Effect of Mg-ADP on the Structure of Actin in the F-Actin-Myosin Subfragment 1 Complex

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Abstract

The orientation and mobility of fluorescent probes phalloidin-rhodamine and 1,5-IAEDANS located in various parts of actin was studied by polarized microfluorimetry techniques. Muscle fibres free of myosin, tropomyosin and troponin (ghost fibres) were used. It was found that the binding of myosin subfragment 1 (S1) to actin induces changes in polarized fluorescence of the fibres. The analysis of the data showed that the formation of actin-S1 and actin-S1-ADP complexes in a muscle fiber resulted in a decrease of the angle between the thin filament and the emission dipole of phalloidin-rhodamine as well as an increase in the mobility of this dye. In the experiments with the 1,5-IAEDANS label the angle of emission dipole increased while the label's mobility decreased. It is assumed that the changes in orientation and mobility of some regions of actin or subdomains of actin take place when a myosin head interacts with actin. The subdomains orientation in actomyosin complex changes, when influenced by Mg-ADP. The data obtained allow to propose the involvement of interdomain motions of some parts of actin monomer in mechanisms of muscle contraction.

Key words: Ghost fibre, polarized fluorescence, conformational changes in F-actin, myosin subfragment 1, muscle contraction.

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It is known that muscle contraction is induced by the interaction of myosin, actin and ATP. The hydrolysis of ATP with myosin ATPase results in the formation of several intermediate complexes of myosin with substrate and with products of ATPase reaction [28]. It has been shown that the formation of some intermediates in active center of myosin ATPase is accompanied by changes in structure of myosin and actin [14, 15, 27]. It remains obscure, however, which conformational changes are associated with force generation in muscle. To gain some insight into this problem, it might be useful to study the movement of domains of myosin head and actin subdomains at different stages of ATP hydrolysis cycle [12, 16, 18]. The necessary information can be obtained with the use of polarized microfluorimetry [3]. To study the orientation and mobility of fluorescent labels, intrinsic polarized fluorescence of tryptophane and tyrosine residues of protein or extrinsic of fluorescent probes specifically bound to F-actin were regarded. It is assumed that changes in probe orientation are indicative of changes in spatial organization of the whole protein molecule or of its regions [1, 4, 9, 10, 12, 21, 24-26, 34].

As has been earlier shown phalloidin-rhodamine (complex of phalloidin and rhodamine) and 1,5-IAEDANS fluorescent probes bound to different actin regions, during interaction of thin filaments with proteolytic myosin fragments [heavy meromyosin and myosin subfragment 1 (S1)], change their orientation in different ways. It appeared that the pattern of these changes depends on the conformation of the myosin head [6, 8, 9, 21]. Thus, in muscle fibres at the so-called form of "weak binding" of myosin head to actin, characteristic of actin-myosin-ATP complex (A-M-ATP) or actin-myosin-ADP-Pi, changes in the orientation of these fluorophores relative to fiber axis strongly differ from the corresponding changes observed in the case of "strong binding" form of myosin head to actin, characteristic of actin-myosin (A-M) complex. The data obtained enabled us to assume that the spatial orientation and mobility of some actin regions or of its domains change during ATP hydrolysis cycle [9].

Table 1. The effect of Mg-ADP on the extent of fluorescence polarization of phalloidin-rhodamine and 1,5-IAEDANS bound to F-actin of the ghost muscle fiber of rabbit with orientation of fiber parallel ($P_{\parallel}$) and perpendicular ($P_{\perp}$) to the plane of incident light in the absence and presence of myosin subfragment 1 (S1)

Structure	ADP	Fluorescence polarization			
		Phalloidin-rhodamine		1,5-IAEDANS	
		$P_{\parallel}$	$P_{\perp}$	$P_{\parallel}$	$P_{\perp}$
F-actin	-	0.481 ± 0.001	-0.341 ± 0.001	0.253 ± 0.004	0.163 ± 0.002
F-actin-S1	-	0.503 ± 0.003	-0.291 ± 0.001	0.172 ± 0.007	0.220 ± 0.001
F-actin	+	0.475 ± 0.005	-0.312 ± 0.002	0.265 ± 0.004	0.183 ± 0.001
F-actin-S1	+	0.494 ± 0.004	-0.276 ± 0.002	0.235 ± 0.003	0.204 ± 0.001

In this study performed with the use of polarized microfluorimetry the orientation of phalloidin-rhodamine and 1,5-IAEDANS was investigated during modelling the two stages of ATP hydrolysis cycle in muscle fiber: A-M and A-M-ADP.

Materials and Methods

Study was made on single glycerinated muscle fibres subjected to selective extraction of myosin, troponin and tropomyosin (on ghost muscle fibers). Glycerinated fibres were obtained from rabbit psoas muscle by method of Szent-Gyorgy [29]. Myosin, troponin and tropomyosin were removed by immersion of single fiber for 1.5 h into a solution containing: 800 mM KCl, 10 mM ATP, 1 mM $MgCl_2$, 67 mM phosphate buffer, pH 7.0. Protein extraction was controlled as described earlier [21].

Modification of F-actin of the ghost fiber with fluorescent dyes was carried out during incubation of fiber in the solution containing: 40 μM phalloidin-rhodamine, 20 mM KCl, 1 mM $MgCl_2$, 6.7 mM phosphate buffer, pH 7.0 [21], or in the solution containing 100 mM 1,5-IAEDANS, 60 mM KCl, 1mM $MgCl_2$, 10 mM Tris-HCl buffer, pH 8.0 [5]. The unbound dye was removed by washing the fibers in the standard solution: 10 mM KCl, 1 mM $MgCl_2$, 6.7 mM phosphate buffer, pH 7.0.

The localization of fluorescent probes in the fiber was obtained by SDS-polyacrylamide gel electrophoresis [22] with subsequent determination of fluorescence intensity by densitometric scans of the gels. In all the fibres the fluorescence of actin alone was observed.

Myosin was isolated from rabbit skeletal muscles [20]. S1 devoid of regulatory light chains was obtained by digestion of myosin with α-chymotrypsin in the presence of 2 mM EDTA [33].

The decoration of F-actin with S1 was performed by immersion of fiber in the solution with S1 (2 mg/ml) containing 10 mM KCl, 1 mM $MgCl_2$, 25 mM Tris-HCl buffer, pH 7.5 [21] in the presence or absence of 1 mM ADP.

The molar ratio of S1 to actin and protein composition of fiber was determined by electrophoresis in the presence of SDS [22], with subsequent gel densitometry. The molar ratio 1:3 of S1 to actin was the same in all the experiments.

The study has been made by polarized microfluorimetry [19]. The intensity of four components of polarized fluorescence ${}_{\parallel}I_{\parallel}$, ${}_{\parallel}I_{\perp}$, ${}_{\perp}I_{\parallel}$, ${}_{\perp}I_{\perp}$ was measured. The subscripts $_{\parallel}$ and $_{\perp}$ denote the direction of polarization parallel and perpendicular to the fiber axis; the presubscripts refer to the incident light and the postsubscripts to the emitted light propagation. The extent of polarized fluorescence with orientation of fiber parallel and perpendicular to the polarization plane of incident light was determined from equations: $P_{\parallel} = ({}_{\parallel}I_{\parallel}-{}_{\parallel}I_{\perp}) / ({}_{\parallel}I_{\parallel}+{}_{\parallel}I_{\perp})$; $P_{\perp} = ({}_{\perp}I_{\perp}-{}_{\perp}I_{\parallel})/({}_{\perp}I_{\perp}+{}_{\perp}I_{\parallel})$.

The fluorescence of phalloidin-rhodamine and 1,5-IAEDANS was excited at 489 ± 5 nm and 365 ± 5 nm respectively, and recorded at 500-600 nm.

The analysis of experimental data was carried out by the earlier described method [21]. The ratios between the four intensities of polarized fluorescence (${}_{\parallel}I_{\perp}/{}_{\parallel}I_{\parallel}$, ${}_{\perp}I_{\parallel}/{}_{\parallel}I_{\parallel}$, ${}_{\perp}I_{\perp}/{}_{\parallel}I_{\parallel}$) were regarded as functions of Φ_A, Φ_E, Θ angles and the N value, where Φ_A and Φ_E are the angles of absorption and emission dipoles of fluorophores relative to the long axis of F-actin, respectively, N is the relative amount of randomly distributed fluorophores.

Results and Discussion

The binding of phalloidin-rhodamine and 1,5-IAEDANS to F-actin of the ghost muscle fiber results in the appearance of polarized fluorescence, in such fibres $P_{\parallel} \neq P_{\perp}$ (see Table 1). This bears evidence of anisotropic distribution of dyes in the muscle fiber. The share of oriented fluorophores is high, since $P_{\parallel}$ is a high positive, and $P_{\perp}$ is a low positive, or a negative value [3, 21].

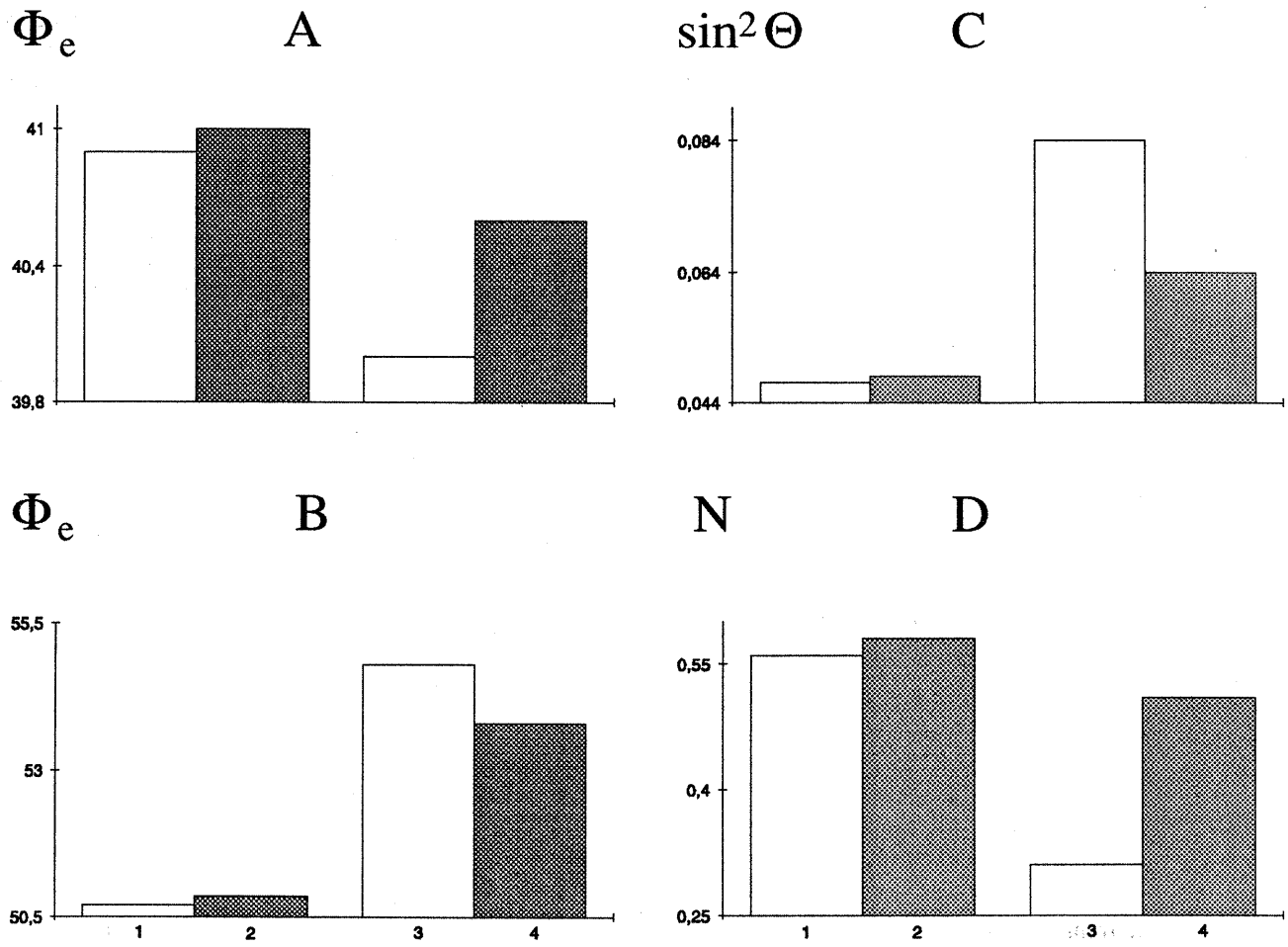

Figure 1. The effect of Mg-ADP on the orientation and mobility of oscillators of phalloidin-rhodamine (a, c) and 1,5-IAEDANS (b, d) specifically bound to F-actin of thin filament of ghost muscle fibre in the absence (1, 2) and in the presence (3, 4) of myosin S1.
Ordinate: Φ_E angle, grade (°) (a, b); $\sin^2\Theta$ (°) (c) and N value, relative units (d). 1, 2- ghost muscle fibre; 3, 4- the same decorated with myosin S1. 1, 3- in the absence of Mg-ADP; 2, 4- in the presence of Mg-ADP. Vertical bars: 95% confidence limits.

The decoration of F-actin with S1 in the absence or in the presence of Mg-ADP causes notable changes in polarized fluorescence (see Table 1). Thus, for phalloidin-rhodamine the $P_{\parallel}$ value increased and the $P_{\perp}$ value decreased, whereas for 1,5-IAEDANS the situation was just the reverse. Since the changes in fluorescence polarization of these fluorophores are indicative of conformational changes in F-actin [5, 21], it can be assumed that the decoration of thin filaments with S1 in the presence or absence of Mg-ADP is accompanied by conformational rearrangement of this protein.

This effect of S1 binding is much lower in the presence of Mg-ADP than in its absence (see Table 1). It is known that ADP when interacting with active center of myosin head, changes the conformation of its heavy chain [28].

On the other hand, the direct effect of Mg-ADP on the structure of F-actin is but slightly expressed [5].Thus, in this study only weak changes in $P_{\perp}$ have been observed and no significant changes in $P_{\parallel}$. Taking these facts into account, it can be easily assumed that weakening of the effect of S1 binding in the presence of Mg-ADP results from the changes in the conformation of S1 heavy chain. Hence, F-actin structure differs in actin-S1 and actin-S1-ADP complexes, the differences being initiated by conformational rearrangements in heavy chain of myosin head.

It should be pointed out that changes in actin conformation occur in at least two different regions of actin monomer. This conclusion is justified since phalloidin-rhodamine and 1,5-IAEDANS are localized in different regions of actin monomer [24, 32], and the polarized

fluorescence of both fluorophores varies by the effect of S1 (see Table 1).

To be able to study the conformational changes of F-actin in muscle fiber, the flexibility of thin filament has been estimated (as characterized by $\sin^2 \Theta$), the angles of orientation of emission dipoles Φ_E of fluorophores have been determined, the mobility of fluorescent probes (judged by N value change) and flexibility of the C-terminal region of actin polypeptide chain (judged by N value calculated after determination of flexibility of thin filament) have been estimated [21, 30, 34].

It appeared that the N values for phalloidin-rhodamine and 1,5-IAEDANS were equal to 0.05 and 0.07, respectively. This implies that the mobility of the former is significantly lower than that of the latter. Since the fluorophores are bound to different regions of actin monomer, these regions are apparently characterized by different mobility within thin filaments.

It is thought that phalloidin-rhodamine, located between large and small domains of actin, bind to Lys-336, Tyr-356 and Tyr-306 of the C-terminal region of the polypeptide actin chain and to amino acid residues 115-118, 121-124 of the N-terminal region of actin, whereas 1,5-IAEDANS binds to Cys-374 [23] of the subdomain 1 of small domain of actin monomer [17]. This enables us to conclude that within thin filaments, subdomain 1 of small domain possesses high mobility [13]. Our data agree with these ideas.

As seen in Fig. 1d, with the same S1/actin molar ratio, N value for 1,5-IAEDANS in the absence of Mg-ADP is notably lower than in its presence. Since 1,5-IAEDANS is located not far from the binding site of myosin head to actin

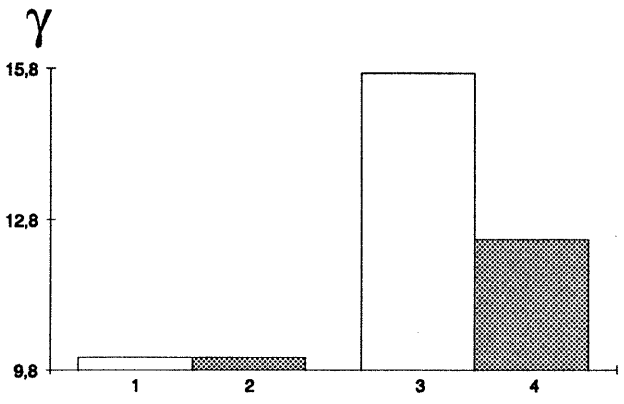

Figure 2. The effect of Mg-ADP on the value of the γ angle between emission dipoles of phalloidin-rhodamine (1, 3) and 1,5-IAEDANS (2, 4) in the absence (1, 2) and in the presence (3, 4) of myosin S1.
Ordinate: γ angle, grade (°); 1, 2- ghost muscle fibre; 3, 4- the same decorated with myosin S1; 1, 3- in the absence of Mg-ADP; 2, 4- in the presence of Mg-ADP. Vertical bars: 95% confidence limits.

[31], a change in N value can be regarded as an evidence of the effect of nucleotide on the interaction of myosin head with subdomain 1 of small domain of actin. Apparently, in the presence of Mg-ADP the flexibility of binding of myosin head to this domain of actin increases. Naturally, an increase in free movement of small domain caused by changes in weakening actin-myosin interaction may result in an increase in randomly distributed fluorophores, i.e. accelerate the increase in flexibility (Fig. 1d).

Changes in actin-myosin interaction affect the flexibility of thin filaments. This follows from the data presented in Fig. 1c. It appeared that in the presence of nucleotide the $\sin^2 \Theta$ value is lower than in its absence. This implies that the flexibility of thin filament, and hence, the flexibility of intermonomer association in thin filaments decreases at the effect of Mg-ADP.

As has earlier been shown in our studies, the flexibility of thin filament depends on the character of actin-myosin interaction. With "strong" form of actin-myosin binding, the flexibility of thin filaments was much higher, whereas the flexibility of actin-myosin interaction notably lower than with "weak" form of binding [5, 9, 21]. Taking these fact into account, a decrease in the flexibility of thin filament in Mg-ADP presence can be regarded as an evidence of weakening actin-myosin binding in A-M-ADP complex [28].

Thus, Mg-ADP affects the flexibility of interdomain and actin-myosin interaction in actomyosin complex, strengthening the former and weakening the latter.

Mathematical modelling of the data obtained also shows that the decoration of actin with S1 in the absence as well as in the presence of Mg-ADP results in a change in Φ_E angle of fluorophores (Fig. 1a, b). Since phalloidin-rhodamine is bound to subdomain 2 of small domain or to large domain, a change in the orientation of fluorophores bears an evidence of a change in the orientation of that of regions of actin in the fiber. It is of interest that the changes in the orientation of phalloidin-rhodamine and 1,5-IAEDANS relative to the fibre axis are of different type: in the former case the Φ_E angle decreases, and in the latter increases (Fig. 1 a, b). This implies that during S1 binding, some of actin regions, move relative to each other. The dislocation of regions is not so significant, since the γ angle formed during S1 binding to F-actin in the absence of Mg-ADP changes by 6°, whereas in its presence by 3° (Fig. 2).

Thus, the decoration of thin filaments with S1 both in the absence and presence of Mg-ADP results in conformational changes of actin. The structure of actin in actin-S1 and actin-S1-ADP complexes corresponding to the A-M- and A-M-ADP stages of ATP hydrolysis cycle, apparently, differs in the flexibility of interdomain and actin-myosin binding, as well as in the location of subdomain 1 of small domain (or their regions) relative to the rest of actin monomer. It is of interest that according to the data of our earlier studies, the location of these monomer regions of actin relative to each other, as well as the flexibility of

interdomain binding, also differs at the A-M and A-M-ATP (A-M-ADP-Pi) stages of the ATP hydrolysis cycle [8, 9]. Taking into account these data, together with those of the present study, it can be concluded that the changes in actin conformation described above take place at least at three stages of ATP hydrolysis: A-M-ATP (A-M-ADP-Pi), A-M-ADP and A-M.

Finally, it should be pointed out that the changes in the location of subdomain 1 in actomyosin complex taking place during ATP hydrolysis cycle are apparently accompanied by the orientation of certain regions of myosin head or its domains. Thus , it has been shown that a dye bound to 20 kda domain of myosin head changes its orientation in the presence of Mg-ADP, the pattern of these changes [1, 9, 11, 24) being similar to that of the changes in the orientation of the fluorophores bound to subdomain 1 of small domain of actin, but radically different from that bound to large domain and subdomain 2 of small domain of actin (Fig. 1, 2). This indicates that the movements of subdomain 1 of small domain of actin and 20 kda domain of myosin are in the same direction and take place relative to the part of actin monomer which is a part of a "core" of thin filament [13]. Movements of domains of myosin and actin, or their regions, in actomyosin complex, as well as the flexibility of interdomain and actin-myosin binding, play apparently an important role in the mechanisms of muscle contraction [18].

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