Fluorescence Polarization Study on Ca^{2+}-Sensitivity of Conformational Changes in F-Actin Induced by the Formation of F-Actin-Subfragment-1 Complex

Yu. S. Borovikov[1] and D. I. Levitsky[2]

1 *Institute of Cytology, Academy of Sciences of the USSR, Tikhoretsky av. 4, Leningrad 194064, USSR*

2 *Moscow State University, Moscow 117234, USSR*

Abstract. Ca^{2+}-dependent conformational changes in F-actin during myosin subfragment-1 binding with thin filament (in the absence of troponin and tropomyosin) were found in myosin-free ghost fibres by polarized UV microscopy. The pattern of the conformational changes in F-actin changed cooperatively within the range of free Ca^{2+} concentrations from 10^{-7} mol/l to 10^{-6} mol/l. It should be suggested that in skeletal muscle of vertebrates there exists a myosin-linked modulation of contraction by Ca^{2+}.

Key words: F-actin conformational changes — Myosin-linked modulation — F-actin-subfragment-1 complex — Ghost fibre — Polarized UV microphotometry

Introduction

It has been established that the regulation of myosin-actin interaction goes via Ca^{2+} binding either with the troponin-tropomyosin system, localized on actin filaments (troponin-linked regulation), or with regulatory light chains of myosin (myosin-linked regulation) (Szent-Györgyi 1975). Myosin-linked regulation is very typical of molluscan muscles but it has not been found in skeletal muscles of vertebrates (Szent-Györgyi 1975; Kendrick-Jones et al. 1976). Nevertheless, Ca^{2+} binding with 5,5′-dithiobis (2-nitrobenzoic acid) (Nbs_2) light chains of skeletal muscles significantly affects the pattern of myosin-actin interaction (Margossian et al. 1975; Haselgrove 1975; Lehman 1978; Borovikov et al. 1982).

In our previous study, it has been shown that Ca^{2+} binding with myosin light chains changes the pattern of conformational changes in F-actin during the formation of F-actin-subfragment-1 complex only at the moment of the myosin subfragment-1 (S1) binding to actin (Borovikov et al. 1982). In the present study, we show that such changes induced by S1 binding occur cooperatively within the range of Ca^{2+} concentrations from 10^{-7} mol/l to 10^{-6} mol/l. The information was

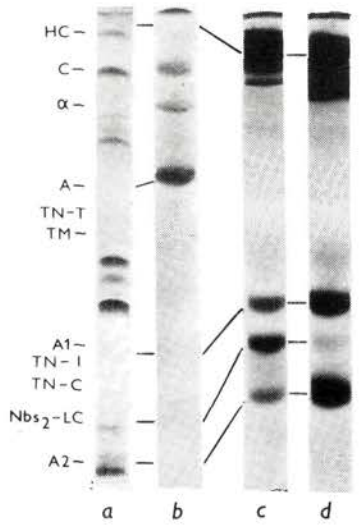

Fig. 1. Sodium dodecyl sulfate gel electrophoresis. (a) Glycerinated muscle fibre (50 μg); (b) Ghost fibre (50 μg); all in 12% polyacrylamide with 2% sodium dodecyl sulfate; (c) Mg-subfragment-1 (100 μg); (d) EDTA-subfragment-1 (200 μg); all in 10% polyacrylamide with 1% sodium dodecyl sulfate. HC, myosin heavy chains; C, C-protein; α, α-actinin; A, actin; TM, tropomyosin; TN-T, TN-I, TN-C, troponin; A1, A2, alkaline light chains; Nbs_2-LC, Nbs_2 light chains.

obtained from studies of intrinsic tryptophan polarized fluorescence of the myosin-free ghost fibres.

Materials and Methods

The study was carried out on single glycerinated muscle fibres of rabbit (Rome 1973), devoid of myosin, troponin and tropomyosin, and containing more than 80% of actin (ghost muscle fibres) (Fig. 1) (Borovikov et al. 1982). S1 was obtained by papain digestion of myosin from rabbit skeletal muscles in the presence of 2 mmol/l $MgCl_2$(Mg-S1) or 2 mmol/l EDTA (EDTA-S1) (Margossian et al. 1975). Mg-S1 contained myosin Nbs_2 light chains, whereas EDTA-S1 was practically free of them (Fig. 1) (Margossian et al. 1975; Borovikov et al. 1982). The protein composition of ghost fibres and the content of light chains in Mg-S1 and EDTA-S1 were determined by sodium dodecyl sulfate gel electrophoresis (Sheludko 1975; Weber and Osborn 1969).

The binding of S1 to F-actin, as described earlier (Borovikov et al. 1982), was performed by one hour incubation of ghost fibre in a solution (in mmol/l^{-1}): 60 KCl; 1 $MgCl_2$; 30 Tris-HCl buffer (pH 7.5); and 2.5 mg/ml S1 at 18 °C. The molar ratio of Mg-S1 or EDTA-S1 to actin in decorated fibres were similar (1:2) (Borovikov et al. 1982). The needed Ca^{2+} concentrations in the solutions were achieved using the Ca-EGTA buffer system (Ogawa 1968).

The degree of polarized tryptophan fluorescence (P) was recorded by a microspectrophotometer with the orientation of fibre parallel ($P_\parallel$) and perpendicular ($P_\perp$) to the polarization plane of the

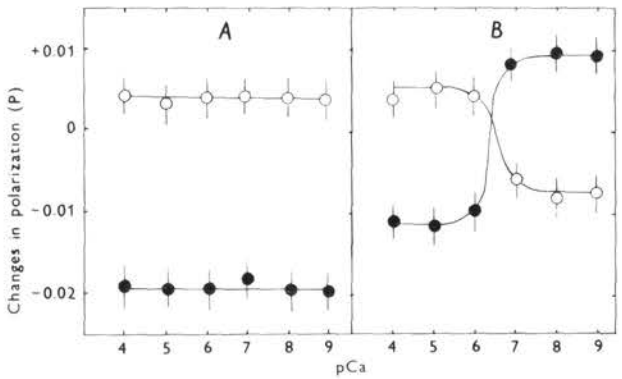

Fig. 2. Changes in the degree of polarization (P) of tryptophan fluorescence of ghost fibres induced by EDTA-S1 (A) or Mg-S1 (B) binding at different Ca^{2+} concentrations (pCa). Measurements were made in a solution containing (in mmol . l^{-1}): 100 KCl; 1 $MgCl_2$; 30 Tris-HCl buffer; pH 7.5 (the required concentration of Ca^{2+} was adjusted by Ca/EGTA buffer). Each point of the curve represents an average of 30—50 measurements, made on 8—10 fibres. Vertical bars indicate standard deviations. ○ — $P_\parallel$; ● — $P_\perp$.

incident light (Borovikov et al. 1982). Fibre fluorescence was excited at 303 ± 4 nm, and registered at 320—380 nm. The analysis of the data obtained was done as described elsewhere (Yanagida and Oosawa 1978).

Results

For muscle fibres devoid of myosin, troponin and tropomyosin (Fig. 1) $P_\perp$ ($P_\perp = 0.207 \pm 0.002$) was higher than $P_\parallel$ ($P_\parallel = 0.191 \pm 0.002$) and the index of anisotropy ($P_\perp/P_\parallel$) exeeded unity (1.08 ± 0.01. $n = 15$). If the thin filaments were damaged, the $P_\perp/P_\parallel$ ratio becomes unity (Kirillina et al. 1979). Consequently, the parameters of tryptophan fluorescence ($P_\perp$, $P_\parallel$ and $P_\perp/P_\parallel$) of ghost fibre are closely connected with F-actin in thin filaments. These parameters are changed significantly during S1 attachment to F-actin. The attachment of EDTA-S1 deficient in Nbs_2 light chains to F-actin, irrespective of Ca^{2+} concentration, resulted in a decrease in tryptophan fluorescence anisotropy, since $P_\perp$ and $P_\perp/P_\parallel$ decreased and $P_\parallel$ increased (Figs. 2A; 3). During Mg-S1 binding (the preparation contained native Nbs_2 light chains) in the presence of Ca^{2+} (from 10^{-4} mol/l to 10^{-6} mol/l) the tryptophan fluorescence anisotropy decreased: $P_\perp$ and $P_\perp/P_\parallel$ diminished and $P_\parallel$ increased, whereas in the absence of Ca^{2+} (from 10^{-7} mol/l to 10^{-9} mol/l) the fluorescence anisotropy increased: $P_\perp$ and $P_\perp/P_\parallel$ increased and $P_\parallel$ decreased (Figs. 2B; 3). It is of interest that tryptophan fluorescence anisotropy showed cooperative changes at Ca^{2+} concentrations from 10^{-7} mol/l to 10^{-6} mol/l (Figs. 2B; 3). These changes

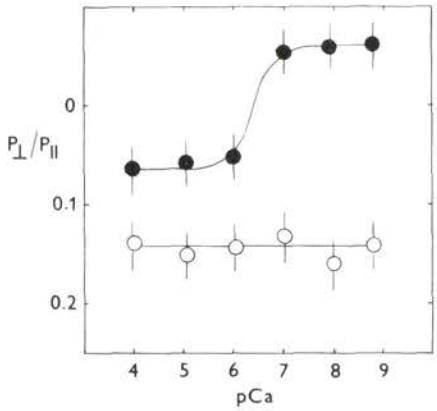

Fig. 3. Changes in tryptophan fluorescence anisotropy index ($P_\perp/P_\parallel$) of ghost fibres induced by EDTA-S1 (○) or Mg-S1 binding (●) at different concentrations of free Ca^{2+} (pCa). For symbols and experiment conditions see legend to Fig. 2.

were not due to Ca^{2+} binding either to the troponin-tropomyosin complex or to F-actin itself, since the ghost fibres and S1 preparations were free of troponin and tropomyosin (Fig. 1) and since no changes in polarized fluorescence parameters of ghost fibres were observed during changes in free Ca^{2+} concentration in the solution. Evidently, Ca^{2+}-dependent changes in $P_\perp$, $P_\parallel$ and $P_\perp/P_\parallel$ (Figs. 2*B*; 3) were due to F-actin binding to the myosin head molecules, whose Nbs_2 light chains are able to bind Ca^{2+}.

It should be pointed out that fibres decorated with Mg-S1 at certain Ca^{2+} concentration lose their sensitivity to further variations in Ca^{2+} concentration. When such fibres are placed into solutions with varying Ca^{2+} concentrations they show no changes in their polarization characteristics; this suggests that the changes initiated by Mg-S1 binding (in the absence of ATP) are irreversible. The reversibility of the effect can be achieved by the addition of ATP (5 mmol/l) to decorated fibres. In this case, the parameters of polarized fluorescence return to their initial values (as in the absence of S1), suggesting dissociation of Mg-S1 from the surface of actin filaments of ghost muscle fibres.

Discussion

It has been shown earlier that changes in polarized tryptophan fluorescence of ghost muscle fibre, resulting from F-actin decoration with S1, reflect conformational changes in F-actin (Borovikov et al. 1982). Ca^{2+} binding to Nbs_2 light chains of Mg-Ca significantly affects the pattern of such changes. Apparently, F-actin is

conformed during the formation of the F-actin-S1 complex depending on the conformation of the actin-binding site in S1 at the moment of binding. The latter, in its turn, depends on whether Nbs_2 light chains contain bound Ca^{2+} or not.

As follows from the present experiments, changes in F-actin conformation pattern during interaction with Mg-S1, induced by Ca^{2+} binding to Nbs_2 light chains, occur within the range of Ca^{2+} concentrations between 10^{-7} mol/l and 10^{-6} mol/l in the presence of 1 mmol/l of Mg^{2+}. Meanwhile, it has been known that Ca^{2+} binding to Nbs_2 light chains of myosin is performed by Ca^{2+}—Mg^{2+} sites (or non-specific metal ion binding sites) in which Ca^{2+} has to compete with Mg^{2+} (Bagshow and Kendrick-Jones 1979). As a result, in the presence of Mg^{2+}, Ca^{2+} binding to Nbs_2 light chains of myosin occurs at rather high Ca^{2+} concentrations (in the order of 10^{-5} mol/l) (Morimoto and Harrington 1974; Bagshow and Kendrick-Jones 1979; Holroyed et al. 1979). Thus, only few molecules of Mg-S1 are capable of binding Ca^{2+} within the range of Ca^{2+} concentrations of 10^{-7} mol/l — 10^{-6} mol/l. Nevertheless, as follows from the results, even such a small number of molecules is quite enough to change radically the pattern of conformational changes in F-actin. Apparently, the changes in F-actin conformation are so radical that its further interaction with other Mg-S1 molecules (both bound and unbound Ca^{2+}) has no effect on F-actin conformation. This is suggested by the data on high positive cooperativity of conformational changes in F-actin at S1 binding (Borovikov et al. 1982; Miki et al. 1982), as well as by the fact that an increase in the number of Mg-S1 molecules bound to Ca^{2+} with the rise in Ca^{2+} concentration in solution from 10^{-6} mol/l to 10^{-4} mol/l initiates almost no further changes in polarized characteristics of F-actin (Figs. 2*B*; 3).

It should be emphasized that our interpretation has been based on the fact that Ca^{2+} binding to Nbs_2 light chains in the presence of Mg^{2+} occurs within the range of Ca^{2+} concentrations of about 10^{-5} mol/l. However, there is evidence that half-maximal conformational changes in myosin spin-labelled Nbs_2 light chains occur in the presence of 1 mmol/l Mg^{2+} at $pCa = 6.8$ (Okamoto and Yagi 1977). If the sensitivity of Nbs_2 light chains to Ca^{2+} in the presence of Mg^{2+} is actually so high, it only facilitates the interpretation of our data; in this case, in the range of Ca^{2+} concentrations from 10^{-7} mol/l to 10^{-6} mol/l more than a half of all the Mg-S1 molecules in the solution would contain bound Ca^{2+}. However, in a majority of studies on Ca^{2+} binding to myosin in the presence of Mg^{2+}, it has been shown that binding occurs within the range of free Ca^{2+} concentrations of the order of 10^{-5} mol/l (Morimoto and Harrington 1974; Bagshow and Kendrick-Jones 1979; Holroyed et al. 1979). If this is actually the case, it can be assumed from the above that the observed Ca^{2+}-dependent conformational changes in F-actin during Mg-S1 binding reflect changes in thin actin filaments at contraction and are involved in the regulation of skeletal muscle contraction by Ca^{2+}.

It is known that Mg^{2+} is substituted for Ca^{2+} in $Ca^{2+} - Mg^{2+}$-sites of Nbs_2 light

chains of myosin at a low rate insufficient to regulate myosin-actin interaction during triggering of contraction (Bagshow and Reed 1977). Calculations show that during contraction triggering when free Ca^{2+} concentration increases rapidly (within several tenth of ms) to 10^{-5} mol/l, the number of myosin heads already bound to Ca^{2+} in the presence of 2.5 mmol/l Mg^{2+} constitutes only 1.5—2% of their total number (Potter et al. 1981). However, as shown above, even such a small number of Ca^{2+}-bound myosin heads might be sufficient to change the conformation of actin filaments. Apparently, such a change in the conformation of actin filaments reflects their transfer to some "switched-on" state (a possibility of the existence of actin filaments in two possible conformational states — "switched-on" and "switched-off" has been assumed earlier (Lednev and Frank 1977; Weber and Murray 1973; Murray et al. 1982; Borovikov and Gusev 1983). Subsequent Ca^{2+} binding to myosin heads (e.g. during tetanic contraction) can be of importance in maintaining the "on"-state of actin, which apparently is necessary to maintain the level of tension developed by a single muscle fibre. During complete relaxation of muscle fibre, when free Ca^{2+} concentration decreases to 10^{-8} mol/l, Nbs_2 light chains of all myosin heads lose Ca^{2+}; in this case, myosin heads in their interaction with actin, apparently switch actin filaments to the "off"-state.

It is possible that a myosin-linked regulation of contraction by Ca^{2+} exists in skeletal muscles of vertebrates alongside with troponin-linked regulation. However, in this case, Ca^{2+} binding to Nbs_2 light chains of myosin regulates the pattern of such interaction rather than the possibility of myosin-actin interaction. Such an effect may better be called "modulation" rather than "regulation" of myosin-actin interaction. Depending on Ca^{2+} concentration, myosin heads and actin interact differently and, as a result, they make actin filaments get into one of two possible states: "on" or "off" state. Regulation (or modulation) of this kind may be of importance for both the triggering of contraction and the maintaining of tension of the muscle fibre (e.g. during tetanic contraction) and its relaxation.

Acknowledgements. Authors wish to appreciate the helpful comments of Drs. N. B. Gusev, S. A. Krolenko, V. V. Lednev and B. F. Poglazov.

References

Bagshow C. R., Reed G. H. (1977): The significance of the slow dissociation of divalent metal ions from myosin "regulatory" light chains. FEBS Lett. **81,** 386—390

Bagshow C. R., Kendrick-Jones J. (1979): Characterization of homologous divalent metal ions binding sites of vertebrate and molluscan myosin using electron paramagnetic resonance spectroscopy. J. Mol. Biol. **130,** 317—336

Borovikov Yu. S., Levitsky D. I., Kirillina V. P., Poglazov B. F. (1982): Effect of Ca^{2+} binding to 5,5'-dithiobis (2-nitrobenzoic acid) light chains on conformational changes of F-actin caused by myosin subfragment-1. Eur. J. Biochem. **125,** 343—347

Borovikov Yu. S., Gusev N. B. (1983): Effect of troponin-tropomyosin complex and Ca^{2+} on conformational changes in F-actin induced by myosin subfragment-1. Eur. J. Biochem. **136,** 363—369

Haselgrove J. C. (1975): X-ray evidence for conformational changes in the myosin filaments of vertebrate striated muscle. J. Mol. Biol. **92,** 113—143

Holroyed M. J., Potter J. D., Solaro K. J. (1979): The calcium binding properties of phosphorylated and unphosphorylated cardiac and skeletal myosins. J. Biol. Chem. **254,** 6478—6482

Kendrick-Jones J., Szentkiralyi E. M., Szent-Györgyi A. G. (1976): Regulatory light chains in myosins. J. Mol. Biol. **104,** 747—775

Kirillina V. P., Borovikov Yu. S., Khaitlina S. Yu., Bogdanova M. S. (1979): On the mode of tryptophan residue orientation in myosin and actin of the striated muscle fibre. Tsitologiya **21,** 171—175 (in Russian)

Lednev V. V., Frank G. M. (1977): Structural non-equivalence of F-actin subunits and its possible role in regulating ATP-ase activity and developing stretching in skeletal muscles. Biofizika **22,** 376—388 (in Russian)

Lehman W. (1978): Thick-filament-linked calcium regulation in vertebrate striated muscle. Nature **274,** 80—81

Margossian S. S., Lowey S., Barshop B. (1975): Effect of DTNB light chains on the interaction of vertebrate skeletal myosin with actin. Nature **258,** 163—166

Miki M., Wahl P., Auchet J. C. (1982): Fluorescence anisotropy of labeled F-actin: Influence of divalent cations on the interaction between F-actin and myosin heads. Biochem. **21,** 3661—3665

Morimoto K., Harrington W. F. (1974): Evidence for structural changes in vertebrate thick filaments induced by calcium. J. Mol. Biol. **88,** 693—709

Murray J. M., Knox M. K., Trueblood C. E., Weber A. (1982): Potentiated state of the tropomyosin actin filament and nucleotide-containing myosin subfragment-1. Biochemistry **5,** 906—915

Ogawa Y. (1968): The apparent binding constant of glycoletherdiamine-tetraacetic acid for calcium at neutral pH. J. Biochem. **64,** 255—257

Okamoto Y., Yagi K. (1977): Inhibition by Mg^{2+} of the interaction of Ca^{2+} with spin-labeled G_2 bound to myosin. J. Biochem. **82,** 835—837

Potter J., Robertson D. P., Johnson J. D. (1981): Magnesium and the regulation of muscle contraction. Fed. Proc. **40,** 2653—2658

Rome E. (1973): Structural studies by X-ray diffraction of striated muscle permeated with certain ions and proteins. Cold Spring Harbor Symp. Quant. Biol. **37,** 331—339

Sheludko N. S. (1975): The protein composition of miofibrills of rabbit by method of disk electrophoresis in the presence of sodium dodecyl sulfate. Tsitologiya **17,** 1148—1154 (in Russian)

Szent-Györgyi A. G. (1975): Calcium regulation of muscle contraction. Biophys. J. **15,** 707—723

Weber K., Osborn M. (1969): The reliability of molecular weight determinations by dodecyl sulfate polyacrilamide gel electrophoresis. J. Biol. Chem. **244,** 4406—4412

Weber A., Murray J. M. (1973): Molecular control mechanisms in muscle contraction. Physiol. Rev. **53,** 612—673

Yanagida T., Oosawa F. (1978): Polarized fluorescence from ε-ADP incorporated into F-actin in a myosin-free single fibre: Conformation of F-actin and changes induced in it by heavy meromyosin. J. Mol. Biol. **126,** 507—524

Received March 6, 1984/Accepted August 10, 1984