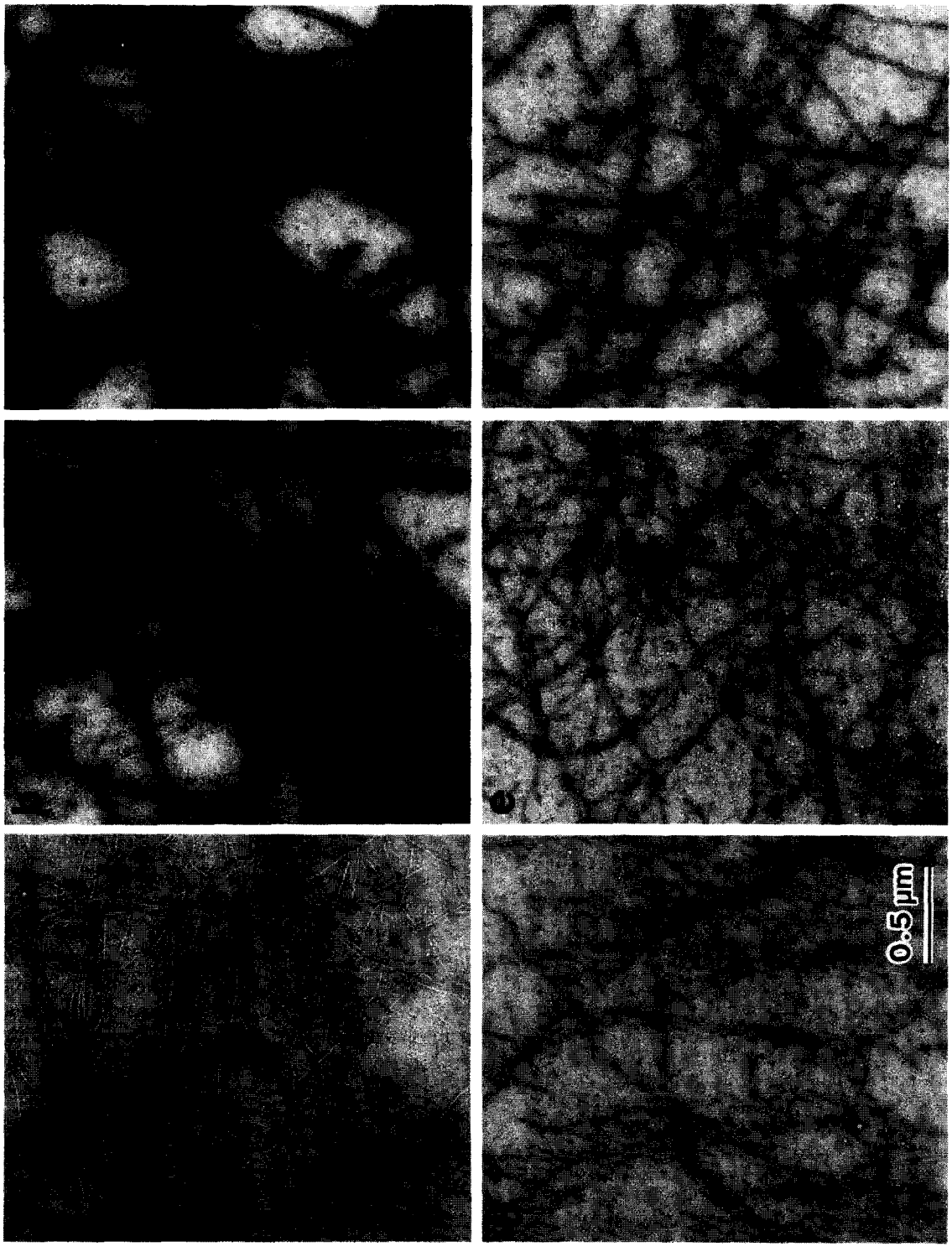

Fig.4. Electron micrographs of negatively stained regulated actin–C-protein complexes. (a–c) Ca^{2+} present, (d–f) Ca^{2+} absent. (a,d) Regulated actin control; (b,e) and (c,f), actin + TT complex + C-protein with a weight ratio of 1:1:1 and 1:1:3, respectively. Images were recorded at a magnification of 20000.

C-protein bound to regulated actin in the absence of Ca^{2+} (fig.2), but the amount of bound C-protein in the presence of Ca^{2+}, like the turbidity, was higher than in the absence of Ca^{2+}. It is known that C-protein tends to aggregate at low ionic strength [1]; however, over 90% of the C-protein remained soluble under this assay condition (50 mM KCl). The remaining actin-TT complex in the supernatant after ultracentrifugation was less than 15% of the total protein. Solubilities of both C-protein and regulated actin controls were not affected by the presence or absence of Ca^{2+}.

SDS-PAGE patterns of the precipitate after ultracentrifugation confirmed that C-protein bound to regulated actin filaments (fig.3). Binding took place even in the absence of Ca^{2+}, but densitometric scanning of these gels confirmed that the amount of C-protein bound to regulated actin was higher in the samples with Ca^{2+} than in the absence of Ca^{2+}. However, the differences were small, as was the case with the spectrophotometric measurements.

Fig.4 shows electron micrographs of negatively stained regulated actin filaments. When C-protein was added to regulated actin in the presence of Ca^{2+}, the actin filaments associated side-by-side into bundles. At higher C-protein concentration, most of the actin filaments associated and large aggregates were sometimes observed. On the other hand, no such thick actin filament bundles were formed in the absence of Ca^{2+}, although some thin bundles were observed.

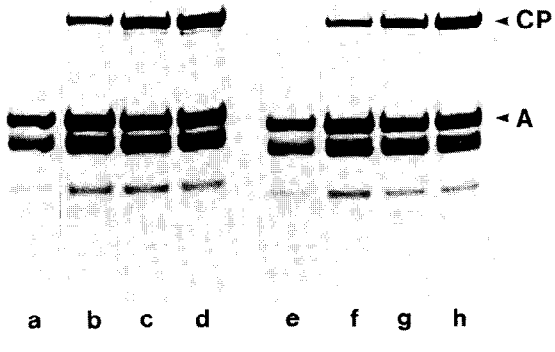

Fig.3. SDS-polyacrylamide gel electrophoretogram of the precipitates of regulated actin plus C-protein in the presence (a–d) or absence (e–h) of Ca^{2+}. (a,e) Regulated actin control; (b,f), (c,g) and (d,h) are actin + TT complex + C-protein with a weight ratio of 1:1:1, 1:1:3, and 1:1:5, respectively. CP, C-protein; A, actin. The bands below actin were the components of tropomyosin-troponin complex. The samples were run on a 7.5% acrylamide gel.

4. DISCUSSION

The data described here show that binding of C-protein to regulated actin is Ca^{2+}-sensitive, but even in the absence of Ca^{2+} a fairly large amount of C-protein can bind to regulated actin. In other words, the binding of C-protein to regulated actin in the presence or absence of Ca^{2+} is not regulated in an all-or-none manner. The binding of C-protein to regulated F-actin filaments causes side-by-side association of the filaments. The appearance of C-protein-bound regulated actin in the presence of Ca^{2+} is quite similar to that of unregulated F-actin filaments [6].

An interesting point is that although the differences between the amounts of bound C-protein in the presence and absence of Ca^{2+} were not large, there were large differences in the morphological appearance. It is known that the structure of regulated actin filaments is changed by Ca^{2+} [17]. In the presence of Ca^{2+}, the position of tropomyosin in the groove of the double-stranded actin filament is shifted and the actin filaments are 'switched on', so that the actin can activate the ATPase of myosin. A possible explanation for side-by-side association of actin filaments is that there are at least two actin-binding sites on a C-protein molecule. In the presence of Ca^{2+}, the configuration of the actin filaments makes both of these sites on the C-protein molecule available for binding to actin. In this case, C-protein can cross-link adjacent actin filaments (fig.4). But in the absence of Ca^{2+}, one of these sites is no longer able to bind to actin, so that no actin filament bundles are formed. Since tropomyosin alone does not substantially affect the binding of C-protein to unregulated F-actin [6], the difference in regulated actin filaments between the presence and absence of Ca^{2+} derives from the regulatory system of tropomyosin and troponin. An alternative possible explanation is that Ca^{2+} acts directly on a C-protein molecule to cause a conformational change and allow cross-linking of actin filaments.

However, since C-protein itself has no Ca^{2+}-binding ability [1], it is unlikely that a conformational change in the C-protein molecule takes place.

The present results indicate that the binding of C-protein to regulated actin filaments depends on the state of the actin filament and support a regulatory role of C-protein in actin-myosin interaction.

ACKNOWLEDGEMENTS

I wish to thank Dr C. Moos for his helpful discussion and Drs T. Yasui and K. Samejima for their encouragement.

REFERENCES

[1] Offer, G., Moos, C. and Starr, R. (1973) J. Mol. Biol. 74, 653–676.
[2] Yamamoto, K. and Moos, C. (1983) J. Biol. Chem. 258, 8395–8401.
[3] Moos, C., Offer, G., Starr, R. and Bennett, P. (1975) J. Mol. Biol. 97, 1–9.
[4] Starr, R. and Offer, G. (1978) Biochem. J. 171, 813–816.
[5] Koretz, J.F. (1979) Biophys. J. 27, 433–446.
[6] Moos, C., Mason, C.M., Besterman, J.M., Feng, I.M. and Dubin, J.H. (1978) J. Mol. Biol. 124, 571–586.
[7] Moos, C. (1981) J. Cell Biol. 90, 25–31.
[8] Moos, C. and Feng, I.M. (1980) Biochim. Biophys. Acta 632, 141–149.
[9] Bennett, P., Starr, R., Elliott, A. and Offer, G. (1985) J. Mol. Biol. 184, 297–309.
[10] Swan, R.C. and Fischman, D.A. (1986) J. Muscle Res. Cell Motil. 7, 160–166.
[11] Hartzell, H.C. and Sale, W.S. (1985) J. Cell Biol. 100, 208–215.
[12] Hartzell, H.C. (1985) J. Mol. Biol. 186, 185–195.
[13] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[14] Hartshorne, D.J. and Mueller, H. (1969) Biochim. Biophys. Acta 175, 301–319.
[15] Yamamoto, K. (1984) J. Biol. Chem. 259, 7163–7168.
[16] Estes, J.E. and Moos, C. (1969) Arch. Biochem. Biophys. 132, 388–396.
[17] Huxley, H.E. (1973) Cold Spring Harbor Symp. Quant. Biol. 37, 361–376.