

Lactate Dehydrogenase-induced Conformational Changes of F-Actin in Myosin-Free Ghost Single Fibres

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Abstract. The changes in conformation of F-actin induced by the binding of the glycolytic enzyme lactate dehydrogenase were studied in myosin-free single ghost muscle fibres. The formation of the lactate dehydrogenase-F-actin complex was accompanied by changes in the parameters of intrinsic (tryptophan) and extrinsic (rhodaminyl-phalloin) polarized fluorescence of ghost muscle fibre F-actin. Lactate dehydrogenase stimulated actin-activated Mg^{2+} -ATPase of myosin subfragment 1 by 30%. F-actin of ghost fibres depressed lactate dehydrogenase activity to 20% of the initial values. It is suggested that the energy-providing mechanism is coupled with that of muscle contraction through conformational changes in F-actin.

Key words: Ghost fibre — Polarized fluorescence — Conformational changes of F-actin — Lactate dehydrogenase

Introduction

It is known that the mechanism of muscle contraction includes the interaction of myosin and actin filaments (Huxley 1969). Monomers of the major protein of thin filaments, F-actin, show two-domain organization (Suck et al. 1981). The monomers are oriented perpendicularly to the long axis of thin filaments and form right-handed two-start helices (Egelman 1985). The flexibility of thin filaments (Fujime et al. 1972; Oosawa 1980) is possibly the result of the existence of slits between the domains. It is assumed that actin monomers in a thin filament have at least two different structural and functional states: "switched-on" and "switched-off" (Bremel et al. 1972; Lednev 1980). These different states apparently are associated with different spatial organization of domains and different rigidity of interdomain junctions (Yanagida and Oosawa 1978; Borovnikov et al. 1982; Borovnikov and Lebedeva 1987). The actin monomers, being polar aggregated and following a "head-tail" pattern, determine the packing and localization of other proteins in thin filaments (Higashi 1963). Thus,

tropomyosin (TM) molecules are located along the thin filament, each TM molecule being in contact with seven actin monomers (Hanson 1967; Matsumura and Lin 1981). The troponin (TN) complex consists of 3 subunits and it interacts with F-actin sites distributed with a periodicity of 38.5 nm (Ohtsuki et al. 1961; Pato et al. 1981). Apart from major contractile proteins (myosin, TM and TN) at least 40 minor proteins, including glycolytic enzymes, interact with F-actin (Larsson 1985; Pollard and Cooper 1986). Electron microscopy and histochemistry data have shown that glycolytic enzymes, traditionally considered as freely dissolved in the sarcoplasm (such as phosphofructokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase and lactate dehydrogenase) are located in I-disc of the sarcomere (Pette and Brandau 1962; Siegel and Pette 1969; Dölken et al. 1975). As demonstrated in experiments with isolated proteins, these enzymes are capable of interacting with F-actin (Arnold and Pette 1969, 1971; Clarke and Masters 1976; Walsh et al. 1980; Sugrobova et al. 1983; Freydina et al. 1987). F-actin have been shown to influence the activity and catalytic properties of aldolase (Arnold and Pette 1969, 1971; Clarke et al. 1974), phosphofructokinase (Liou and Anderson 1980), phosphorylase kinase (Poglasov et al. 1982), glyceraldehyde-3-phosphate dehydrogenase (Eronina et al. 1983) and lactate dehydrogenase (Sugrobova et al. 1983). Hence an isolated enzyme may behave differently from that adsorbed to F-actin. The integration of the metabolic enzymes in muscle cell structures allows coupling regulation of energy metabolism with muscle contraction. The capacity of F-actin to adsorb glycolytic enzymes supports the view that F-actin not only is involved in mechanical contraction of muscle, but is also responsible for the regulation of the enzymatic mechanism of the cell (Poglasov 1983). Glycolytic enzymes when bound to F-actin may affect the structure of the latter and hence modify cell motility. Conformational changes of F-actin induced by binding of enzymes involved in energy metabolism have not been studied so far.

A better understanding of the role of enzyme adsorption to F-actin in metabolic regulation and its association to muscle contraction requires a clear-cut knowledge of the effects of binding of glycolytic enzymes to F-actin on structural and functional characteristics of each of the components of the F-actin-enzyme complex. The present study investigates the relations between metabolism and the mechanisms of cell motility.

To obtain information on lactate dehydrogenase-induced conformational changes in F-actin of ghost single fibre, intrinsic (tryptophan residues of F-actin) as well as extrinsic (rhodaminyl-phalloin bound to F-actin) polarized fluorescence was measured. The effect of ghost fibre F-actin on enzymatic activity of lactate dehydrogenase and that of lactate dehydrogenase on Mg^{2+} -ATPase activity of myosin subfragment 1 were studied.

Material and Methods

Preparation of proteins

Smooth muscle tropomyosin (TM) was purified according to the procedure described elsewhere (Galazkiewicz et al. 1987). Rabbit skeletal muscle myosin was prepared according to the method of Perry (1955). Myosin subfragment 1 (S₁) devoid of regulatory light chain was obtained by digestion of myosin with α -chymotrypsin (Okamoto and Sekine 1985). G-actin was prepared according to Spudich and Watt (1971).

Preparation of ghost fibres

Ghost fibres were prepared from single glycerinated fibres of rabbit psoas muscle by extraction of myosin, tropomyosin and troponin as described previously (Kirillina et al. 1979; Borovikov and Gusev 1983). Polarized fluorescence of intrinsic tryptophan residues in F-actin and/or rhodaminyl-phalloin specifically bound to F-actin was measured prior to and after the addition of lactate dehydrogenase.

Fluorescence labeling of F-actin

F-actin of ghost fibres was labeled with rhodaminyl-phalloin for 20 min as described earlier (Kakol et al. 1987; Galazkiewicz et al. 1987). Excess label was removed by washing the fibres with a solution containing (in mmol/l): KCl 100, MgCl₂ 1, phosphate buffer, pH 7.0, 67.

Binding of lactate dehydrogenase, tropomyosin and myosin subfragment 1 to F-actin of ghost fibres

Incorporation of lactate dehydrogenase to F-actin in ghost fibres was allowed by immersion of fibres in a solution containing 5 mg/ml lactate dehydrogenase and (in mmol/l): MgCl₂ 1, tris-acetate buffer, pH 6.0, 25 ($\mu = 0.05$ — solution of low ionic strength) or MgCl₂ 1, KCl 100, phosphate buffer, pH 6.0, 6.7 ($\mu = 0.13$ — solution of physiological ionic strength) for 18 h at 4°C. The unbound lactate dehydrogenase was removed by washing with one of the above solution. Tropomyosin was bound to F-actin in ghost fibres as described previously (Galazkiewicz et al. 1987). Myosin subfragment 1 was obtained according to Okamoto and Sekine (1985). To allow binding of myosin subfragment 1 to F-actin in ghost fibres, the fibres were immersed in a solution containing (in mmol/l): MgCl₂ 1, NaN₃ 1, KCl 20, Tris-HCl buffer, pH 7.5, 25, and 1 mg/ml myosin subfragment 1. The unbound myosin subfragment 1 was removed by washing with same solution without subfragment 1.

Lactate dehydrogenase activity

Lactate dehydrogenase (isoenzyme H₄) from pig myocardium (the electrophoretic pattern is shown in Figure 1A) was purchased from Reanal, dialyzed for 18 h at 4°C against (mmol/l): MgCl₂ 1, NaN₃ 1, tris-acetate buffer, pH 6.0, 5. Ghost fibres containing F-actin were mounted to glass plates, 5 fibres (10 mm long) on each. Prior to incubation with lactate dehydrogenase the ghost fibres were washed three times with 20 ml each of a solution containing 5 mmol/l tris-acetate buffer, pH 6.0, for 18 h at 4°C, to remove factors which might affect the actin and lactate dehydrogenase activity. The

incubation of F-actin fibres with lactate dehydrogenase (82.5 μg) was performed in a total volume of 3 ml of tris-acetate buffer (5 mmol/ml, pH 6.0) for 2 h at room temperature. After the incubation the unbound enzyme was washed out. The medium was assayed for lactate dehydrogenase activity both prior to and after the incubation with ghost fibres as were the ghost fibres after the incubation with lactate dehydrogenase. The lactate dehydrogenase activity was estimated by the rate of NAD reduction in a lactate-containing medium (Emery 1967) of the following composition (mmol/l): sodium lactate 80, NAD 5, tris-acetate buffer, pH 6.0, 5, to which the assayed enzyme solution or ghost fibres were added. The total volume of the sample was 3 ml. The rate of NADH formation was measured with a spectrophotometer as adsorption changes at 340 nm at room temperature.

ATPase assays

The actin-activated Mg^{2+} -ATPase of myosin subfragment 1 (S_1) was determined in the presence and absence of lactate dehydrogenase using the following media (mmol/l): KCl 40, MgCl_2 3, ATP 2, tris-HCl buffer, pH 7.5, 40, G-actin 50 μg /ml, myosin 200 μg /ml, lactate dehydrogenase 130 μg /ml. The reaction was stopped by the addition of sodium dodecylsulphate (SDS) to a final concentration of 1% (Dabrowska et al. 1985). The enzyme activity was expressed as $\mu\text{mol Pi mg myosin min}$. The amount of inorganic phosphate (Pi) liberated was measured by the method of Fiske and Subbarow (1925).

Protein determination

G-actin, lactate dehydrogenase, tropomyosin, myosin and subfragment 1 were quantitated spectrophotometrically using the microbiuret method (Itzhaki and Gill 1964).

Polyacrylamide SDS-gel electrophoresis

The protein composition of ghost fibres and the lactate dehydrogenase-F-actin molar ratio were determined by disc-SDS-electrophoresis (Laemmli 1970) with subsequent densitometry of the gels.

Fluorescence polarization measurements

The intensity (I) of intrinsic (tryptophan) and extrinsic (rhodaminyl-phalloin) fluorescence of F-actin was recorded with a polarized microfluorimeter (Loffe et al. 1974). Four components of polarized fluorescence, $I_{\parallel}, I_{\perp}, I_{\perp\perp}, I_{\parallel\parallel}$, were measured with the subscripts \parallel and \perp denoting the direction of polarization (parallel and perpendicular to the fibre axis); the left subscript concerns the incident light and the right one the emitted light. The degree of polarized fluorescence (P, P_{\perp}) was determined from equations:

$$P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp}); P_{\perp} = (I_{\perp\perp} - I_{\parallel\parallel}) / (I_{\perp\perp} + I_{\parallel\parallel})$$

Tryptophan fluorescence was excited at 303 ± 4 nm and emitted light was recorded at 320–380 nm.

Fluorescence of rhodaminyl-phalloin bound to F-actin in ghost fibres was excited at 479 ± 5 nm and recorded at 500 ± 6 nm.

Experimental data were analyzed in terms of mathematical models described earlier (Kakol et al. 1987). The models have been based on the assumption that anisotropy of F-actin of muscle fibre fluorophores aligns parallel to the long axis of thin filament and the randomly arranged fluorophores. The four components of polarized fluorescence were expressed as functions of angles Φ_A, Φ_E , and Θ value, where Φ_A, Φ_E are angles between the long axis of F-actin and the adsorption

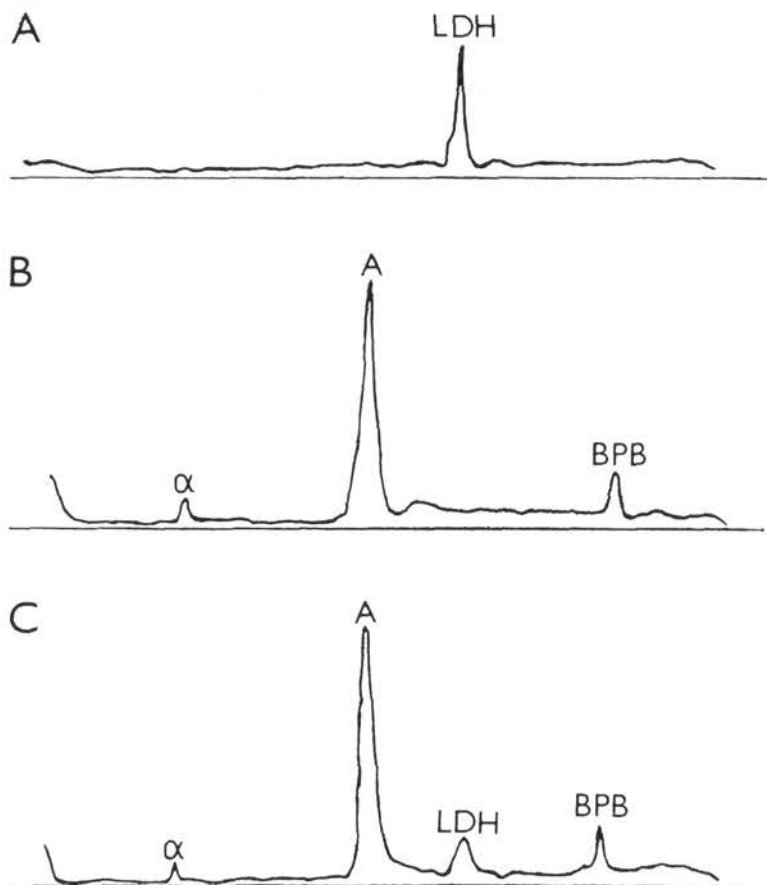


Fig. 1. Densitograms of slab SDS-gel electrophoresis of lactate dehydrogenase (A), ghost muscle fibre (B), complex of F-actin with lactate dehydrogenase (C). Abbreviations: α — α -actinin, LDH — lactate dehydrogenase, A — actin, BPB — bromphenol blue.

and the emission dipoles, respectively; Θ is angle between the long axis of F-actin and the fibre axis. Angle γ (between Φ_A and Φ_E) was 32° and 28° for tryptophan and rhodaminyl-phalloin, respectively. The modulus of elasticity for bending, or flexion rigidity (ϵ) (Yanagida and Oosawa 1978) of thin filaments was obtained from: $\sin^2 \Theta = 0.8(kT/\epsilon)L$, where k and T are the Boltzman constant and absolute temperature, respectively, and L is the length of the filament.

Results and Discussion

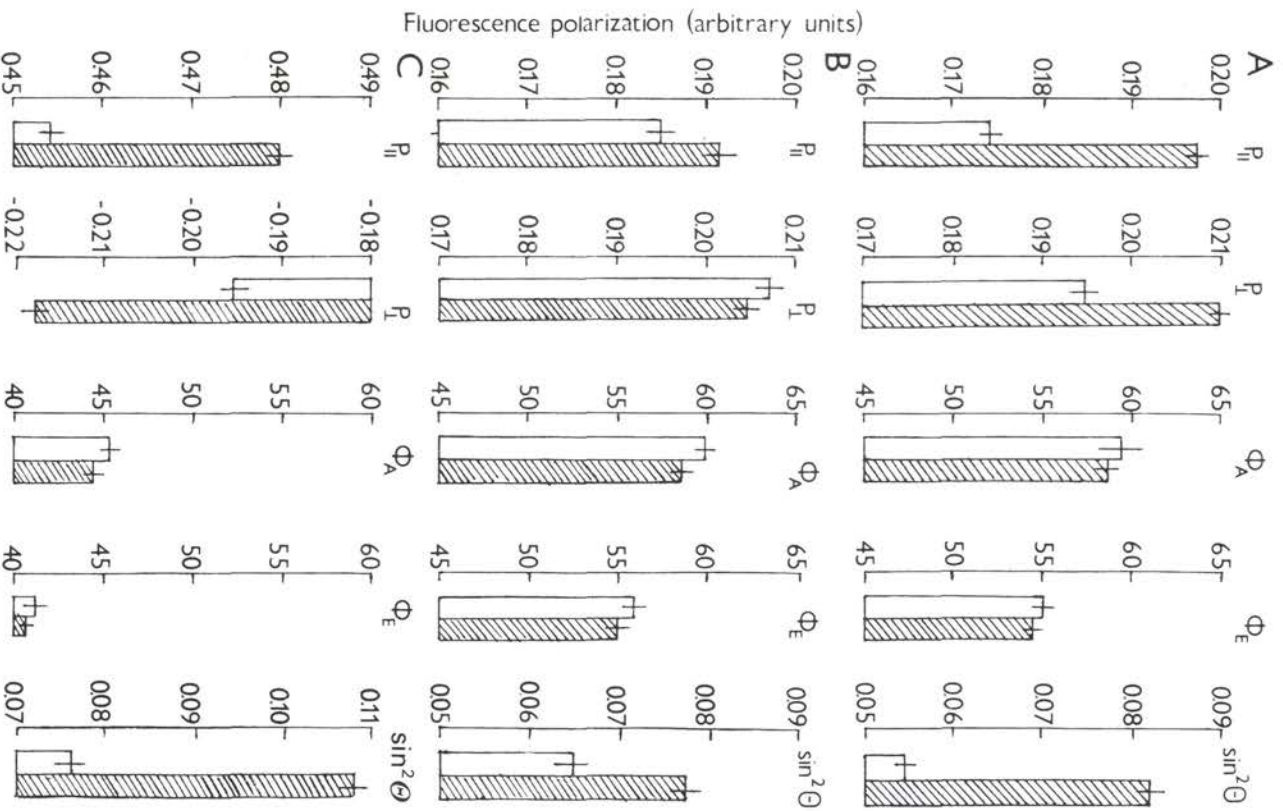
F-actin of ghost muscle fibre is present in thin filaments regularly packed in

sacromeres (Tawada et al. 1976). Electrophoretic analysis of ghost muscle fibres revealed that more than 80% of the protein was F-actin, and no lactate dehydrogenase was present (Fig. 1B). During incubation of ghost muscle fibres in a lactate dehydrogenase-containing solution the enzyme was adsorbed to F-actin of thin filaments. This is suggested by electrophoretic densitogram of ghost muscle fibres after the incubation with lactate dehydrogenase. The molar ratio of lactate dehydrogenase to F-actin depends on the ionic strength of the solution. With increasing ionic strength from $\mu = 0.05$ to $\mu = 0.13$ the ratio decreases from 1 : 5 to 1 : 10. This is in accordance with the data from literature on F-actin binding to lactate dehydrogenase solutions (Sugrobova et al. 1983). It should be mentioned that an abrupt decrease in lactate dehydrogenase adsorption to ghost fibre was observed after disintegration and partial removal of F-actin from thin filaments. On the other hand, an increase in the relative content of myosin in ghost muscle fibres did not significantly change the amount of lactate dehydrogenase adsorbed to F-actin.

The formation of lactate-dehydrogenase-F-actin complexes (Fig. 1C) both at $\mu = 0.05$ and at $\mu = 0.13$ of the washing solution significantly affects intrinsic (tryptophan) and extrinsic (rhodaminy-phalloin) polarized fluorescence of ghost muscle fibres (Fig. 2A and 2B). During lactate dehydrogenase binding to F-actin, the values of P_{\parallel} and P_{\perp} for tryptophan fluorescence increase. P_{\perp} increases less than does P_{\parallel} (Fig. 2A). As a result, the calculated parameters Φ_A and Φ_E decrease, but $\sin^2 \Theta$ increases. Since the pattern of changes in recorded and calculated parameters of polarized fluorescence of F-actin during F-actin-lactate dehydrogenase interaction remains the same regardless of ionic strength (P_{\parallel} increases, P_{\perp} changes but slightly, Φ_A , Φ_E decrease, $\sin^2 \Theta$ increases), it can be concluded that ionic strength has no significant effect on the conformational changes in F-actin filaments during lactate dehydrogenase binding (Fig. 2A and 2B).

As shown in Fig. 2A, the binding of lactate dehydrogenase to F-actin induced a decrease in Φ_A and Φ_E and an increase in $\sin^2 \Theta$. Since increase of $\sin^2 \Theta$ is indicative of higher flexibility of thin filaments, whereas decreased Φ_A and Φ_E reflect changes in orientation of F-actin monomers in thin filaments

Fig. 2. The effect of lactate dehydrogenase on parameters of intrinsic (tryptophan) polarized fluorescence of F-actin (A, B — for $\mu = 0.05$ and $\mu = 0.13$, respectively) and extrinsic (rhodaminy-phalloin) polarized fluorescence of F-actin (C). The angle between absorption and emission dipoles in tryptophan molecule and rhodaminy-phalloin molecule, $\gamma = 32^\circ$ and 28° , respectively. The angles of absorption (Φ_A) and emission (Φ_E) dipoles, $\sin^2 \Theta$, where Θ is the angle between the filament axis and the fibre axis, the degrees of polarized fluorescence (P_{\parallel} , P_{\perp}) were determined as described elsewhere (Kakol et al. 1987). The molar ratio of lactate dehydrogenase to F-actin was 1 : 5 (A, C) and 1 : 10 (B) for $\mu = 0.05$ and $\mu = 0.13$, respectively. \square — F-actin, \boxplus — F-actin + LDH



(Yanagida and Oosawa 1978; Kakol et al. 1987), it might be assumed that lactate dehydrogenase induces conformational changes in F-actin, which in turn result in changes of the structural state of thin filaments.

Similar changes in recorded and calculated parameters of polarized fluorescence were observed in experiments with rhodaminyl-phalloin, specifically bound to F-actin-lactate dehydrogenase complex (increase in P_{\parallel} , decrease in P_{\perp} , Φ_A and Φ_E , increase in $\sin^2 \Theta$) (Fig. 2C).

It is of interest that the interaction of S_1 with F-actin of thin filaments is accompanied with an increase in P_{\parallel} , a decrease in P_{\perp} , Φ_A , Φ_E and an increase in $\sin^2 \Theta$ (Fig. 3A). As suggested earlier, the decrease in Φ_A and Φ_E values and the increase in $\sin^2 \Theta$ during binding to myosin heads are indicative of the transition of monomers to the "switched-on" state, essential for power generation in muscle fibre (Borovikov et al. 1982; Kakol et al. 1987; Galazkiewicz et al. 1987). A comparison of these reports with the data obtained with lactate dehydrogenase binding to F-actin allows to assume that lactate dehydrogenase induces "switching-on" of thin filaments.

If our assumption holds lactate dehydrogenase can be supposed to activate actomyosin Mg^{2+} -ATPase. Indeed, an approximately 30% increase of activation was observed (0.33 $\mu\text{mol Pi/mg myosin/min}$ compared with the control level of 0.25 $\mu\text{mol Pi/mg myosin/min}$). It is of interest that the effects of lactate dehydrogenase on F-actin conformation and actomyosin Mg^{2+} -ATPase activity are identical with those of smooth muscle tropomyosin. As shown in Fig. 3B the interaction of smooth muscle tropomyosin with F-actin changes parameters of polarized fluorescence, similar to those induced by lactate dehydrogenase binding to F-actin. In both cases an essential increase of $\sin^2 \Theta$ is observed. According to our interpretation this suggests an increase of amount of "switched-on" monomers of F-actin. After the addition of tropomyosin the parameters of F-actin are restored (P_{\parallel} changes but slightly, P_{\perp} increases, Φ_A , Φ_E , $\sin^2 \Theta$ reach their initial values); this apparently points to the competitive nature of tropomyosin and lactate dehydrogenase binding to F-actin (Fig. 3B). Lactate dehydrogenase as well as smooth muscle tropomyosin may affect the pattern of the myosin-actin interaction.

During lactate dehydrogenase binding to F-actin the enzyme activity changes. It has been shown in previous studies that actomyosin is isolated from muscles together with lactate dehydrogenase, and the enzyme cannot be removed either by reprecipitation or with large volume of washing solution. This permits us to assume that in muscle lactate dehydrogenase is bound to proteins of the actomyosin complex.

Prior to incubation with lactate dehydrogenase the ghost fibres showed no activity. During incubation with lactate dehydrogenase the enzyme is adsorbed to F-actin as reflected by a decrease in lactate dehydrogenase activity in the

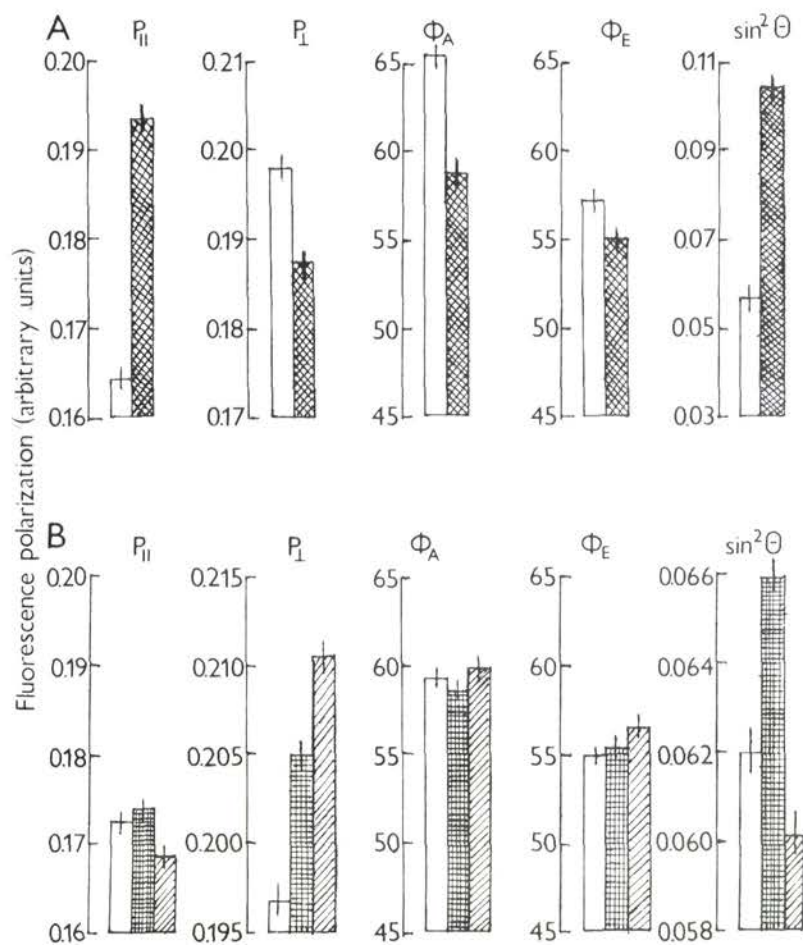


Fig. 3. The comparison of changes of intrinsic (tryptophan) polarized fluorescence of F-actin during the binding with myosin subfragment 1 (A), smooth muscle tropomyosin and lactate dehydrogenase (B). For symbols see legend to Fig. 2. The molar ratio of lactate dehydrogenase to F-actin was 1 : 5. A: \square — F-actin, \otimes — F-actin + S_1 . B: \square — F-actin, $\#$ — F-actin + TM, ▨ — F-actin + TM + LDH.

incubation media and by the appearance of lactate dehydrogenase activity in ghost fibres (Table 1). Lactate dehydrogenase activity in incubation media decreases to one fifth of the preincubation values. The absence of lactate dehydrogenase activity in the washing solution allows to assume that the 5-fold decrease in lactate dehydrogenase activity is due to the enzyme being adsorbed to F-actin. A similar pattern of changes in lactate dehydrogenase activity was

Table 1. The effect of ghost muscle fibre F-actin on lactate dehydrogenase activity

Lactate dehydrogenase activity*			
in lactate dehydrogenase solution		in ghost muscle fibre	
Prior to incubation with ghost fibre	After incubation with ghost fibre	Prior to incubation with lactate dehydrogenase	After incubation with lactate dehydrogenase
188.3 ± 16.1	118.0 ± 8.4	—	13.8 ± 1.8

* $\mu\text{mol NAD}/\text{min}$ (measured in a total volume of incubation medium of 3 ml)

Lactate dehydrogenase: F-actin molar ratio was 1 : 10

observed in earlier experiments with F-actin polymerized from G-actin (Sugrobova et al. 1983). These authors observed that lactate dehydrogenase binding to F-actin inhibited the enzyme activity 2.5 times. The difference in inhibition may be explained by regular packing of actin filaments in sacromeres of ghost muscle fibres.

Adsorption of an enzyme to biological compartment is known to be associated with changes in structural and functional state of the complex (Kurganov et al. 1986). The present study shows that lactate dehydrogenase adsorption to F-actin of thin filaments of ghost muscle fibres induced structural changes in F-actin as well as in catalytic properties of the enzyme itself. The formation of the F-actin-lactate dehydrogenase complex in muscle cell can be expected to activate motility response and to modulate regulation of catalytic activity of glycolytic enzymes. We believe conformational changes in F-actin are the coupling site of energy-providing mechanisms with that of muscle contraction.

Acknowledgements. The authors are thankful to Prof. Theodor Wieland of Heidelberg University, FRG, for his kind gift of rhodaminy-phalloin, to Dr. B. Galazkiewicz, Nencki Institute of Experimental Biology, Warsaw, Poland, for her kind gift of smooth muscle tropomyosin, and to Dr. S. A. Krolenko for stimulating discussion.

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