The Detection of Denervation-Induced Structural Changes in Actin by Phalloidin

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It has been shown earlier, that actin from denervated muscles is not readily polymerized and does not actively combine with heavy meromyosin (Kalamkarova et al. 1976; Moskalenko and Strankfeld 1980; Kofman et al. 1981). It has been suggested that these changes are due to local changes in the actin structure taking place at the sites of actin-actin bonds in the F-actin structure (Borovikov et al. 1983). Since phalloidin which binds specifically to F-actin affects these bonds (Dancker et al. 1975), it was of interest to use this reagent to study changes in the actin structure occurring during denervation.

Single ghost fibres of normal and denervated m . soleus of rabbit were used. Denervation was performed as described earlier (Kalamkarova et al. 1976). Intact and denervated rabbit muscles (after 30 days) were taken from 5 animals. Single ghost fibres were prepared as described in Borovikov et al. (1983). The fibres were free of myosin, troponin and tropomyosin and contained more than 80% of F-actin (Yanagida and Oosawa 1978; Borovikov et al. 1983). Some of the ghost fibres were treated with 40 μg/ml mushroom toxin phalloidin for 20 min (Bukatina and Morozov 1979). The unbound toxin was removed from the fibre by washing with a standard solution containing (in mmol . l^{-1}): 100 KCl; 1 $MgCl_2$; 4 EGTA; 67 phosphate buffer; pH 7.0. Measurements were made in both the standard solution and the standard solution supplemented with 5 mmol . l^{-1} ATP. The degrees of tryptophan fluorescence (P) polarization and birefringence were measured by a microphotometer (Ioffe et al. 1974). P was recorded at fibre orientations both parallel ($P_{\parallel}$) and perpendicular ($P_{\perp}$) to the exciting light plane. The data on polarized fluorescence were analyzed as suggested previously (Rozanov et al. 1971; Yanagida and Oosawa 1978). The birefringence of fibres was evaluated as described by Yanagida and Oosawa (1978).

The value of $P_{\perp}$ for ghost fibres was higher than that of $P_{\parallel}$ and the ratio $P_{\perp}/P_{\parallel} > 1$ (Table 1). The attachment of both phalloidin and ATP to the sites of localization of slow-reacting thiol groups of F-actin monomers (Blackholm and Faulstich 1981) in normal ghost muscle fibres results in an increase in the anisotropy of tryptophan fluorescence, since the anisotropy index ($P_{\perp}/P_{\parallel}$) increases

Table 1. Polarization of tryptophan fluorescence of myosin-free ghost fibres

Muscle fibres	$P_\perp$	$P_\parallel$	$P_\perp/P_\parallel$
Normal	0.207 ± 0.002	0.195 ± 0.001	1.06 ± 0.02
Denervated (30 days)	0.219 ± 0.003	0.197 ± 0.002	1.11 ± 0.01

Measurements were carried out using a standard solution. No less than 8 fibres taken from muscles of 5 rabbits were taken for a single experiment.

(Fig. 1). In similar experiments, the anisotropy of tryptophan fluorescence of denervated fibre decreased ($P_\perp/P_\parallel$ diminished) (Fig. 1).

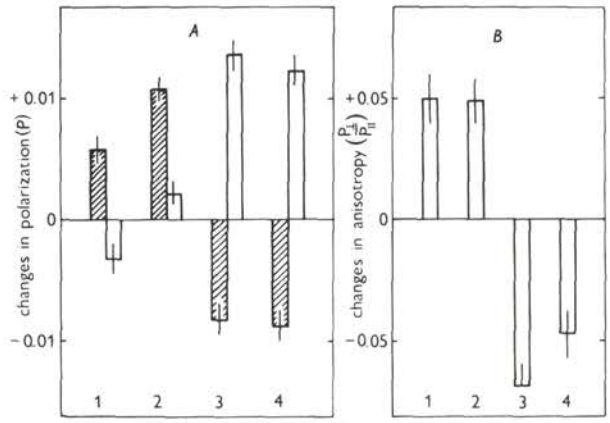

Fig. 1. The effect of ATP (1—3) and the toxin phalloidin (2—4) on the degree of polarization (*A*) and anisotropy (*B*) of tryptophan fluorescence of ghost muscle fibres free of myosin, troponin and tropomyosin, obtained from normal (1—2) and denervated (3—4) rabbit muscles. Average values and standard deviation from 50 measurements on 5 different fibres are given. In 1*A*, dashed columns correspond to $P_\perp$ and the open ones to $P_\parallel$.

As shown earlier, following the removal of myosin, troponin and tropomyosin, the absorption and the emittance oscillators of tryptophan residues of F-actin are preferably oriented perpendicular to the long axis of the fibre, i.e. anisotropically (Yanagida and Oosawa 1978; Borovikov and Chernogriadskaya 1979). On the other hand, the tryptophan residues of all other proteins are isotropic and their contribution to the total emission of fibres is significantly lower than that of the F-actin fluorescence (Yanagida et al. 1974; Borovikov and Chernogriadskaya 1979); thus $P_\perp > P_\parallel$ and $P_\perp/P_\parallel$ exceed unity. $P_\perp$, $P_\parallel$ and $P_\perp/P_\parallel$ depend on optical properties of tryptophan residues of F-actin, which are localized in a hydrophobic environment (Vedenkina et al. 1972; Elzinga and Collins 1973). Changes in $P_\perp$, $P_\parallel$ and $P_\perp/P_\parallel$ are therefore sensitive to conformational changes in F-actin (Borovikov et al. 1982, 1983; Borovikov and Chernogriadskaya 1979).

It can be assumed that both phalloidin and ATP induce conformational changes in F-actin of normal and denervated muscles. Using polarized fluorescence analysis (Rozanov et al. 1971; Yanagida and Oosawa 1978) and a mathematical model of the data obtained, it could be assumed that the flexibility of thin filaments decreased in all experiments. Due to the effect of phalloidin and ATP, the birefringenge of normal and denervated fibres increased by $8 \pm 2\%$ and $12 \pm 3\%$, respectively ($N = 15$); this could also be due to a decrease in the flexibility of thin filaments.

From the analysis of our data it also follows that similar changes in the flexibility of thin filaments of normal and denervated fibres are accompanied by different changes in the angles of absorption and emission dipoles of tryptophan residues of F-actin. These angles decrease in denervated muscles, whereas they increase in normal ones. F-actin of denervated and normal muscles hence slows different conformational changes in response to the same reagents. This, in turn, points to the fact that F-actin of normal apparently differs in a structure from that of denervated muscles. Since both phalloidin and ATP bind to slow-reacting thiol groups localized close to the N-terminal peptide (Blackholm and Faulstich 1981) involved in actin polymerization (Elzinga and Collins 1973) and they affect the actin-actin bonds in the F-actin structure (Dancker et al. 1975), it seems possible that at least these peptide regions change during denervation. This assumption is in accordance with the recently obtained data (Ibragimov et al. 1981; Borovikov et al. 1983).

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References

Blackholm H., Faulstich H. (1981): Protection of one of the two reactive thiol groups in F-actin by ATP and phalloidin. Bioch. Biophys. Res. Comm. **103,** 125—130

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

Borovikov Yu. S., Levitskii D. I., Kirillina V. P., Poglazov B. 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. J. Biochem. **125**, 343—347

Borovikov Yu. S., Moskalenko I. E., Kirillina V. P., Strankfeld I. G. (1983): Study of the state of F-actin in denervated muscle fibre by polarized UV fluorescence microscopy. Gen. Physiol. Biophys. **2**, 1—11

Bukatina A. E., Morozov V. N. (1979): Effect of phalloidin on contractile properties of glycerinated skeletal muscle of the rabbit. Biofizika **26**, 437—438 (in Russian)

Dancker P., Löw I., Hasselbach W., Wieland T. H. (1975): Interaction of F-actin with phalloidin: polymerization and stabilization of F-actin. Biochim. Biophys. Acta **400**, 407—414

Elzinga M., Collins J. H. (1973): The amino acid sequence of rabbit skeletal muscle actin. Cold Spring Harbor Symp. Quant. Biol. **37**, 1—7

Ibragimov R. N., Moskalenko I. E., Strankfeld I. G. (1981): Changes in structural and functional properties of actin in denervated rabbit muscle. Biofizika **26**, 437—438 (in Russian)

Ioffe V. A., Borovikov Yu. S., Barskyi I. Ya., Rozanov Yu. M. (1974): A two-channel polarization microfluorimeter. Tsitologiya **16**, 112—116 (in Russian)

Kalamkarova M. B., Moskalenko I. E., Podlubnaya Z. A., Strankfeld I. G. (1976): The effect of denervation upon the functional properties of myosin and its fragments. Physiol. Bohemoslov. **25**, 543—550

Kofman E. B., Moskalenko I. E., Strankfeld I. G. (1981): Effect of denervation on the properties of actin (According to superprecipitation data). Acta Biochim. Biophys. Acad. Sci. Hung. **16**, 83—87.

Moskalenko I. E., Strankfeld I. G. (1980): Effect of denervation on actin and myosin interaction. In: Structural Basis and Regulation of Biological Motility (Ed. G. R. Ivanitskii) pp. 336—341, Nauka, Moscow (in Russian)

Rozanov Yu. M., Chernogriadskaya N. A., Barskyi I. Ya., Borovikov Yu. S., Shudel M. S. (1971): Polarized ultra-violet fluorescence of muscle fibers and some other cytologycal anizotropic objects. Tsitologiya **13**, 190—200 (in Russian)

Vedenkina N. S., Burshtein E. A., Gotberg M. I. (1972): Study on actin by method of fluorescence. In: Mechanisms of Muscle Contraction (Ed. G. M. Frank) pp. 4—9, Nauka, Moscow (in Russian)

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

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

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