and doublet band that has a much stronger radioactive intensity with a mol.wt of about 70,500 and 58,000.

Apart from the protein of mol.wt of about 58,000, this pattern appears to consist of a number of bands of the human MAC even though the molecular weights of each band are higher than the published mol.wt of isolated high purified human MAC components (C_{5b}, 173,000; C_7, 110,000; C_6, 99,000; $C_{8\alpha/\gamma}$, 93,000; C_9, 76,000; $C_{8\beta}$, 70,000)[3,4].

It is interesting to note the *Xenopus* high molecular weight band which did not penetrate in 5–10% gradient gel (arrow in fig. 2, track D) and was resistant to reduction by 2-mercapthoethanol and boiling in SDS (data non shown). It may represent, as in man[18], the polymerized C_9 (poly C_9) responsible for the oligomeric structures observed in the lyzed membranes at the electron microscope.

Structural information on highly purified *Xenopus* MAC, extracted from complement lyzed cells and from the fluid-phase, is needed to establish a more precise relationship with human MAC.

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2 To whom correspondence should be sent. Present address: Istituto di Zoologia, via Archirafi 18 I-90138 Palermo, Italy.

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Effect of phosphorylation of myosin light chains on interaction of heavy meromyosin with regulated F-actin in ghost fibers

D. Szczesna, Yu. S. Borovikov*, N. N. Lebedeva* and I. Kakol

*Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, 02093 Warszawa (Poland) and *Institute of Cytology, Academy of Sciences of the USSR, Leningrad 194064 (USSR), 5 May 1985*

Summary. The binding of phosphorylated heavy meromyosin to regulated F-actin in ghost fibers at high Ca^{2+} concentration increases, and at low Ca^{2+} concentration decreases, the anisotropy of intrinsic tryptophan fluorescence of F-actin. The effect is opposite to the effect of the binding of dephosphorylated heavy meromyosin.

Key words. Phosphorylated heavy meromyosin; regulated F-actin; ghost muscle fiber.

The binding of soluble fragments of myosin, e.g. heavy meromyosin (HMM) and subfragment 1 (S-1) induces significant changes of F-actin conformation[1–7]. The Ca^{2+}-dependent changes of polarized fluorescence of intrinsic tryptophan residues and F-actin-bound 1:N^6-ethenoadenosine 5-diphosphate induced by the binding of heavy meromyosin and subfragment 1 were found to be related to the presence of intact 18-kDa light chains[8]. Recently, the effect of phosphorylation of 18-kDa light chains on the changes of polarized fluorescence of intrinsic tryptophan residues and unregulated F-actin-bound rhodamin-phalloidin in ghost fibers, induced by the binding of heavy meromyosin, was observed to be dependent on the concentration of free calcium ions[9–11].

In the present study changes of polarization of intrinsic tryptophan fluorescence of ghost-fibers F-actin containing the rebound troponin tropomyosin complex, induced by the binding of phosphorylated and dephosphorylated heavy meromyosin at low and high concentrations of calcium ions, were investigated. The anisotropy of tryptophan fluorescence was used as an indicator of F-actin conformational changes induced by the binding of phosphorylated and dephosphorylated heavy meromyosins.

Materials and methods. The study was carried out on myosin-free ghost single fibers of skeletal muscle of rabbit, containing more than 80% of actin (for determination of F-actin in ghost fibers see below). Phosphorylated and dephosphorylated HMM containing 98 ± 2% and 0–2% of total 18-kDa light chains in phosphorylated form, respectively, were prepared as described by Stepkowski et al.[12]. Phosphorylated and dephosphorylated myosin (10 mg/ml) prepared according to Stepkowski et al.[13] was dissolved in 0.5 M KCl, 20 mM phosphate buffer pH 7.0, 2 mM $CaCl_2$. Digestion was carried out at 22–24 °C for 4 min in the presence of trypsin-free α-chymotrypsin with enzyme substrate ratio 1:260. Phenylmethylsulphonyl fluoride (8.7 mg/ml in ethanol) was added to the final concentration of 0.36 mM to stop the reaction. The solution was dialyzed against 20 mM phosphate buffer pH 7.0, to precipitate undigested myosin and all proteolytic fragments insoluble at low ionic strength. The precipitate was removed by centrifugation. HMM was purified by precipitation with ammonium sulphate or by ultracentrifugation of heavy meromyosin complexed with actin, followed by dissociation of the acto-heavy meromyosin complex in the presence of 5 mM magnesium chloride and 5 mM pyrophosphate, and removal of actin by repeated ultracentrifugation[14].

HMM was checked by sodium dodecyl sulphate gel electrophoresis[15] (fig. 1d, e), whereas ghost fibers were checked on minislab gradient gels (5–20%) according to the method of Matsudaira and Burgess[16] (fig. 1a, b, c). The amount of actin in ghost fibers was calculated from densitometric measurements of electrophoretic patterns of parallel samples of ghost fibers used for sodium dodecyl sulphate electrophoresis in 6–8% gels[5,17]. The amount of phosphorylated 18-kDa light chains was calculated from densitometric data of the electrophoretic patterns of phosphory-

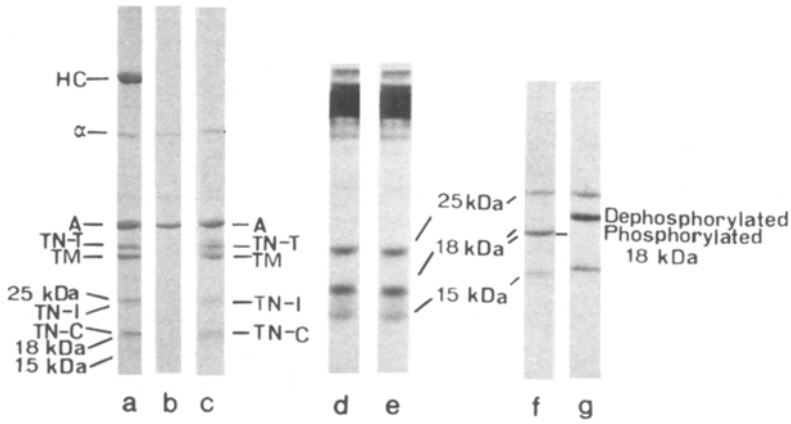

Figure 1. Electrophoretic patterns of glycerinated muscle fiber (a), ghost fiber (b), ghost fiber decorated with troponin-tropomyosin complex (c), phosphorylated (d, f) and dephosphorylated (e, g) heavy meromyosins, SDS gel (a–e), 8 M urea gel (f, g). HC, myosin heavy chains; 25 kDa, 18 kDa, 15 kDa myosin light chains; α, α-actinin; A, actin; TM, tropomyosin; TN-T, TN-I, TN-C, troponin (for details see methods).

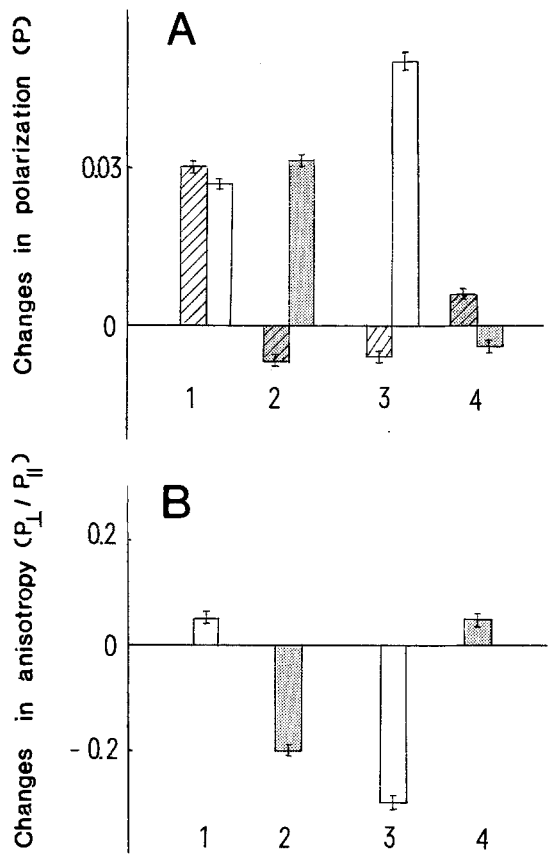

Figure 2. Effect of dephosphorylated (1, 2) and phosphorylated (3, 4) heavy meromyosins binding to regulated F-actin at low (1, 3) and high (2, 4) calcium-ion concentrations on the degree of polarization (A) and anisotropy (B) of intrinsic tryptophan fluorescence of thost fibers. In 2A, dashed columns correspond to $P_\perp$ and open to $P_\parallel$.

lated and dephosphorylated HMM obtained in gel electrophoresis in the presence of 8 M urea[18] (fig. 1f, g).

Troponin and tropomyosin were obtained by a modification of the Greaser and Gergely method[19]. The tropomyosin-troponin complex was incorporated into thin filaments as described by Borovikov and Gusev[17] (fig. 1c).

Phosphorylated or dephosphorylated HMM were bound to regulated F-actin by incubation of ghost fibers with 1.5–3 mg/ml of HMM dissolved in 1 mM $MgCl_2$, 20 mM Tris-acetate buffer pH 7.0 and 1 mM EGTA or 0.1 mM $CaCl_2$ (low and high calcium-ion concentrations, respectively).

The degree of polarization of intrinsic tryptophan fluorescence (P) of F-actin in ghost fiber was measured with a microspectrophotometer[17]. P was registered at fiber orientations both parallel ($P_\parallel$) and perpendicular ($P_\perp$) to the plane of exciting light. The anisotropy index of fluorescence was determined as $A = P_\perp/P_\parallel$. Changes in the degree of polarization were estimated as the difference between the degree of polarization before and after HMM binding to regulated F-actin in ghost fibers at low and high calcium-ion concentrations.

Results and discussion. The value of $P_\perp$ for ghost fiber containing regulated F-actin is higher than $P_\parallel$, hence the anisotropy $A = P_\perp/P_\parallel$ exceeds unity[17]. Decoration of regulated F-actin with phosphorylated and dephosphorylated heavy meromyosins induces Ca^{2+}-dependent changes of tryptophan fluorescence anisotropy of F-actin in myosin-free single ghost fibers. At low concentrations of calcium ions, phosphorylated HMM decreases the anisotropy of tryptophan fluorescence, and at high concentrations of calcium ions it increases it (fig. 2). Conversely, the binding of dephosphorylated heavy meromyosin to regulated F-actin induces a decrease of anisotropy in the presence of Ca^{2+} and its increase in the absence of Ca^{2+} (fig. 2). It has been shown that the anisotropy of tryptophan fluorescence of myosin-free ghost fibers is due to the fluorscence of F-actin tryptophan residues predominantly oriented perpendicularly to the axis of actin filaments. On the other hand, tryptophan residues of troponin and HMM are practically arranged isotropically, tropomyosin being free of tryptophan[17,20,21]. Therefore, the observed changes of anisotrophy of intrinsic tryptophan fluorescence reflect the conformational changes of F-actin induced by the binding of heavy meromyosin. Thus, the dependence of these conformational changes on Ca^{2+} concentration is different for the phosphorylated and dephosphorylated forms of heavy meromyosin (opposite direction of changes has been found). As found previously[9–11], in myosin and tropomyosin-troponin complex-free ghost fibers the anisotropy of intrinsic tryptophan fluorescence of F-actin also decreases, when phosphorylated HMM is bound at low Ca^{2+}-concentrations, and when dephosphorylated HMM is bound at high Ca^{2+} concentrations. Conversely, this anisotropy increases when the binding of phosphorylated and dephosphorylated HMM takes place at high and low Ca^{2+} concentrations, respectively. Since the conformational changes observed in the present studies are analogous with those obtained on ghost fibers free from the troponin-tropomyosin complex[9–11], it seems possible that the latter complex does not significantly affect the character of conformational changes of F-actin, induced by the binding of heavy meromyosin to ghost fibers. Consequently, it can be concluded that the modulation of myo-

sin-actin interaction depends both on the phosphorylation of myosin heads and the binding of the Ca^{2+} by myosin.

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Retinoic acid enhances the proliferation of smooth muscle cells

M. M. Peclo and O. Yu. Printseva*

USSR Cardiology Research Center, 3rd Cherepkovskaya 15 A, Moscow 121552 (USSR), 9 December 1985

Summary. Retinoic acid (RA, $10^{-5} - 10^{-7}$ M) is shown to enhance the proliferation of cultured rat aortic smooth muscle cells (SMC). This effect is not connected with a synergistic action of RA together with serum mitogens. Moreover, the expression of L1, a surface antigen specific for modulated SMC entering the cell cycle, is amplified by RA treatment.
Key words. Smooth muscle cells; proliferation; surface antigen; retinoic acid.

The loss of the contractile phenotype and of the proliferation capability of smooth muscle cells (SMC) are considered to be early events in the development of some vascular diseases[1,2]. Screening of drugs able to influence these processes is therefore of considerable importance. On the other hand, derivatives of vitamin A, in particular retinoic acid (RA), have been reported to inhibit the proliferation of normal fibroblasts and of several lines of malignant cells, as well as preventing, in some cases, neoplastic transformation of cells[3,4].

With this in mind, we applied retinoic acid to long-term cultured SMC originally isolated from rat aortic tunica media. As shown previously[5], such cultures retain some characteristics of vascular SMC. In particular, the most remarkable property of the cells appear to be the continual synthesis of the vascular smooth muscle type of actin. The morphology exhibits a series of features that have been considered to be typical of SMC (e.g. typical surface invagination, numerous 'dark bodies'). The results we have obtained show that the proliferation of SMC in vitro is enhanced by RA treatment. Moreover, RA treatment leads to an increase in the expression of L1 antigen which, as shown earlier[6,7], is a specific surface antigen of SMC entering the cell cycle.

Material and methods. Cell cultures. Cell cultures were obtained from the aortic tunica media of adult Wistar rats (250 g b. wt) and from the skin of 17-day-old embryos as described earlier[5]. SMC and skin embryo fibroblasts (SEF) were grown in Dulbecco's modification of Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS, Flow), 4 mM glutamine and 100 µg/ml kanamycine (Gibco), in an atmosphere containing 5% CO_2. Subcultures of SMC (3rd–30th passages) and of SEF (3rd passage) were used for the two following types of experiments:

1) Cells were seeded in 24-well trays (Falcon) at a density of 2.5×10^4 cm^{-2}. When subconfluency was reached, the growth medium was replaced by serum-free DMEM medium (SF medium) and 24 h later by growth medium containing $10^{-5} - 10^{-7}$ M RA ('all trans', type XX, Sigma) from a stock solution made in absolute ethanol. Wells with growth medium and 0.1% of etha-

Table 1. RA effect on ^{14}CT incorporation in SMC and SEF in the presence of 10% FCS. Results presented in percentage: ^{14}CT incorporation (200.000–500.000 dpm during 1–3 days of experiment) in control wells (without RA treatment) was taken as 100%. The cells were prepared according to protocol 1. The level of ^{14}CT non-specific absorption was about 100 dpm. Each value is the mean of three separate determinations

RA treatment	Duration of treatment, days 1	2	3
SMC			
10^{-5}M	157 ± 13^b	166 ± 6^a	565 ± 25^a
10^{-6}M	159 ± 2^a	146 ± 9^b	181 ± 100 (ns)
10^{-7}M	129 ± 6^c	101 ± 10 (ns)	67 ± 45 (ns)
SEF			
10^{-5}M	74 ± 3^b	58 ± 1^a	56 ± 3^a
10^{-6}M	84 ± 5^a	61 ± 4^a	61 ± 3^a
10^{-7}M	86 ± 6^c	72 ± 4^a	68 ± 3^a

^{a,b,c} Significant differences according to Student's t-test with $p < 0.001$, 0.01, 0.05 respectively; (ns) not significant.

Table 2. RA effect on ^{14}CT incorporation in SMC and SF medium (percentage). ^{14}CT incorporation (50.000–75.000 dpm during 1-4 days of experiment) in control wells, without RA treatment, was taken as 100%. The cells were prepared using protocol II. The level of ^{14}CT non-specific absorption was about 100 dpm. Each value is the mean of three separate determinations. All differences were significant using Student's t-test with $p < 0.001$.

RA treatment	Duration of treatment, days 1	2	3	4
10^{-5}M	116 ± 1	126 ± 2	219 ± 1	179 ± 3