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Influence of Mg^{2+} and Ca^{2+} bound to 1,5-IAEDANS-labeled phosphorylated and dephosphorylated heavy meromyosin complexed with F-actin on polarized fluorescence of the fluorophore

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Dephosphorylated and phosphorylated heavy meromyosin, fluorescently labeled with 1,5-IAEDANS attached at the SH_1 group, was introduced into myosin-free ghost fibres and the polarized fluorescence of the bound label was measured. The results depended on whether the divalent cation binding sites on heavy meromyosin were saturated with Mg^{2+} or Ca^{2+}. The calculated angles of absorption and emission dipoles and the amount of random fluorophores were significantly changed, indicating that the random mobility and orientation of the fluorophores of phosphorylated and dephosphorylated heavy meromyosin heads complexed with F-actin in the ghost fibre depend on saturation of heavy meromyosin with Ca^{2+} or Mg^{2+}. The presence of bound Ca^{2+} has an opposite effect on the polarized fluorescence of phosphorylated and dephosphorylated 1,5-IAEDANS-heavy meromyosin.

Heavy meromyosin; Polarized fluorescence; Ghost fiber; Ca^{2+} regulation

1. INTRODUCTION

The problem of structural changes in the cross-bridges during cyclic interaction with F-actin in the course of ATP hydrolysis still remains controversial [1–5]. A series of investigations centering around measurements of polarized fluorescence emitted by both intrinsic (tryptophan [6–9]) and extrinsic (5-(iodoacetamidonaphthalene)-1-sulfonic acid [10–12] and 1-N^6-ethenoadenosine-5′-diphosphate [4,9,12]) fluorophores have shown that the alteration in the orientation of the myosin heads and actin monomers can be distinguished by such measurements. It was found that the binding of phosphorylated heavy meromyosin to F-actin in ghost fibres at high Ca^{2+} concentration increases the anisotropy of intrinsic tryptophan fluorescence of F-actin, whereas the binding of phosphorylated heavy meromyosin to F-actin at low Ca^{2+} concentration decreases it. Binding of dephosphorylated heavy meromyosin causes opposite effects [13,14]. Similar results were obtained when the tropomyosin-troponin complex was rebound to F-actin in the ghost fibre [15]. In the present study, the changes in polarized fluorescence of 1,5-IAEDANS attached to phosphorylated and dephosphorylated heavy meromyosin complexed with F-actin in ghost fibre were investigated under conditions where the divalent cation binding sites of heavy meromyosin were saturated with Ca^{2+} or Mg^{2+}. A preliminary report of some of these

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results has appeared at the 15th European Conference on Muscle and Motility [16].

2. MATERIALS AND METHODS

Phosphorylated and dephosphorylated heavy meromyosin was obtained as described by Stepkowski et al. [17] and the myosin-free ghost fibres as described by Borovikov et al. [8]. Heavy meromyosin was treated with 1,5-IAEDANS in conditions described by Borejdo and Putnam [10]. An about 2.5-fold increase of Ca^{2+}-ATPase activity of 1,5-IAEDANS modified heavy meromyosin as compared with the activity of unmodified heavy meromyosin was observed, indicating the attachment of 1,5-IAEDANS to the SH_1 group of heavy meromyosin. Ca^{2+}-ATPase activity of both modified and unmodified heavy meromyosin was determined in conditions described by Margossian and Lowey [18]. Heavy meromyosin preparations were examined electrophoretically. SDS electrophoresis [19] and electrophoresis in 8 M urea [20] were performed, and then the density of the electrophoretic pattern was measured. The amount of phosphorylated heavy meromyosin regulatory light chains was about 95–100% in phosphorylated and less than 1% in dephosphorylated heavy meromyosin preparations used in these studies. The SDS electrophoretic pattern of 1,5-IAEDANS-modified heavy meromyosin enabled additional control showing that the fluorophore was attached to the heavy chain of heavy meromyosin only. 1,5-IAEDANS-modified heavy meromyosin was incubated in darkness for about 12 h at 0°C with the ghost fibre in the presence of 20 mM Tris-acetate, pH 7.0, 10 mM KCl, 1 mM $MgCl_2$, 3 mM NaN_3 and 0.1 mM $CaCl_2$ or 1 mM EGTA for saturation of heavy meromyosin divalent ion binding sites with Ca^{2+} or Mg^{2+}, respectively. The concentration of 1,5-IAEDANS-modified heavy meromyosin was 3 mg/ml. The unbound heavy meromyosin was then removed by washing the fibre with 20 mM Tris-acetate, pH 7.0, 10 mM KCl and 1 mM $MgCl_2$. The fluorescence of 1,5-IAEDANS was excited with polarized light at 365 ± 5 nm and registered at 480–600 nm. Four intensities of polarized fluorescence were measured in parallel ($_{\parallel}I_{\parallel}$, $_{\parallel}I_{\perp}$) and perpendicular ($_{\perp}I_{\perp}$, $_{\perp}I_{\parallel}$) orientation of the fibre axis to the polarization plane of the exciting light. I denotes intensities of four components of polarized fluorescence. The direction of polarization of the exciting and of the emitted light relative to the fibre axis is indicated on the left and on the right side of I, respectively. The degree of fluorescence polarization $P_{\perp}$ and $P_{\parallel}$ was determined from the equation:

$$P_{\perp} = ({}_{\perp}I_{\perp} - {}_{\perp}I_{\parallel})/({}_{\perp}I_{\perp} + {}_{\perp}I_{\parallel}),$$

$$P'_{\parallel} = ({}_{\parallel}I_{\parallel} - {}_{\parallel}I_{\perp})/({}_{\parallel}I_{\parallel} + {}_{\parallel}I_{\perp}).$$

The angle of absorption (ϕ_A) and emission (ϕ_E) dipoles of the fluorophore relative to the F-actin axis and the number of random fluorophores (N) were obtained by computer simulation. For calculation of ϕ_A, ϕ_E and N the previously obtained values of $\sin^2\theta$ [14] characterizing the flexibility in thin filaments were used. The theoretical basis for the calculations is given in [10–12].

3. RESULTS AND DISCUSSION

The experiments were performed on ghost fibres in which F-actin was the major protein constituent

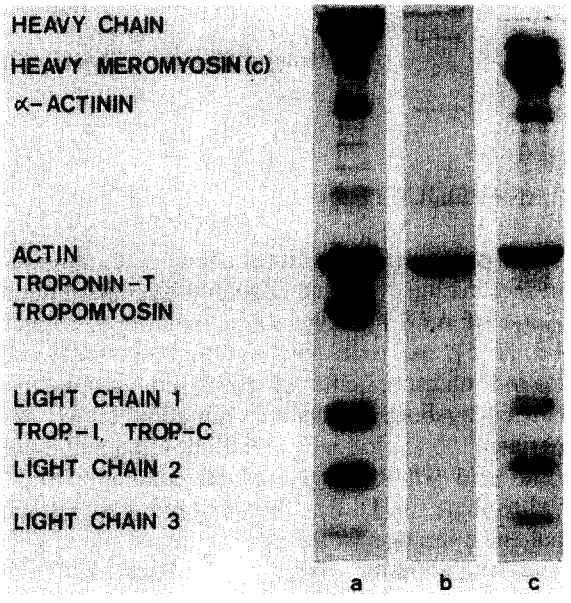

Fig.1. SDS-electrophoretic patterns of glycerinated muscle fibres (from psoas major) (a), ghost fibres (b), and ghost fibres decorated with 1,5-IAEDANS-modified heavy meromyosin (c). SDS-electrophoresis was performed according to the method of Laemmli [19].

(80% of total protein). The F-actin in the fibre was decorated with 1,5-IAEDANS-heavy meromyosin. The amount of the bound heavy meromyosin, calculated on the basis of densitometry of the SDS-polyacrylamide gels (fig.1), was about 0.3 mol per mol of actin monomer, showing saturation of actin monomers with heavy meromyosin. No difference was found between the amounts of bound phosphorylated and dephosphorylated heavy meromyosin depending on the presence of either 1 mM EGTA (low Ca^{2+} concentration) or 0.1 mM $CaCl_2$ (high Ca^{2+} concentration) in the medium (20 mM Tris-acetate, pH 7.0, 10 mM KCl, 1 mM $MgCl_2$ and 3 mg/ml of 1,5-IAEDANS-modified heavy meromyosin). In view of earlier studies [21–23] one may expect that in the presence of 1 mM EGTA and 1 mM $MgCl_2$ the divalent ion binding sites of heavy meromyosin were saturated with Mg^{2+}, whereas in the presence of 1 mM Mg^{2+} and 0.1 mM $CaCl_2$ they were saturated with Ca^{2+}. The degree of fluorescence polarization $P_\perp$ and $P_\parallel$ of 1,5-IAEDANS attached to phosphorylated or dephosphorylated heavy meromyosins complexed with F-actin in ghost fibres, the calculated angle of absorption (ϕ_A) and emission (ϕ_E) dipoles and the number of random fluorophores (N) are shown in table 1.

As seen, the values of all parameters are dependent both on the phosphorylation of the heavy meromyosin regulatory light chain and on the kind of divalent cation (Ca^{2+} or Mg^{2+}) bound to heavy meromyosin before forming the rigor bonds with F-actin.

When 1,5-IAEDANS-modified dephosphorylated heavy meromyosin containing bound Ca^{2+} was complexed with F-actin in the ghost fibre, the degree of fluorescence polarization P was almost 7-times higher than that of the fluorophore in heavy meromyosin containing bound Mg^{2+}. When phosphorylated heavy meromyosin was complexed with F-actin in the ghost fibre, the values of $P_\perp$ were not sensitive to the kind of cation bound. The values of $P_\parallel$ were higher than those of $P_\perp$. However, the differences in $P_\parallel$ dependent on both phosphorylation of heavy meromyosin and on the kind of the bound divalent cation were small. Moreover, in contrast to $P_\perp$, a significant difference depending on the bound cation was observed only when the heavy meromyosin regulatory light chains were in the phosphorylated form. As shown previously, the flexibility of F-actin filaments in ghost fibres characterized by the values of $\sin^2\theta$ (where θ is the angle between the filament axis and the fibre axis) is different in the

Table 1

Fluorescence polarization of 1,5-IAEDANS-modified phosphorylated and dephosphorylated heavy meromyosin bound to F-actin in a ghost fibre in the presence of 1 mM EGTA or 0.1 mM $CaCl_2$ and calculated angles of absorption (ϕ_A) and emission (ϕ_E) dipoles relative to the fibre axis

Additions		$P_\perp$	$P_\parallel$	ϕ_A	ϕ_E	N
1,5-IAEDANS dephosphorylated HMM bound to F-actin in ghost fibre	1 mM EGTA	−0.006 ± 0.003 (85)	0.416 ± 0.007 (85)	48.0 ± 0.2	42.6 ± 0.1	0.38 ± 0.02
	0.1 mM $CaCl_2$	−0.041 ± 0.004 (80)	0.424 ± 0.004 (80)	48.4 ± 0.1	43.1 ± 0.1	0.27 ± 0.01
1,5-IAEDANS phosphorylated HMM bound to F-actin in ghost fibre	1 mM EGTA	−0.060 ± 0.003 (68)	0.385 ± 0.004 (68)	58.5 ± 0.4	46.3 ± 0.1	0.12 ± 0.01
	0.1 mM $CaCl_2$	−0.056 ± 0.002 (70)	0.370 ± 0.005 (70)	51.4 ± 0.4	45.9 ± 0.1	0.16 ± 0.01

The calculation was performed as described in [9,11]. The values of $\sin^2\theta$ characterizing the flexibility of thin filaments calculated on the basis of the experimental data in [14] were 0.053, 0.068 and 0.060, 0.060 for F-actin complexed with dephosphorylated and phosphorylated heavy meromyosin bound in the presence of 1 mM EGTA or 0.1 mM $CaCl_2$, respectively. The number of measurements are given in parentheses. For details see section 2. HMM, heavy meromyosin

binding of phosphorylated and dephosphorylated heavy meromyosin. Moreover, the character of those changes depended on the saturation of heavy meromyosin divalent ion binding sites by Mg^{2+} or Ca^{2+} ions [14]. Therefore, the respective values of $\sin^2\theta$ [14] were used for calculation of the angles of absorption ϕ_A and emission ϕ_E dipoles and random fluorophores (N) of the dye. The calculated values of ϕ_A, ϕ_E and N were different for 1,5-IAEDANS-heavy meromyosin complexed with F-actin in ghost fibres in the presence of 1 mM EGTA or 0.1 mM $CaCl_2$. Decrease of values of ϕ_A and ϕ_E and increase of N were observed when phosphorylated 1,5-IAEDANS-heavy meromyosin with bound Ca^{2+} was complexed with F-actin in the ghost fibre, while in the case of dephosphorylated 1,5-IAEDANS-heavy meromyosin the changes of values of ϕ_A, ϕ_E and N were inverted (table 1). Thus, the structural organization of phosphorylated and dephosphorylated heavy meromyosin heads in rigor binding seems to be different, depending on whether the divalent ion binding sites were saturated with Mg^{2+} or Ca^{2+}, this showing a possible alteration in orientation and in the random movement of heavy meromyosin heads. In contrast to the dephosphorylated heavy meromyosin, the orientation of phosphorylated heavy meromyosin heads rather than randomisation seems to be influenced by the kind of bound divalent cation.

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REFERENCES

[1] Huxley, H.E., Simmons, R.M., Faruqi, A.R., Kress, M., Bordas, J. and Koch, M.H.J. (1983) J. Mol. Biol. 169, 469–506.
[2] Cooke, R., Crowder, M.S. and Thomas, D.D. (1982) Nature 300, 776–778.
[3] Yanagida, T. (1980) in: Muscle Contraction: Its Regulatory Mechanism (Ebashi, S. et al. eds) pp.173–180, Japanese Scientific Press, Springer, Tokyo/Berlin.
[4] Yanagida, T. (1981) J. Mol. Biol. 146, 539–560.
[5] Yanagida, T. (1984) in: Cross-Bridge Mechanisms in Muscle Contraction (Pollack, G.H. and Sugi, H. eds) pp.397–411, Plenum, New York.
[6] Aronson, J.F. and Morales, M.F. (1969) Biochemistry 8, 4517–4522.
[7] Dos Remedios, C.G., Millikan, R.G.C. and Morales, M.F. (1972) J. Gen. Physiol. 59, 103–120.
[8] Borovikov, Yu.S., Levitsky, D.I., Kirillina, V.P. and Poglazov, B.F. (1982) Eur. J. Biochem. 125, 343–347.
[9] Treager, R.T. and Mendelson, R.A. (1975) Biophys. J. 15, 455–467.
[10] Borejdo, J. and Putnam, S. (1977) Biochim. Biophys. Acta 459, 578–595.
[11] Wilson, M.G. and Mendelson, R.A. (1983) J. Muscle Res. Cell Motil. 4, 671–693.
[12] Yanagida, T. and Oosawa, F. (1978) J. Mol. Biol. 126, 507–524.
[13] Borovikov, Yu.S., Kakol, I., Szczęsna, D., Kirillina, V.P. and Levitsky, D.I. (1986) Biochimia 51, 691–694.
[14] Kakol, I., Borovikov, Yu.S., Szczęsna, D., Kirillina, V.P. and Levitsky, D.I. (1987) Biochim. Biophys. Acta 913, 1–9.
[15] Szczęsna, D., Borovikov, Yu.S., Lebedeva, N.N. and Kakol, I. (1987) Experientia 43, 194–196.
[16] Borovikov, Yu.S., Wrotek, M., Aksenova, N.N., Lebedeva, N.N. and Kakol, I. (1987) J. Muscle Res. Cell Mot. 8, 64.
[17] Stępkowski, D., Szczęsna, D., Wrotek, M. and Kakol, I. (1985) Biochim. Biophys. Acta 830, 321–329.
[18] Margossian, S.S. and Lowey, S. (1982) Methods Enzymol. 85, 55–71.
[19] Laemmli, U.K. (1970) Nature 277, 680–685.
[20] Perrie, W.T. and Perry, S.V. (1970) Biochem. J. 119, 31–38.
[21] Morimoto, K. and Harrington, W.F. (1974) J. Mol. Biol. 88, 693–709.
[22] Holroyed, M.J., Potter, J.D. and Solara, K.J. (1979) J. Biol. Chem. 254, 6478–6482.
[23] Bagshaw, C.R. and Kendrick-Jones (1979) J. Mol. Biol. 130, 317–336.