The effect of phallotoxins on the structure of F-actin in myosin-free ghost muscle fibres of rabbit

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Received 3 September 1984

Using polarized UV fluorescent microscopy it has been shown that phallotoxins (phalloidin-sulfone, phalloidin-sulfoxide-B, phalloidin-sulfoxide-A and dithio-phalloidin) cause an increase in tryptophan fluorescence anisotropy of F-actin myofilaments in myosin-free ghost muscle fibres of rabbit. The results obtained are considered to be evidence of conformational changes in F-actin, induced by phallotoxins. These changes are irreversible to a significant extent, which points to a high degree of actin binding to both toxic and non-toxic phallotoxins.

F-Actin conformational change *Phallotoxin* *Ghost fiber* *Polarized UV microphotometry*

1. INTRODUCTION

The phallotoxins [1], which stabilize the structure of F-actin, are capable of changing the mechanism of Ca^{2+}-activation of muscle fibres: they lead to the disappearance of 'slow bridges', characterized by a low rate of development of delaying tension, and to the functioning of muscle at low degrees of Ca^{2+}-activation [2].

In terms of their effects on the mechanical properties of muscle, the phallotoxins form a series: Ph = PhS > PhSB ≃ DTPh > PhSA > SPh. This series correlates with the degree of stabilization of F-actin structure in solutions for all substances, except DTPh, which is similar to SPh.

To elucidate the mechanism of the effect of phallotoxins on the contractility of striated muscles, we studied their effect on tryptophan fluorescence anisotropy and birefringence of single muscle fibres, free of myosin, troponin and tropomyosin (ghost muscle fibres).

Abbreviations: Ph, phalloidin; PhS, phalloidin-sulfone; PhSB, phalloidin-sulfoxide-B; DTPh, dithio-phalloidin; PhSA, phalloidin-sulfoxide-A; SPh, seco-phalloidin

2. MATERIALS AND METHODS

The glycerinization of psoas muscle of rabbit was carried out in a solution containing 50% glycerol, 1 mM $MgCl_2$, 67 mM phosphate buffer, pH 7.0. Ghost fibres were obtained by extraction of myosin, troponin and tropomyosin from single glycerinated muscle fibres by a solution containing 800 mM KCl, 10 mM ATP, 67 mM phosphate buffer, pH 7.0 [3]. The protein composition of glycerinated fibres and ghost fibres was determined by a modified method of [4]. A separating gel of 12% acrylamide and stacking gel of 2.5% acrylamide were used. Phallotoxins were synthesized by Professor T. Wieland (Max-Planck-Institute for Medical Research, Heidelberg, FRG). Studies were carried out in standard solution containing 140 mM KCl, 50 mM imidazole, 5 mM $MgCl_2$, 5 mM EGTA, pH 7.0. Tryptophan fluorescence polarization was recorded with a microspectrophotometer [5]. Four components of polarized tryptophan fluorescence were registered: ${}_{\parallel}I_{\parallel}$, ${}_{\parallel}I_{\perp}$, ${}_{\perp}I_{\parallel}$, ${}_{\perp}I_{\perp}$, where I is the fluorescence intensity and $\parallel$ and $\perp$ refer to the direction of polarization, parallel and perpendicular to the axis of the fibre; the first

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

subscript refers to the exciting light, the second to the emitted light. The degree of polarization of fluorescence during orientation of fibres parallel ($P_{\parallel}$) and perpendicular ($P_{\perp}$) to the polarization plane of the exciting light is expressed by the ratios:

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

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

Fluorescence was excited at 303 ± 4 nm and detected at 320–380 nm.

2.1. *Experimental procedure*

The ghost fibre was glued to a glass with a quick-drying glue, put into standard solution, then ${}_{\parallel}I_{\parallel}$, ${}_{\parallel}I_{\perp}$, ${}_{\perp}I_{\parallel}$, and ${}_{\perp}I_{\perp}$ were measured and the fibre was put back into standard solution at room temperature for 20 min (the standard solution contained 40 μg/ml phallotoxin). The phallotoxin was washed out by standard solution 2–3 times and the measurements repeated. In some experiments changes in birefringence of the fibre caused by phallotoxins were recorded [6].

2.2. *Processing of the data*

Changes in degree of polarization were estimated as the difference between the degree of polarization before and after treatment. The $P_{\perp}/P_{\parallel}$ ratio served as an index of anisotropy of tryptophan fluorescence.

3. RESULTS AND DISCUSSION

According to data from SDS gel electrophoresis, the ghost fibres (fig.1) do not contain myosin, troponin or tropomyosin. They contain more than 80% of native actin [7].

As shown earlier, following the removal of myosin, troponin and tropomyosin, the absorption, and emission oscillators of tryptophan residues of F-actin are preferably oriented perpendicular to the long axis of the fibre, i.e., anisotropically [3,6]. 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 F-actin fluorescence [3]; thus $P_{\perp} > P_{\parallel}$, and $P_{\perp}/P_{\parallel}$ exceed unity. $P_{\perp}$, $P_{\parallel}$ and $P_{\perp}/P_{\perp}$ depend on the optical properties of tryptophan residues of F-actin, which are localized in a hydrophobic environment [8]. Changes in $P_{\perp}$, $P_{\parallel}$ and $P_{\perp}/P_{\parallel}$ are therefore sensitive to conformational changes in F-actin [3,7].

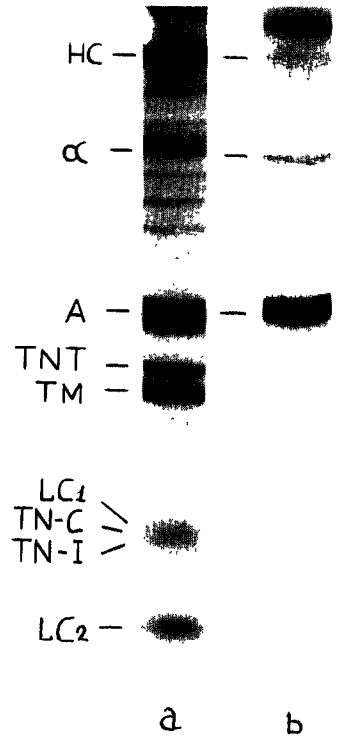

Fig.1. SDS gel electrophoresis. (a) Glycerinated muscle fibre (50 μg); (b) ghost fibre (50 μg). HC, myosin heavy chains; α, α-actinin; A, actin; TM, tropomyosin; TN-T, TN-I, TN-C, troponin; LC_1, LC_2, light chains.

Changes in polarized tryptophan fluorescence of ghost fibres induced by phallotoxins are shown in fig.2. It can be seen that after treatment with each of the phallotoxins which changes the mechanical properties of fibres [2], changes were observed in the polarization degree of tryptophan fluorescence of actin in ghost fibres, although the values of $P_{\perp}$ and $P_{\parallel}$ varied from one phallotoxin to another (fig.2A). Nevertheless, almost all phallotoxins caused an increase in tryptophan fluorescence anisotropy of F-actin (fig.2B). SPh, which did not have any effect on Ca^{2+}-sensitive parameters of contraction [2], was ineffective in producing changes in anisotropy (fig.2). As to birefringence, the measurement showed a 12 ± 3% increase caused by phalloidin (8 fibres).

Thus, all the phallotoxins, including DTPh, which change the mechanism of Ca^{2+}-activation of

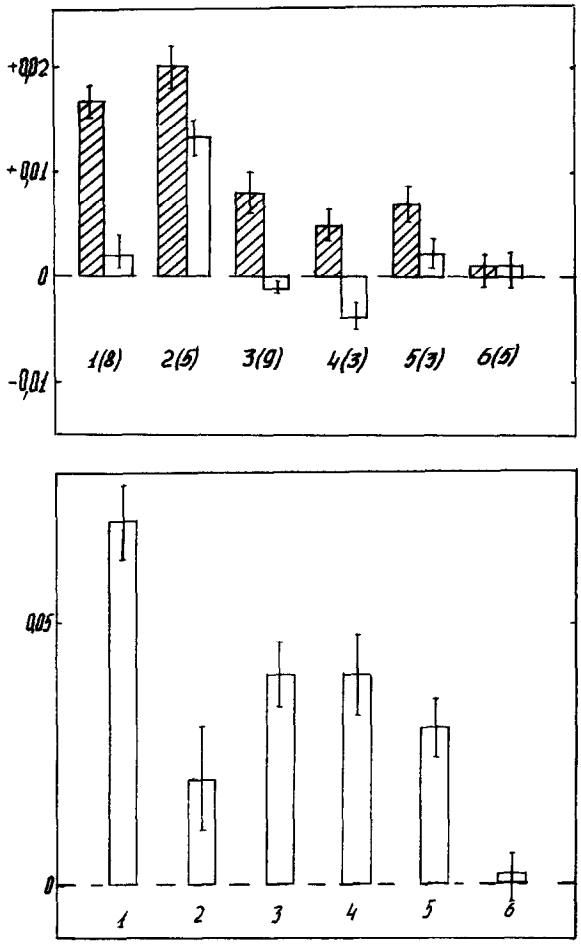

Fig.2. The effect of phallotoxins on polarization degree (A) and anisotropy (B) of tryptophan fluorescence of myosin-free ghost muscle fibres of rabbit. Changes in the above parameters produced by phallotoxins (differences in their values before and after phallotoxin treatment) are shown. 1, phalloidin; 2, phalloidin-sulfone; 3, dithio-phalloidin; 4, phalloidin-sulfoxide-A; 5, phalloidin-sulfoxide-B; 6, seco-phalloidin. In (A) shaded columns show changes in polarization degree during the orientation of fibre perpendicular ($P_\perp$) and unshaded columns parallel ($P_\parallel$) to the polarization plane of exciting light. Average values and deviation from the means are given for the number of fibres indicated in brackets.

fibres, can bind rather tightly to actin of thin filaments and change its conformation. An increase in tryptophan fluorescence anisotropy and birefringence of ghost fibres are apparently due to a decrease in thermal fluctuations in thin filaments caused by phallotoxins, which might result from fixation of F-actin structure. Therefore, changes in Ca^{2+}-sensitive parameters of concentration induced by phallotoxins might point to the importance of the lability of F-actin structure for the mechanism of muscle contraction at low levels of Ca^{2+}-activation.

ACKNOWLEDGEMENT

The authors are very grateful to Professor T. Wieland for his kind gift of phallotoxins.

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