Short communication

The Effect of Glutaraldehyde and Phalloidin on the Conformation of F-Actin

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Recent data allow the assertion that conformational changes in F-actin regulate actin-myosin interaction in muscle fibre (Adelstein and Eisenberg 1980; Lednev 1980; Yanagida and Oosawa 1980; Borovikov et al. 1982; Borovikov and Gusev 1983; Borovikov and Karandashov 1983). However, the exact mechanism of such regulation cannot be established without further experimental evidence. The study of conformational changes in F-actin induced by glutaraldehyde or phalloidin (mushroom toxin phalloidin) seems to be of interest. These reagents cause no disturbances in actomyosin functions in a solution (Dancker et al. 1975; Mikawa et al. 1979), inhibiting, however, contraction in glycerinated muscle fibres (Bukatina and Morozov 1979; Prochniewicz-Nakayama and Yanagida 1982; Borovikov 1984). In the present study, we have shown that glutaraldehyde — and phalloidin — induced modifications of F-actin decrease the flexibility of thin filaments. Polarization UV fluorescent microscopy and birefringence methods were used.

Myosin, troponin and tropomyosin were selectively extracted from single glycerinated muscle fibres. Single muscle fibres were prepared from glycerinated psoas muscle of rabbit (Rome 1973). Myosin, troponin and tropomyosin were removed by maintaining the single fibre in extracting solution containing (in mmol/l): 800 KCl; 10 ATP; 5 $MgCl_2$; and 67 sodium phosphate buffer, pH 7.0 for 30—40 min at 4 °C. Such fibres (ghost fibres) contained more than 80% of actin (Borovikov et al. 1982). Measurements were performed in two solutions: one, standard solution, containing (in mmol/l): 100 KCl; 5 $MgCl_2$; 2 EGTA; 67 phosphate buffer, pH 7.0; the other solution differed from the standard one by the content of 5 mmol/l of ATP. Some of the fibres were treated with a standard solution containing either 40 μg/ml of mushroom toxin phalloidin (Bukatina and Morozov 1979) or 0.05% glutaraldehyde (Borovikov 1984) for 20 min at 18 °C. Non-bound reagents were washed out with standard solution. The degree of polarization tryptophan fluorescence (P) was recorded during the orientation of fibres parallel ($P_{\parallel}$) and perpendicular ($P\perp$) to the polarization plane of the incident light. Fluorescence was excited at 303 ± 5 nm and recorded at 320—380 nm (Borovikov and Chernogriadskaia 1979; Borovikov et al. 1982). The analysis of

Table 1. Polarization of tryptophan fluorescence and birefringence Δn of F-actin filaments of myosin-free ghost fibres. Average value and standard deviations from 80 measurements on 8 different fibres.

	$P_\perp$	$P_\parallel$	$P_\perp/P_\parallel$	$\Delta n(\%)$
F-actin	0.207 ± 0.002	0.196 ± 0.001	1.05 ± 0.01	100
F-actin — ATP	0.213 ± 0.002	0.192 ± 0.001	1.11 ± 0.01	108 ± 3
F-actin — phalloidin	0.218 ± 0.002	0.197 ± 0.002	1.11 ± 0.02	112 ± 5
F-actin — phalloidin — ATP	0.217 ± 0.001	0.196 ± 0.001	1.11 ± 0.01	113 ± 4
F-actin — glutaraldehyde	0.218 ± 0.003	0.193 ± 0.002	1.12 ± 0.03	105 ± 3
F-actin — glutaraldehyde-ATP	0.218 ± 0.003	0.192 ± 0.002	1.12 ± 0.03	105 ± 3

polarized fluorescence was performed as described elsewhere (Rozanov et al. 1971; Yanagida and Oosawa 1978). Changes in the position of the emission spectrum of F-actin were determined from the changes in the ratio between the values of the fluorescence intensity at 320 nm and 365 nm, respectively during excitation at 297 nm (Turoverov and Shchelchkov 1970). The birefringence Δn of fibres was evaluated as described earlier (Yanagida and Oosawa 1978). The value of $P\perp$ for ghost fibres is higher than that of $P_\parallel$, and the ratio $P_\perp/P_\parallel > 1$ (see Table 1) (Borovikov and Chernogriadskaia 1979; Borovikov et al. 1982). The interaction of F-actin with either glutaraldehyde or phalloidin or ATP changes $P_\perp$ and $P_\parallel$ and increases the anisotropy of tryptophan fluorescence of thin filaments, since $P_\perp/P_\parallel$ increases (Table 1). It has been shown earlier that, following the extraction of myosin, troponin and tropomyosin, the tryptophan residues of F-actin remain in ghost fibres whose absorption and emission oscillators are oriented predominantly perpendicular to the long axis of the fibre (Yanagida and Oosawa 1978; Borovikov and Chernogriadskaia 1979), i. e. anisotropically. On the other hand, tryptophan residues of all the other proteins of ghost muscle fibres are only weakly anisotropic and their contribution to the total emission of fibres is much lower than that of the F-actin ffuorescence. Therefore, $P_\perp > P_\parallel$ and $P_\perp/P_\parallel$ exceed unity (Borovikov and Chernogriadskaia 1979; Borovikov et al. 1982). $P_\perp$, $P_\parallel$ and $P_\perp/P_\parallel$ depend on the optical properties of tryptophan residues of F-actin which are located in hydrophobic sites inaccessible to the solvent inside F-actin protomers (Elzinga and Collins 1973; Vedenkina et al. 1972). Hence, $P_\perp$, $P_\parallel$ and $P_\perp/P_\parallel$ are sensitive to conformational changes in F-actin (Borovikov and Chernogriadskaia 1979; Borovikov et al. 1982). Thus, it can be assumed that glutaraldehyde, phalloidin and ATP induce conformational changes in F-actin. An analysis of the data obtained showed that, in all the experiments, the conformational changes resulted in a decrease in the flexibility of thin filaments. The changes could be due to both intra- and intermonometric conformational changes in F-actin. Therefore taking into consideration internal conservatism of the actin structure (Elzinga and Collins 1973) and the data of the present study, these reagents can be assumed to bring about

conformational changes in the sites of actin-actin bonds in F-actin. This is apparently accompanied by a decrease in the flexibility of thin filaments. An increase in birefringence of ghost fibres observed in the present work (Table 1) does not contradict such conclusions and is in accordance with the data obtained by other methods (Dancker et al. 1975; Löw et al. 1975; Blackholm and Faulstich 1981). The stabilization of the F-actin structure can, evidently, affect myosin-actin interaction. Thus, it has been shown that Ca^{2+}-free troponin-tropomyosin complex stabilizes the F-actin structure (Oosawa et al 1973; Yanagida and Oosawa 1980; Borovikov and Gusev 1983) and inhibits muscle contraction. It is assumed that thin filaments with low flexibility contain mainly "switched-off" monomers, i. e. monomers, with which myosin forms cross bridges but fails to generate tension (Borovikov and Gusev 1983). Possibly, the stabilization of the structure caused by ATP is also accompanied by "switching-off" of some of the monomers in F-actin and is responsible for the relaxation of the fibre (Borovikov and Chernogriadskaia 1979). In contrast to ATP, phalloidin and glutaraldehyde irreversibly stabilize the structure of F-actin and the modified actin loses its ability to respond to ATP by decreasing the flexibility of thin filaments (Table 1), i. e. to change its conformation in response to the effect of ATP. Since phalloidin and glutaraldehyde cause no disturbances in the function of actomyosin systems in a solution, such as the actin ability to activate ATP-ase activity of myosin (Dancker et al. 1975; Mikawa 1979), it is very probable that the irreversible stabilization of the F-actin structure is the reason for the observed decrease in isometric tension developed by muscle fibres whose F-actin is modified by glutaraldehyde (Prochniewicz-Nakajama and Yanagida 1982; Borovikov 1984), or phalloidin (Bukatina and Morozov 1979; Borovikov 1984). Apparently, the irreversible "switching-off" of a certain number of functional units (Lednev 1980) from the contractile system affects the isometric tension developed by such fibres.

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