Conformational Changes of Contractile Proteins Accompanying Modulation of Skeletal Muscle Contraction. Polarized Microfluorometry Investigations.

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Abstract. Results of studies on the modulation of skeletal muscle contraction by phosphorylation of myosin regulatory light chains and by exchange of magnesium for calcium in myosin heads were reviewed. The polarized fluorescence method was used in these studies, and conformational changes of contractile proteins accompanying modulation of skeletal muscle contraction were investigated. It was found that both the exchange of bound magnesium for calcium on myosin heads and the phosphorylation of myosin regulatory light chains control the ability of myosin heads to induce, upon binding to actin, conformational changes of thin filament leading to decrease or increase of its flexibility. The changes in actin filament flexibility may be caused by alteration of both the inter- and the intramonomer structural organization.

Key words: Modulation of contraction — Skeletal muscle — Conformational changes of contractile proteins

Introduction

As generally assumed, the interaction of myosin filamets with actin filaments coupled with ATP hydrolysis is the basis of muscle contraction (Huxley and Niedergereke 1954; Huxley and Hanson 1954; Huxley 1957; Huxley 1969; Huxley and Simmons 1971). During these processes globular regions of myosin molecules (myosin heads) undergo conformational changes concomitant with the movement of thin filaments of actin past thick filaments of myosin (Huxley and Simmons 1971 and the references therein).

However, it remains unclear whether the movement of myosin cross-bridges or that of actin monomers, or both, are involved in the process of force generation. On the basis of ultrastructural studies of muscle Huxley (1969)

proposed that the myosin head bound to actin changes in orientation with respect to the axis of the thin filament, and pulls the myosin filament forward. This rotating myosin head model was tested in time-resolved X-ray difraction studies (Huxley and Faruqi 1983; Huxley and Kress 1985), EPR experiments (Thomas and Cooke 1980; Cooke et al 1982, 1984; Goody and Holmes 1983), and using polarized fluorescence methods (Aronson and Morales 1969; Borovikov et al. 1971; Borejdo et al. 1979; Yanagida 1981; Ajtai and Burghardt 1986).

Harrington (1971, 1979) and Ueno and Harrington (1986) proposed an alternative hypothesis of force generation, namely, a helix-coil transition model. They suggested that the conformational change in the "hinge" portion of the myosin molecule is the force-generating mechanism. However, Hynes et al. (1987) reported that short heavy meromyosin lacking the "hinge" region and incapable of forming thick filaments nevertheless moves along Nitella actin cables. Toyoshima et al. (1987) showed that myosin subfragment 1 is sufficient to cause sliding movement on actin filaments *in vitro*.

Moreover, tension development can be observed during interaction of an actin filament with myosin subfragment 1, and the force exerted is comparable to that developed in the presence of intact myosin molecules (Kishino and Yanagida 1988). For these reasons the Harrington theory should be abandoned.

Thus, it seems to be more likely that the movement of myosin filaments along actin filaments may be caused by changes of reorientation of myosin heads (or their segments) to the axis of thin filaments (Huxley and Kress 1985; Hynes et al. 1987; Toyoshima et al. 1987), and/or by structural alteration of actin filaments (Yanagida et al. 1974, 1978; Borovikov et al. 1974, 1990; Borovikov and Chernogriadskaya 1979; Yanagida 1984) as postulated by several authors.

In vertebrate skeletal muscle, muscle contraction at the molecular level seems to be regulated by calcium-dependent changes in the thin filament, and in smooth and scallop muscles, it seems to be due to conformational changes in myosin light chains induced by calcium-dependent phosphorylation of these light chains, and/or by calcium binding (Ebashi and Endo 1968; Adelstein and Conti 1975; Aksoy et al. 1983; Sobieszek and Small 1976, 1977; Kendrick-Jones et al. 1976; Chantler and Szent-Györgyi 1980).

However, some recent studies have suggested the presence in vertebrate skeletal muscle of an additional calcium-dependent system linked to the thick filament, influencing the interaction of myosin cross-bridges with actin thin filaments (Haselgrove 1975). The nature of this thick filament-linked skeletal muscle contraction modulating system is at present not clear. There have been some observations suggesting the influence of calcium binding to the regulatory light chains on the contractile properties of skeletal muscle (Haselgrove 1975;

Lehman 1978). The regulatory role of Ca^{2+} binding to myosin was postulated on the basis of Ca^{2+}-dependent changes of the myosin-F-actin interaction (Werber and Oplatka 1974; Borovikov et al. 1982; Borovikov and Karandashov 1983).

According to the studies of Bagshaw and Reed (1977) the slow dissociation of divalent metal ions from myosin regulatory light chains indicates that the divalent metal binding site of skeletal muscle regulatory light chains cannot be involved in the regulation on the known time scale of activation.

The conformational changes of regulatory light chains due to Ca^{2+} binding (Alexis and Gratzer 1978) may, however, induce some structural changes in the myosin heads.

The existence of calcium and calmodulin dependent myosin light chains kinase in skeletal muscle cells (Pires and Perry 1977) stimulated studies concerning myosin light chain phosphorylation and the actin-myosin interaction. However, the modulatory role of myosin phosphorylation remains unclear. Manning and Stull (1982), Klug et al. (1982), and Moore and Stull (1984) have shown a correlation between the potentiation of isometric twich tension and myosin phosphorylation. However, the correlation between the shortening velocity of intact skeletal muscle and myosin phosphorylation was not confirmed by Butler et al. (1983).

A modulating effect of myosin phosphorylation on myosin-actin interaction was shown by the observation of the "arrowhead" structure of actin filaments decorated by phosphorylated and dephosphorylated heavy meromyosin (Stepkowski et al. 1985a). Claims concerning the influence of myosin phosphorylation on the interaction of isolated contractile proteins have not been fully convincing (Perry 1979; Pemrick 1980; Kąkol et al. 1982; Michnicka et al. 1982; Cardinaud and Kąkol 1985).

The effect of myosin phosphorylation seems to depend on several factors (Stepkowski et al., 1985b).

Pulliam et al. (1983) have found a correlation between calcium sensitivity of Mg^{2+}-ATPase of myosin complexes with pure actin and the phosphorylation of myosin regulatory light chains.

Similarly, changes of some contractile properties of skeletal muscle seem to be dependent on Ca^{2+} binding and myosin phosphorylation (Persechini et al. 1985; Sweeney and Stull 1986; Wrotek et al. 1989).

We shall show in this paper that the cooperation of Ca^{2+} binding and myosin light chain phosphorylation in modulating myosin cross-bridge interaction with actin filaments can be confirmed by studying conformational changes of contractile proteins using the polarized fluorescence method.

Conformational changes of contractile proteins and modulation of myosin-actin interaction by myosin phosphorylation and by the exchange of Ca^{2+} for bound Mg^{2+} in myosin heads

Measurements of polarized fluorescence emitted by intrinsic and extrinsic fluorophores located in myosin heads or in actin filaments enabled the observation of conformational changes of contractile proteins in various physiological states of the muscle (Aronson and Morales 1969; Dos Remedios et al. 1972; Borejdo et al. 1979; Borovikov and Chernogriadskaya 1979; Yanagida 1984, 1985; Borovikov et al. 1971, 1974, 1978; Borejdo and Putnam 1977; Borovikov 1980; Wilson and Mendelson 1983; Prochniewicz-Nakayama et al. 1983; Miyanishi and Borejdo 1989; Ajtai and Burghardt 1986, 1989).

Studies of polarized fluorescence from intrinsic tryptophans of F-actin and that from extrinsic fluorophores bound to actin or to myosin heads in single "ghost" fibres complexed with phosphorylated or dephosphorylated heavy meromyosin in the presence of Mg^{2+}, and at low or high concentrations of Ca^{2+}, provide further information about the modulation of the actomyosin interactions under phosphorylation and Ca^{2+} binding (Borovikov et al. 1986a; Kąkol et al. 1987; Borovikov et al. 1987; Szczęsna et al. 1987; Borovikov et al. 1988).

Mathematical models proposed for the analysis of experimental data obtained by fluorimetrical methods have enabled the characterization of conformational changes in terms of polarized fluorescence parameters (Rozanov et al. 1971; Tregear and Mendelson 1975; Mendelson and Morales 1977; Yanagida and Oosawa 1978; Morales 1984; Burghardt 1984).

The models assume that absorption ($\vec{A}$) and emission ($\vec{E}$) transitions of either intrinsic or extrinsic fluorophores incorporated into sarcomeric structures behave like dipolar oscillators, and that the incorporation is either into a regular assay (e. g. a helix) or is totally random. It is further supposed that the oscillators can be excited and observed along well-defined Cartesian directions $\underset{\sim}{i}$ and $\underset{\sim}{j}$, so that the observed intensity is ${}_{\underset{\sim}{i}}I_{\underset{\sim}{j}}$. The Z-axis is taken as the fiber axis.

In Fig. 1*A* the excitation is represented along the positive Y-axis. The directions of the parallel ($\parallel$) and penpendicular ($\perp$) components of this light are shown. It is assumed that the fiber lies along the Z-axis, and the actin filament along the W-axis, which is at a polar angle Θ with the Z-axis. Fig. 1*B* shows the coordinate system U-V-W, related to the X-Y-Z system by Euler rotation. In the U-V-W system, the absorption and emission dipoles have polar angles Φ_A, Φ_E and the angle between them is γ (Tregear and Mendelson 1975; Mendelson and Morales 1977; Yanagida and Oosawa 1978).

Two empirical functions can be obtained from the measurements: $P_\perp = ({}_\perp I_\perp - {}_\perp I_\parallel)/({}_\perp I_\perp + {}_\perp I_\parallel)$ and $P_\parallel = ({}_\parallel I_\parallel - {}_\parallel I_\perp)/({}_\parallel I_\parallel + {}_\parallel I_\perp)$. The "anisotropy index", $P_\perp/P_\parallel$ may be taken as a characteristic of the F-actin filaments flexibility (Borovikov and Gusev 1983).

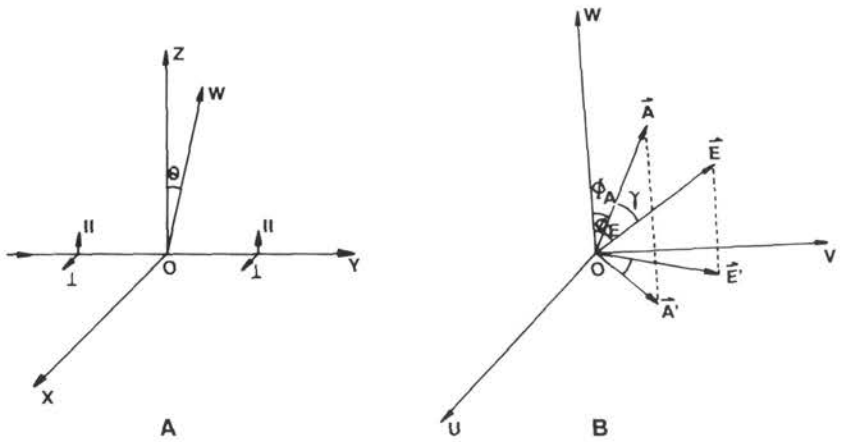

Figure 1. Diagrams explaining the calculation of the polarized fluorescence componets (Yanagida and Oosawa 1978). *A*: Θ is the angle between the filament axis of F-actin (OW) and fiber axis (OZ) at parallel (||) or penpendicular ($\perp$) direction of exciting light. *B*: Explanation of the calculation of parameters of polarized fluoroscence; $\Phi_A\Phi_E$ are angles between the F-actin long axis and the absorption ($\vec{A}$) and emission ($\vec{E}$) dipoles in F-actin respectively, γ is the angle between $\vec{A}$ and $\vec{E}$ dipoles.

a. Conformational changes of actin filaments and changes of orientation and random motion of myosin heads in ghost fibre decorated by soluble myosin fragments.

Ghost fibres have been shown to be very convenient for the study of conformational changes of actin filaments induced by the binding of both the tropomyosin-troponin complex and the myosin heads (Yanagida and Oosawa 1978; Borovikov et al. 1978, 1982; Borovikov and Karandashov 1983; Borovikov and Gusev 1983; Borovikov et al. 1986a, b; Kąkol et al. 1987; Szczęsna et al. 1987; Borovikov et al. 1987, 1988; Gałązkiewicz et al. 1987; Nowak et al. 1990).

The question arises whether the conformational changes of actin filaments induced by the binding of skeletal muscle myosin heads are dependent on the type of the divalent cation bound to the heads and/or on the phosphorylation of regulatory light chains? In other words, whether the interaction of actin with myosin in skeletal muscle is influenced by Ca^{2+} binding to myosin heads and/or by phosphorylation of myosin regulatory light chains.

The studies of changes of polarized fluorescence of intrinsic and extrinsic fluorophores bound to actin filaments in ghost fibres, induced by the binding of

phosphorylated and dephosphorylated myosin heads in the absence of ATP (rigor binding) at low or high Ca^{2+} concentrations, clearly showed that these changes depend on both the type of the divalent cation bound and that of the phosphorylated or dephosphorylated form of myosin regulatory light chains. In these studies, actin filament flexibility was observed to increase upon binding to actin of dephosphorylated heavy meromyosin with bound Ca^{2+} or phosphorylated meromyosin with bound Mg^{2+}. The actin filament flexibility could be decreased by binding dephosphorylated heavy meromyosin with bound Mg^{2+} or phosphorylated meromyosin with bound Ca^{2+} (Kąkol et al. 1987). The effect of the binding was enhanced in the presence of tropomyosin-troponin complex rebound to the ghost fibres Szczęsna et al. 1987).

The polarization of intrinsic F-actin tryptophan fluorescence was dependent on Ca^{2+} concentration when soluble fragments of myosin containing intact regulatory light chains were bound to the actin filaments (Borovikov et al. 1982; Borovikov and Karandashov 1983). The binding of phosphorylated or dephosphorylated heavy meromyosin containing regulatory light chains without 2 kDa N-terminal fragments induced an increase of the flexibility of actin filaments regardless of whether Ca^{2+} or Mg^{2+} were present (Szczęsna 1989).

Szczęsna et al. (1989) could show that the conformational changes of actin filaments induced by the binding of phosphorylated and dephosphorylated myosin heads influenced the structural organization of tropomyosin on the actin filaments. Thus, the kind of tropomyosin binding to the actin filament is influenced by the modulation of binding of myosin crossbridges with the actin filament.

These observations may be summarized as follows:

In the absence of ATP myosin heads seem to be able to form at least two kinds of complexes with actin filaments, differing in their structural organization and in actin filament flexibility.

Myosin heads consist of three domain-like fragments, designed according to the molecular weight of their heavy chains as 50K, 27K and 20K (Mornet et al. 1981, 1984). The structural organization of a domain may be determined by several factors. The myosin light chains are close to both the 27 and the 20K domain (Audemard et al. 1988 and the references therein).

Depending on the phosphorylation of myosin regulatory light chains and on whether Ca^{2+} or Mg^{2+} are bound, myosin may exist in two conformational states able to form two types of complexes with actin. These states differ in the structure of myosin regulatory light chains, very probably involving changes of the 20K domain structure and the interdomain organization of myosin heads. Thus, the ability to form two different complexes with actin monomer depends on the internal organization of the domain-like regions of myosin heads. This

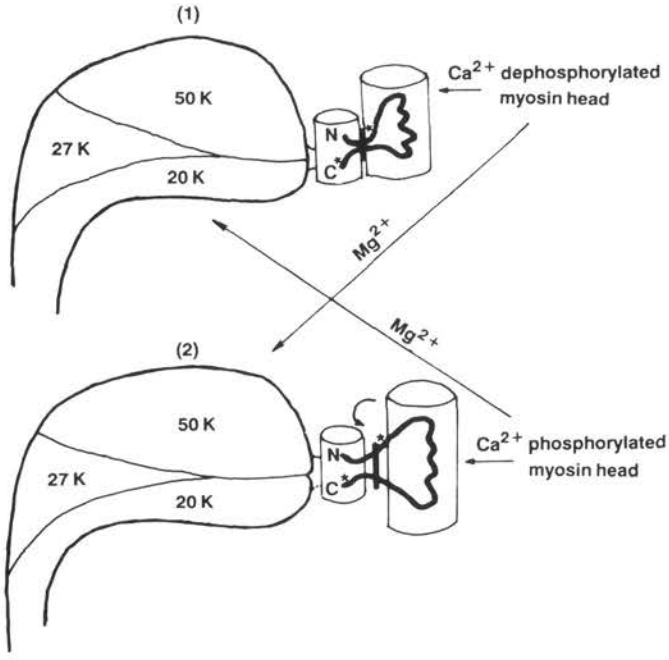

Figure 2. Schematic representation of myosin heads-actin complexes formed depending on the phosphorylation of myosin heads and on the type of divalent cations bound. State 1 represents the case with the heads bound with the actin filament, inducing an increase of actin filament flexibility. State 2 leads to a decrease of actin filament flexibility. The sites of fluorophore attachment to actin monomers are marked by asterisks.

ability may be influenced by phosphorylation of regulatory light chains and by the type of ion (Mg^{2+} or Ca^{2+}).

Dependent on the type of myosin heads binding to actin monomer, the intermolecular organization of actin changes and the actin filament flexibility increases or decreases.

Two peptide chain segments (10 kDa and 35 kDa) may be recognized in actin monomer. The segments form two-domain-like structures (Holmes et al. 1989) and the domains may differ in their organization.

Assuming that the organizations of the two actin monomers are different in rigid and flexible actin filaments, the above described findings concerning the influence of different forms of myosin heads on the flexibility of actin filaments may be summarized as follows:

At least two different forms of actin monomer-myosin head complexes exist in rigor binding. The difference concerns the kind of the myosin heads binding to actin monomer and the structural organization of the myosin heads and the domains of actin monomers.

Fig. 2 is a schematic representation of these complexes. State (1) may represent the kind of the binding of myosin heads resulting in an increase, and state (2) in a decreasse of the actin filament flexibility.

Myosin heads with the regulatory light chains lacking the 2kDa N-terminal fragments are able to form only one type of complexes, witch result in an increase of the actin filament flexibility.

Polarized fluorescence studies with 1,5 IAEDANS-labeled phosphorylated and dephosphorylated heavy meromyosin complexed with F-actin in ghost fibre showed differences in polarized fluorescence parameters dependent on the type of the bound cation (Mg^{2+} or Ca^{2+}) (Borovikov et al. 1987, 1988).

The changes in orientation and random motion of myosin heads complexed with actin filaments were dependent on phosphorylation of the regulatory light chains as well as on the kind of the divalent cation bound to the heads.

The observations described above comply with the assumption that the structural alterations of actin filaments induced by the binding of myosin heads depend on the kind of the binding limited by the conformation of myosin heads.

b. *Conformational changes of actin filaments decorated by modified soluble myosin fragments in ghost fibres.*

When myosin heads are modified by N, N′-phenylenedimaleimide (pPDM) in the presence of MgADP, interthiol cross-link forms between the reactive SH_1 and SH_2 sulfhydryl groups (Cys 707 — Cys 697) leading to the entrapment of MgADP at the active site (Wells and Yount 1979, 1982). The conformation of pPDM(SH_1—SH_2) modified myosin heads simulates an MATP and/or MADP-Pi state (King and Greene 1986). However, when SH_2 and SH_x (Cys 697 — Cys 540) are cross-linked, pPDM ($SH_2 - SH_x$) modified myosin simulate an MADP state (Chaussepied et al. 1988).

The polarized fluorescence studies of the effect of binding of pPDM-modified heavy meromyosin to phalloidin-rhodamine-labeled F-actin in ghost fibres disclosed significant differences between the phosphorylated and dephosphorylated modified heavy meromyosin, and the native one.

The values of all parameters of phalloidin-rhodamine fluorescence are strongly dependent on whether SH_1 — SH_2 or SH_2 — SH_x were crosslinked in the presence of MgADP.

Fig. 3 shows the changes of the values of the angle between the actin filament long axis and the fibre axis expressed as $\Delta \sin^2 \Theta$, and of the angle between the emission dipoles and the F-actin axis expressed as $\Delta \Phi_E$.

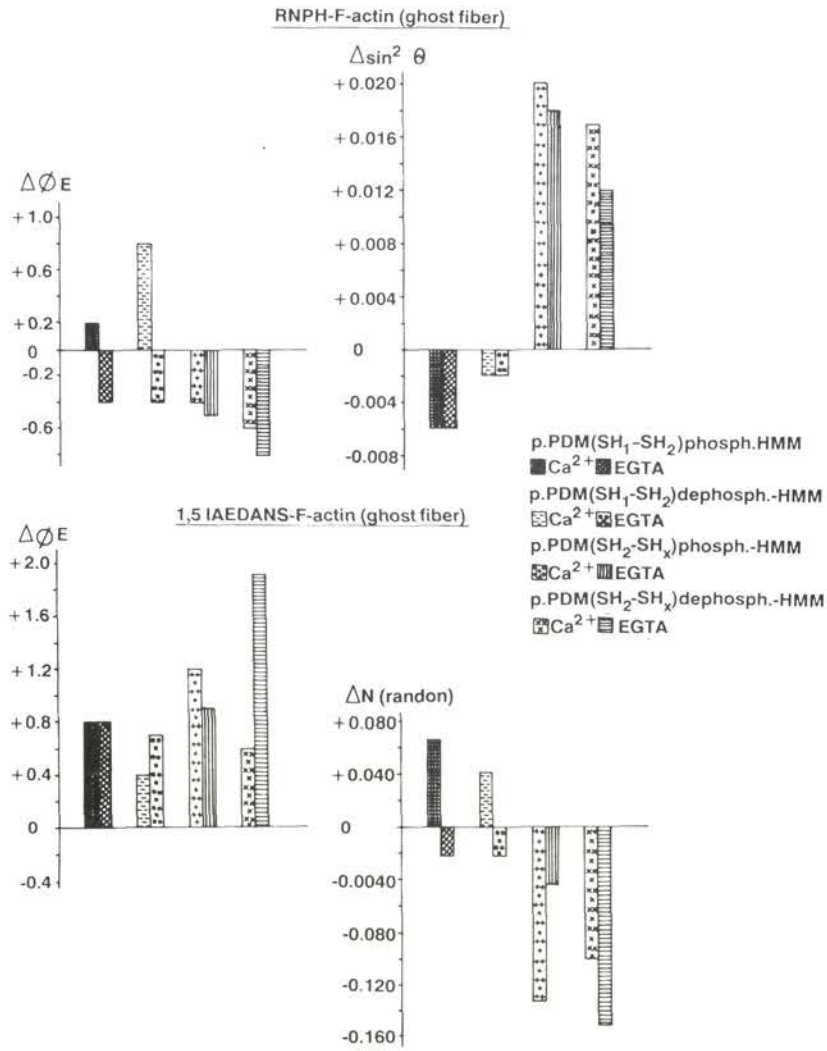

Figure 3. Changes of the angle between the actin filament long axis and the fibre axis (expressed as $\sin^2 \Theta$), and the angle between the actin filament long axis and the emission dipoles ($\Delta\Phi_E$) induced by the binding of phosphorylated and dephosphorylated pPDM-modified heavy meromyosin to phalloidin-rhodamine and 1,5-IADEANS-labeled actin filament in ghost fibres in dependence on the type of the divalent cation bound with the myosin heads. The binding of myosin heads was performed in a solution containing 30mmol/phosphate buffer (pH 7.0), 1 mmol/l $MgCl_2$ and 1mmol/l EGTA or 0.1 mmol/l$CaCl_2$ (EGTA or Ca^{2+}, respectively); pPDM(SH_1—SH_2) — phosphorylated and dephosphorylated heavy meromyosin modified by cross-linking of Cys 707 and Cys 697 in the presence of MgADP; pPDM(SH_2 — SH_x) — phosphorylated and dephosphorylated heavy meromyosin modified by cross-linking of Cys 697 — Cys 540 by pPDM in the presence of MgADP.

The flexibility of actin filaments induced by the binding of phosphorylated heavy meromyosin with SH_1—SH_2 cross-linking trapped was lower than that of actin filaments without bound myosin heads, independent of whether the divalent binding sites of myosin heads were saturated by Mg^{2+} or Ca^{2+}. The effect of the binding of dephosphorylated pPDM (SH_1—SH_2) modified myosin on the flexibility of actin filaments was strongly diminished as compared to that of native dephosphorylated heavy meromyosin.

However, when MgADP was trapped by cross-linking of SH_2—SH_x of phosphorylated and dephosphorylated heavy meromyosin, a highly significant increase of the actin filament flexibility was observed (Fig. 3) with myosin heads saturated with Mg^{2+} or Ca^{2+} ions.

The binding of phosphorylated and dephosphorylated pPDM (SH_2—SH_x) modified heavy meromyosin to actin filaments decreases the angle between the emission dipole and the fibre axis, independent of the type of the divalent cation bound and the form (phosphorylated or dephosphorylated) of the heavy meromyosin.

On the other hand, when actin filaments in ghost fibres were specifically labeled with 1,5-IAEDANS, the pPDM modified heavy meromyosin induced an increase of the angle between the emission dipole of fluorophores and the F-actin axis. This angle was smaller when the bound heavy meromyosin was modified by cross-linking of SH_1—SH_2 than by SH_2—SH_x groups in the presence of MgADP (Fig. 3).

The conformational changes of F-actin were reflected in changes of polarized fluorescence parameters of phalloidin-rhodamine bound probably to the cleft between the two domains of the actin monomers (Vandekerckhove et al. 1985; Miki 1987), or in those of 1,5-IAEDANS attached to Cys-375 of F-actin.

The angles of the emission dipoles of different fluorophores were observed to change with respect to the axis of the actin filament (Φ_E) in opposite direction (compare Fig. 3)

Since the fluorophores are attached to different regions of actin monomer the above observation might suggest that some intramolecular conformational changes of actin monomer occur (compare Fig. 2).

Thus it seems reasonable to assume that during ATP hydrolysis the loose binding of myosin heads complexed with ATP (MATP) or ADPPi (MADPPi) with actin filaments decreases the flexibility of the actin filaments and after the release of Pi (complexed with ADP (MADP)) the flexibility of the actin filaments increases. The changes of the actin filament flexibility seem to be connected with some alteration of the actin monomer organization in the actin filament and/or with intermonomer structural changes.

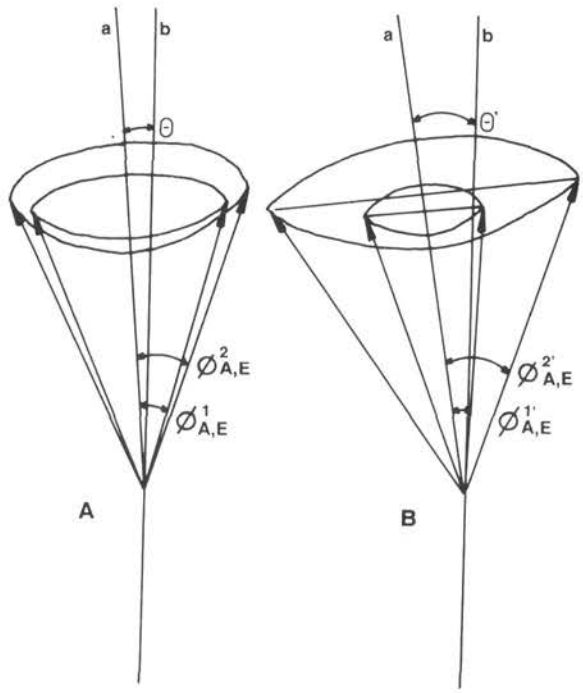

Figure 4. Diagrams explaining the changes of polarized fluorescence parameters of phalloidin-rhodamine and 1,5-IAEDANS attached to actin filaments in ghost fibres accompanying "loose" (*A*) and „tight" (*B*) binding of myosin heads to actin monomer. Φ^1_{AE}, Φ^2_{AE} and $\Phi^{1'}_{AE}$, $\Phi^{2'}_{AE}$ are the angles between the actin filament long axis (a) and the absorption and emission dipoles of phalloidin-rhodamine and 1,5-IADEDANS respectively Θ and Θ' are the angles between the fibre axis and the actin filament long axis for "loose" or "tight" binding of myosin heads to actin monomer respectively. $\Theta < \Theta'$; $\Phi^1_{AE} > \Phi^{1'}_{AE}$; $\Phi^2_{AE} < \Phi^{2'}_{AE}$.

General model of conformational changes of actin thin filaments and cross-bridges of myosin thick filament, and modulation of skeletal muscle contraction.

Let us assume that Fig. 4 is a true representation of changes of polarized fluorescence parameters of F-actin fluorophores induced by loose (*A*) and tight (*B*) binding of myosin heads to actin monomers. Dependent on the localization of the fluorophore in the actin monomer, various changes of angles between the actin filament axis and the absorption and emission dipoles. ($\vec{A}$, $\vec{E}$) are obtained. The diagram illustrates the spatial organization of the absorption and emission dipoles ($\vec{A}$, $\vec{E}$) as cones around the fibre axis, different for phalloidin-rhodamine and 1,5-IAEDANS attached to actin monomer.

In the case of heavy meromyosin bound loosely to actin, the angles between the actin filament axis and the absorption and emision dipoles of phalloidin-

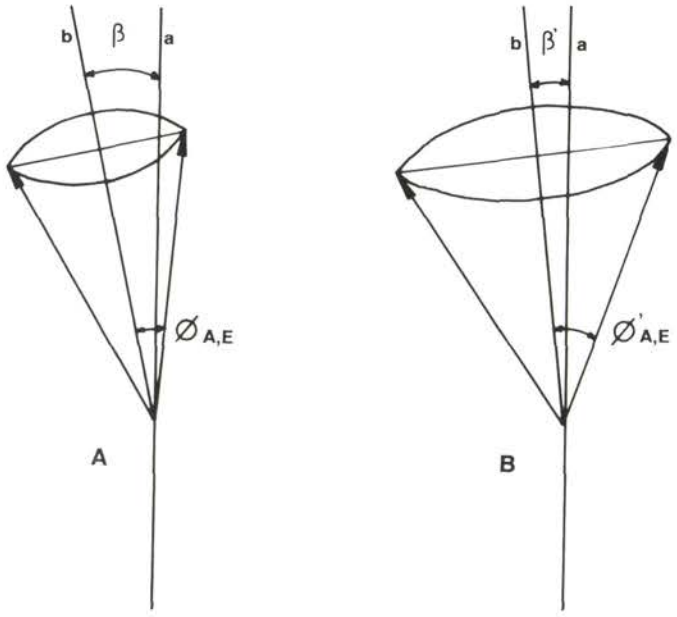

Figure 5. Diagrams explaining the changes of polarized fluorescence parameters of 1,5-IAEDANS attached to Cys 707 in myosin heads bound to actin monomers "loosely" (*A*) and "tightly" (*B*). The spatial organization of absorption and emission dipoles of the fluorophore are represented as cones around the axis. β, β' are the angles between the actin filament long axis and the fibre axis; Φ_{AE} and Φ'_{AE} are the angles between the absorption and emission dipoles of the fluorophore and the myosin head long axis $\beta > \beta'$; $\Phi_{AE} < \Phi'_{AE}$.

rhodamine (Φ^{l}_{AE}) are lower than for tight binding ($\Phi^{l'}_{AE}$). The opposite is true for 1,5-IAEDANS.

In both cases the flexibility of the actin filament is higher when myosin heads are bound tightly than when they are bound loosely (compare Fig. 3).

That means, that the various parts of actin (in the case of phalloidin-rhodamine the cleft between the two domains and in the case of 1,5-IAEDANS around the Cys-375) changed differently.

The spatial organization of absorption and emission dipoles of 1,5-IAEDANS bound to the SH_1 group (Cys-707) of myosin heads is shown on diagram (Fig. 5) as cones on myosin head long axis. It changed at tight and loose binding similarly as in the case of IAEDANS attached to Cys-375 on actin filament (Borovikov et al. 1990). The angle $\Phi_{AE} < \Phi'_{AE}$, and that between the myosin head long axis and the actin filament axis $\beta > \beta'$.

Thus, the conformational changes of myosin cross-bridges and actin filaments induced by the binding of heavy meromyosin to F-actin in ghost fibres

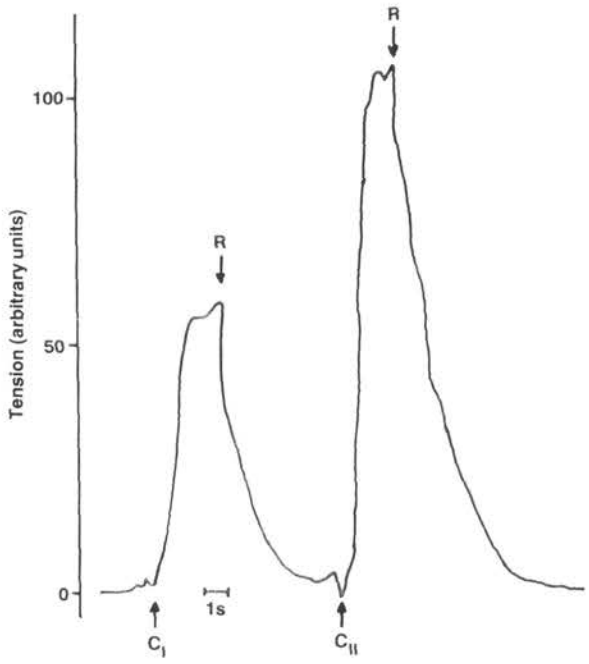

Figure 6. The influence of ionic strenght of "contracting" solution on isomeric tension development by glycerinated rabbit skeletal (psoas) muscle fibre. The contracting solution contained: 10 mml/l stock $CaCl_2$ solution calculated as described by Persechini et al. (1985) was added to obtain a Ca^{2+} concentration of 10 μmol/l. The concentration of KCl was 120 mmol/l (C_I) and 50 mmol/l (C_{II}). The arrows indicate additions of relaxation solution (R), i. e. solutions C_I and C_{II} without added Ca^{2+}.

may be described as follows. The flexibility of actin filaments decreases when myosin cross-bridges are loosely bound to actin filaments, and the random motion of the cross-bridges and the amount of randomly oriented fluorophores on myosin heads increases. The opposite is true for tight binding. The spatial arrangement of several regions of actin monomer and myosin head changed.

Conformational changes of actin filaments and cross-bridges of thick filaments upon the transition from relaxation to contraction and rigor of skeletal muscle fibres.

The polarization of tryptophan fluorescence in muscle depends on the physiological state of the latter (Aronson and Morales 1969; Dos Remedios et al. 1972; Borovikov et al. 1971, 1974). Güth (1980) suggested, that the degree of the tryptohan fluorescence polarization in muscle is insensitive to the orientation of the cross-bridges.

With muscle fibres at constant (MgATP), the tension developed in the

Table 1 Polarized fluorescence of tryptophan residues of F-actin in glycerinated muscle fibre. The degrees of fluorescence polarization $P_\perp$ and $P_\parallel$ are expressed as $(_\parallel I_\perp - {}_\perp I_\perp):({}_\parallel I_\perp + {}_\perp I_\perp)$ and $({}_\parallel I_\parallel - {}_\parallel I_\perp):({}_\parallel I_\parallel + {}_\parallel I_\perp)$ respectively, where I stands for the intensities of the four components of polarized fluorescence. The directions of the polarization planes of the exciting and the emitted light relative to the fibre axis are indicated on the left and on the right side, respectively. The fluorescence anisotropy index was determined as $A = P_\perp/P$

Tension (arbitrary units)	A_{rigor}	A_{relax}	A_{rigor}-A_{relax}	Ionic strength (mol/l)
102	2.55	2.15	0.4	0.07
52	2.35	2.15	0.2	0.14

presence of Ca^{2+} depended on the ionic strength (Borovikov and Lebedeva 1987). As shown in Fig. 6 the tension increased with the decreasing ionic strength of the "contracting" solution. Assosiated with this increase in tension, there was an increase in the anisotropy index of intrinsic polarized tryptophan fluorescence (Table 1).

The anisotropy of the tryptophan fluorescence of muscle fibres in "relaxing" solution was independent of the ionic strenght, and the increase of anisotropy index was correlated with the increase in tension and the decrease of ionic strength of the "contracting" solution (Table 1, Fig. 6).

The observed changes of anisotropy of tryptophan fluorescence of muscle fibres, dependent on physiological state, may be connected with some alteration of the actin filament structure induced by the binding of myosin heads. The effect of myosin heads binding to the actin filaments manifested as an increase in their flexibility has been demonstrated using various methods (Yanagida and Oosawa 1978 and the references therein).

When the polarization plane of the exciting light is directed parallel or perpendicular to the fibre axis, and the emission polarization from an extrinsic fluorophore specifically bound to actin or the myosin heads is investigated, it can be assumed that changes in polarization are caused by movements of the ligated protein or of its ligated domain.

As mentioned above, the four components of polarized fluorescence, ${}_\parallel I_\parallel$, ${}_\parallel I_\perp$, ${}_\perp I_\perp$, ${}_\perp I_\parallel$ can be employed to calculate $P_\perp$ and $P_\parallel$ and the angles between the absorption and emission dipoles and the fibre axis. Therefore, the changes in the structural organization of the labelled protein may be characterized by the values of these parameters of polarized fluorescence.

When F-actin in glycerinated muscle fibre containing phosphorylated myosin was specifically labelled by phalloidin-rhodamine, changes of the parameters which characterize the structural changes of the actin filament occurred

upon the transition from the relaxed state to contraction. Our previous results (Wrotek et al., 1989) have shown a significant decrease of the angle between the emission dipoles and the actin filament axis in contracted glycerinated muscle fibre. Also, the flexibility of actin filaments increased as indicated by the increased values of $\sin^2 \Theta$. The changes of the parameters of polarized fluorescence of phalloidin-rhodamine were correlated to the changes of tension developed by glycerinated muscle fibres; the tension development showed dependence on both the phosphorylation of myosin heads and the concentration of Ca^{2+} ions.

The changes described above are probably influenced by phosphorylation of myosin heads and by the exchange of calcium ions for magnesium ions in myosin heads. Thus, it seems reasonable to assume that muscle contraction is modified by myosin phosphorylation and the exchange of divalent ions, which affect the kind of the binding of myosin heads to actin by allowing a proper conformation of myosin heads, i.e. a proper organization of their inter-domain structure.

Finally, it can be concluded that: 1. The flexibility of actin filaments is increased or decreased depending on the kind of the binding of heavy meromyosin to actin. 2. When phosphorylated myosin heads are bound to actin filaments the flexibility remained increased at low concentrations of free calcium. 3. The conformation of myosin heads allowing to induce a decrease of actin filament flexibility by exchanging Ca^{2+} for Mg^{2+} ions requires the presence of regulatory light chains with intact N-terminal fragments containing phosphorylable serine. 4. Both, the changes of thin filaments flexibility and the alteration of structural organization of myosin heads depend on the type of the divalent cation bound with the myosin heads, and on the form (phosphorylated or dephosphorylated) of myosin regulatory light chains. 5. Upon the transition of the skeletal muscle fiber from rigor to the relaxed state, the flexibility of the thin filaments decreases and the structural inter-domain organization of myosin heads changes.

Thus, it seems reasonable to suggest that the modulation of skeletal muscle contraction by phosphorylation and by the exchange of Ca^{2+} bound with myosin heads for Mg^{2+} is due to changes of the inter-domain organization of the myosin heads which influence their ability to from complexes with actin, and to increase or decrease actin filament flexibility.

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Reference

Adelstein R. S., Conti M A. (1975): Phosphorylation of platelet myosin increases actin activated myosin ATPase activity. Nature **256,** 597—598

Ajtai K., Burghardt T. P. (1986): Observations of two orientations of rigor cross-bridges in glycerinated muscle fibres. Biochemistry **25,** 6203—6207

Ajtai K., Burghardt T. P. (1989): Fluorescent modification and orientation of myosin sulfhydryl 2 in skeletal muscle fibres. Biochemistry **28,** 2204—2210

Aksoy M. O., Mras S., Kamm K. E., Murphy R. A. (1983): Ca^{2+}, cAMP and changes in myosin phosphorylation during contraction of smooth muscle. Amer. J. Physiol. **295,** C255—C270

Alexis M. N., Gratzer W. B. (1981): Interaction of skeletal myosin light chains with calcium ions. Biochemistry **17,** 2319—2325

Aronson J. F., Morales M. F. (1969): Polarization of tryptophan fluorescence in muscle. Biochemistry **8,** 4517—4522

Audemard E., Bertrand R., Bonet A., Chaussepied P., Mornet D. (1988): Pathway for the communication between the ATPase and actin sites in myosin. J. Muscle Res. Cell Motil. **9,** 197—218

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

Borejdo J., Putnam S. (1977): Polarization of flourescence from single skinned glycerinated rabbit psoas fibres in rigor and relaxation. Biochim. Biophys. Acta **459,** 578—595

Borejdo J., Putnam S., Morales M. F. (1979): Fluctuations in polarized fluoroscence: evidence that muscle cross-bridges rotate repetitively during contraction. Proc. Nat. Acad. Sci. USA **76,** 6346—6350

Borovikov Yu. S. (1980): Studies on structural changes of F-actin and myosin in living, intact and damaged muscle fibres by means of polarized ultraviolet fluorescence microscopy. Microscopic Acta **82,** 379—388

Borovikov Yu. S., Vdovina I. B., Khoroshev M. I., Kirillina V. P. (1990): The orientation of fluorescent probes attached to actin and myosin subfragment-1 at the strong and the weak binding of these proteins in ghost fiber. In: XIXth European Conference on Muscle Contraction and Cell Motility, September 10th-13th, 1990, p. 35, Brussels, Belgium

Borovikov Yu. S., Chernogriadskaya N. A. (1979): Studies on conformational changes in F-actin of glycerinated muscle during relaxation by means of polarized ultraviolet fluorescence microscopy. Microscopic. Acta **81,** 383—392

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

Borovikov Yu. S., Karandashov E. A. (1983): Effect of Ca^{2+} on conformation of actin in the F-actin heavy meromyosin complex. Biochem. Int. **7,** 319—328

Borovikov Yu. S., Chernogriandskaia N. A., Rosanov Yu. M. (1974): A study of structural changes in myosin and actin filaments of muscle fibre by the polarized ultraviolet fluorescence microscopy. Tsitologiya **16,** 977—982 (in Russian)

Borovikov Yu. S., Kirillina V. P., Chernogriadskaya N. A. (1978): A study of structural changes in muscle contractile proteins by the polarized ultraviolet fluorescent microscopy. Tsitologiya **20,** 1384—1389 (in Rusian)

Borovikov Yu. S., Lebedeva N. N. (1987): Studies of structural changes in muscle contractile proteins by the polarized ultraviolet fluorescent microscopy. Effects of ATP, Ca^{2+}, changes in pH and ionic strength of washing solution on the state of thick filaments. Tsitologiya **11,** 1270—1274 (in Rusian)

Borovikov Yu. S., Levitsky D. I., Kirillina V. P., Poglazov E. 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. Biochem. **125,** 343—347

Borovikov Yu. S., Shudel M. S., Chernogriadskaya N. A., Rozanov Yu. M., Barskii I. Ya. (1971): Study on changes in azimutal characteristics of polarized ultra violet fluorescence of single muscle fibers at streching and contraction. Dokl. Acad. Nauk USSR **194,** 962 (in Russian)

Borovikov Yu. S., Kąkol I., Szczęsna D., Kirillina V. P., Levitsky D. J. (1986a): Effect of phosphorylation of rabbit skeletal muscle myosin light chains on the nature of conformational changes of F-actin induced by heavy meromyosin. Biokhimiya **51,** 691—694 (in Russian)

Borovikov Yu. S. Kąkol I., Levitsky D. J. (1986b): SH-group binding in myosin heads alters the character of conformational changes of F-actin, induced by myosin subfragment-1 or heavy meromyosin. Dokl. Akad. Nauk SSSR **287,** 216—219 (in Russian)

Borovikov Yu. S., Wrotek M., Aksenova N. B., Lebedeva N. N., Kąkol I. (1987): Influence of Mg^{2+} and Ca^{2+} bound 1.5- IAEDANS labeled phosphorylated and dephosphorylated heavy meromyosin complexed with F-actin on polarized fluorescence of the fluorophore. FEBS Lett. **223,** 409—412

Borovikov Yu. S., Wrotek M., Aksenova N. B., Lebedeva N. N., Kąkol I.) (1988): Effect of Ca^{2+} on myosin head motility in the F-actin-heavy meromyosin complex. Biokhimiya **53,** 97—100 (in Russian)

Borovikov Yu. S., Szczęsna D., Khoroshev M. I., Kąkol I. (1990): "Freezing" of heavy meromyosin with a bifunctional reagent inhibits dependence of the actin-myosin interaction on phosphorylation of myosin light chains. Tsitologiya **32,** 481—488 (in Russian)

Burghardt T. P. (1984): Model-independent fluorescence polarization for measuring order in a biological assembly. Biopolymers **23,** 1383—1406

Butler T. M., Siegman M. J., Mooers S. V., Barsotti R. J. (1983): Myosin light chain phosphorylation does not modulate cross-bridge cycling rate in mouse skeletal muscle. Science **220,** 1167—1169

Cardinaud R., Kąkol I. (1985): Influence of the regulatory light chain of fast skeletal muscle on its interaction with actin in the presence and absence of ATP. Biochim. Biophys. Acta **832,** 80—88

Chantler P. D., Szent-Györgyi A. G. (1980): Regulatory light-chains and scallop myosin: full dissociation, reversibility and cooperative effects. J. Mol. Biol. **138,** 473—480

Chaussepied P., Morales M. F., Kassab R. (1988): The myosin SH_2-50-kilodalton fragment cross-link: location and consequences. Biochemistry **27,** 1778—1785

Cooke R., Crowder M. S., Thomas D. P. (1982): Orientation of spin labeles attached to cross-bridges in contracting muscle fibres. Nature **300,** 776—778

Cooke R., Crowder M. S., Wendt C. H., Bernett V. A., Thomas D. (1984): Muscle cross-bridges: do they rotate? In: Contractile Mechanism in Muscle Ed. G. H. Pollack and S. Hasur) pp. 413—427, Plenum, New York

Dos Remedios D., Millikan C. G., Morales M. F. (1972): Polarization of tryptophan fluorescence from single striated muscle fibres. A molecular probe of contraction. J. Gen. Physiol. **59,** 103—120

Ebashi S., Endo M. (1968): Calcium ion and muscle contraction. Prog. Biophys. Mol. Biol. **18,** 123—183

Gałązkiewicz B., Borovikov Yu. S., Dąbrowska R. (1987): The effect of caldesmon on actin-myosin interaction in skeletal muscle fibres. Biochim. Biophys. Acta **916,** 368—375

Goody R. S., Holmes K. C. (1983): Cross-bridges and the mechanism of muscle contraction. Biochim. Biophys. Acta **726,** 13—39

Güth K. (1980): Polarization of tryptophan fluorescence measurement in muscle. Biophys. Struct. Mech. **6,** 81—93

Harrington W. F. (1971): A mechano-chemical mechanism for muscle contraction. Proc. Nat. Acad. Sci. USA **68,** 685—689

Harrington W. F. (1979): On the origin of the contractile force in skeletal muscle. Proc. Nat. Acad. Sci. USA **76,** 5066—5070

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

Holmes K. C., Popp D., Gebhard W., Kabsch W. (1989): The Structure of F-actin Calculated From x-ray Fibre Diagrams and the 0.6 nm Crystal Structure. Cytoskeletal and Extracellular Proteins (Ed. U. Aebi and J. Engel) pp. 48—50 Springer-Verlag

Huxley A. F. (1957): Muscle structure and theories of contraction. Progr. Biophys. **7,** 255—318

Huxley A. F., Niedergerke R. (1954): Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. Nature (London) **173,** 971—973

Huxley A. F., Simmons R. M. (1971): Proposed mechanism of force generation in striated muscle. Nature (London) **233,** 533—538

Huxley H. E. (1969): The mechanism of muscular contraction. Science **164,** 1356—1365

Huxley H. E., Faruqi A. R. (1983): Time resolved X-ray diffraction studies on vertebrate striated muscle. Ann. Rev. Biophys. Chem. **12,** 381—417

Huxley H. E, Hanson J. (1954): Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature (London) **173,** 973—976

Huxley H. E., Kress M. (1985): Crossbridge behaviour during muscle conctraction. J. Muscle Res. Cell Motil. **6,** 153—161

Hynes T. R., Block S. M., White B. T., Spudich J. A. (1987): Movement of myosin fragments in vitro: domains involved in force production. Cell **48,** 953—963

Kąkol I., Kasman K., Michnicka M. (1982): The phosphorylation process as a myosin-linked regulation of superprecipitation of fast skeletal muscle actomyosin. Biochim. Biophys. Acta **704,** 437—443

Kąkol I., Borovikov Yu. S., Szczęsna D., Kirillina V. P., Levitsky D. I. (1987): Conformational changes of F-actin in myosin-free ghost single fibre induced by either phosphorylated or dephosphorylated heavy meromyosin. Biochim. Biophys. Acta **913,** 1—9

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

King T. R., Greene L. E. (1986): The conformation of crosslinked actin S-1 in the presence and absence of ATP. J. Biol. Chem. **262,** 6128—6134

Kishino A., Yanagida T. (1988): Force measurements by micromanipulation of a single actin filament by glass needles. Nature **334,** 74—76

Klug G. A., Botterman B. R., Stull J. T. (1982): The effect of low frequency stimulation on myosin light chain phosphorylation in skeletal muscle. J. Biol. Chem. **257,** 4688—4690

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

Manning D. R., Stull J. T. (1982): Myosin light chain phosphorylation-dephosphorylation in mammalian skeletal muscle. Amer. J. Physiol. **242,** C234—C241

Mendelson R. A., Morales M. F. (1977): Appendix to Borejdo J., Putnam, S. M.: Polarization of fluorescence from single skinned glycerinated rabbit psoas fibres in rigor and relaxation. Biochim. Biophys. Acta **459,** 578—595

Michnicka M., Kasman K., Kąkol I. (1982): The binding of actin to phosphorylated and dephosphorylated myosin. Biochim. Biophys. Acta **704,** 470—475

Miki M. (1987): The recovery of the polymerizability of Lys-61-labelled actin by the addition of phalloidin. Fluorescence polarization and resonance-energy-transfer. Eur. J. Biochem. **164,** 229—235

Miyanishi T., Borejdo S. (1989): Differential behavior of two residues of the myosin head in muscle fibres. Biochemistry **28,** 1287—1294

Moore R. L., Stull J. T. (1984): Myosin light chain phosphorylation in fast and slow skeletal muscles in situ. Amer. J. Physiol. **247,** C462—C471

Morales M. F. (1984): Calculation of the polarized fluorescence from a labeled fiber. Proc. Nat. Acad. Sci. USA **81,** 8145—8156

Mornet D., Ue K., Morales M. F. (1984): Proteolysis and the domain organization of myosin subfragment. Proc. Nat. Acad. Sci USA **81,** 736—739

Mornet D., Pantel P.,Bertrand R., Audemard E., Kassab R. (1981): Isolation and characterization of the trypsin modified S-1 derivatives. FEBS Lett. **123,** 54—58

Nowak E., Borovikov Yu. S., Dąbrowska R. (1989): Caldesmon weakens the binding between myosin heads and actin in ghost fibres. Biochim. Biophys. Acta **999,** 289—292

Pemrick S. M. (1980): The phosphorylated L_2 light chain of skeletal myosin is a modifier of the actomyosin ATPase. J. Biol. Chem. **225,** 8836—8841

Perry S. V. (1979): The regulation of contractile activity in muscle. Biochem. Soc. Trans. **7,** 593—617

Persechini A., Stull J. T., Cooke R. (1985): The effect of myosin phosphorylation on the contractile properties of skinned rabbit skeletal muscle fibres. J. Biol. Chem. **260,** 7951—7954

Pires J. M. V., Perry S. V. (1977): Purification and properties of myosin light-chain kinase from fast skeletal muscle. Biochem. J. **167,**137—146

Próchniewicz-Nakayama E., Yanagida T., Oosawa F. (1983): Studies on conformation of F-actin in muscle fibres in the relaxed state, rigor and during contraction using fluorescent phalloidin. J. Cell. Biol. **97,** 1663—1667

Pulliam D. L., Savyna V., Levine R. J. C. (1983): Calcium sensitivity of vertebrate skeletal muscle myosin. Biochemistry **22,** 2324—2331

Rozanov Yu. M., Chernogriadskaya N. A., Barskii J. Ja., Borovikov Yu. S., Shudel M. S. (1971): Polarized ultraviolet fluorescence of muscle fibres and some other cytological anisotropic objects. Tsitologiya **13,** 190—200 (in Russian)

Sobieszek A., Small J. V. (1976): Myosin-linked calcium regulation in vertebrate smooth muscle. J. Mol. Biol. **101,** 75—92

Sobieszek A., Small J. V. (1977): Regulation of the actin myosin interaction in vertebrate smooth muscle: activation via a myosin light-chain kinase and the effect of tropomyosin. J. Moll. Biol. **112,** 559—567

Stępkowski D., Osinska H., Szczęsna D., Wrotek M., Kąkol I. (1985a): Decoration of actin filaments with skeletal muscle heavy meromyosin containing either phosphorylated or dephosphorylated regulatory light chains. Biochim. Biophys. Acta **830,** 337—340

Stępkowski D., Szczęsna D., Wrotek M., Kąkol I. (1985b): Factors influencing interaction of phosphorylated and dephosphorylated myosin with actin. Biochim. Biophys. Acta **831,** 321—329

Sweeney H. L., Stull J. T. (1986): Phosphorylation of myosin in permeabilized mamalian cardiac and skeletal muscle cells. Amer. J. Physiol. **250,** C657—C660

Szczęsna D. (1989): Conformational changes of myosin modulating the interaction of thick and thin filaments in fast skeletal muscle. PhD. Thesis. Nencki Institute of Experimental Biology

Szczęsna D., Borovikov Y u. S., Lebedeva N. N., Kąkol I. (1987) Effect of phosphorylation of myosin light chains on interaction of heavy meromyosin with regulated F-actin in ghost fibres. Experientia **43,** 194—196

Szczęsna D., Borovikov Yu. S., Kąkol I., Sobieszek A. (1989): Interaction of tropomyosin with F-actin-heavy meromyosin complex. Biol. Chem. Hoppe-Seyler **370,** 399—407

Thomas D. D. Cooke R. (1980): Orientation of spin labeled myosin heads in glycerinated muscle fibres. Biophys. J. **32,** 891—906

Tyoshima Y. Y., Korn S. J., McNally S. M., Niebling K. R., Toyoshima Ch. E., Spudich J. A. [1987): Myosin subfragment 1 is sufficient to move actin filament *in vitro*. Nature **328,** 536—539

Tregear R., Mendelson R. (1975): Polarization from a helix of fluorophores and its relation to that obtained from muscle. Biophys. J. **15,** 455—467

Ueno H., Harrington W. F. (1986); Local melting in the subfragment 2 region of myosin in activated muscle and its correlation with contractile force. J. Mol. Biol. **190,** 69—82

Vandekerckhove J., Doboben A., Nassal M., Wieland T. (1985): The phalloidin binding site of F-actin. EMBO **4,** 2815—2818

Wells, J. A., Yount R. G. (1979): Active site trapping of nucleotides by cross-linking two sulfhydryls in myosin subfragment 1. Proc. Nat. Acad. Sci. USA **76,** 4966—4970

Wells J. A. Z., Yount R. C. (1982): Chemical modification of myosin by active-site trapping of metal-nucleotides with thiol cross-linking reagents. In: Methods in Enzymology **85,** (Ed. D. W. Frederiksen, L. W. Cunningham) p. p. 93—115, Acad. Press, New York

Weber M. M., Oplatka A. (1974): Physico-chemical studies on the light chains of myosin. Evidence for a regulatory role of rabbit myosin light chains. Biochem. Biophys. Res. Commun. **57,** 823—830

Wilson M. G. A., Mendelson R. A. (1983): A comparison of order and orientation of cross-bridges in rigor and relaxed muscle fibres using fluoroscence polarization. J. Muscle Res. Cell Motil. **4,** 671—693

Wrotek M., Borovikov Yu. S., Lebedeva N. N., Kąkol I. (1989): Some properties of glycerinated skeletal muscle fibres containing phosphorylated myosin. Gen. Physiol. Biophys. **8,** 569—578

Yanagida T. (1981): Angles of nucleotides bound to crossbridges in glycerinated muscle fiber at various concentrations of epsilon ATP epsilon ADP and epsilon AMPPNP detected by polarized fluorescence. J. Mol. Biol. **146,** 539—560

Yanagida T. (1984): Angles of fluorescently labelled myosin heads and actin monomers in contracting and rigor stained muscle fiber. In: Contractile Mechanisms in Muscle (Ed. G. H. Pollak and H. Sugi). Plenum Press, N. Y. and London

Yanagida T. (1985): Angle of active site of myosin heads in contracting muscle during sudden length changes. J. Muscle Res. Cell Motil. **6,** 43—52

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

Yanagida T., Taniguchi N., Oosawa F. (1974): Conformational changes of F-actin in the thin filament of muscle induced *in vivo* and *in vitro* by calcium ions. J. Mol. Biol. **90,** 509—522

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