Short communication

Mechanical Properties of Skinned Single Muscle Fibers Crosslinked with Glutaraldehyde

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It was shown earlier (Mikawa 1979; Prochniewicz 1979) that crosslinking of thin filaments in vitro by glutaraldehyde can "freeze" their structure either in the activated (in the presence of Ca^{2+}-ions) or depressed (in the absence of Ca^{2+}-ions) state. This treatment does not inhibit the ability of actin to bind to myosin heads and to activate myosin ATPase (Lehrer 1981; Prochniewicz-Nakayama and Yanagida 1982; Poo and Hartshorne 1976).

The aim of the present work was to "freeze" in vivo the "on" and "off" states of thin filaments and to study mechanical properties of these frozen states.

A single skinned fiber from rabbit psoas muscle was incubated with 0.05 % glutaraldehyde for 2—3 min in rigor solution containing (mmol/l) 85 KCl; 5 $MgCl_2$; 5 EGTA; 15 tris-HCl buffer, pH 7.3. The crosslinking was terminated by washing the fiber in rigor solution. Isometric rigor tension and stiffness of the

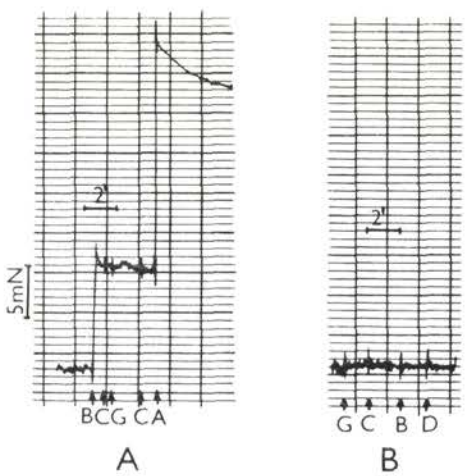

Fig. 1. Isometric tension, developed by single skinned fiber crosslinked with glutaraldehyde in different states: *A* — in rigor state. *B* — in relaxed state. Arrows indicate changes in bathing solutions. *A* — relaxing solution. *B* — rigor solution with EDTA. *C* — rigor solution with EGTA. *G* — buffered 0.05 % glutaraldehyde solution. *D* — Ca-activating solution, pCa 4.6.

crosslinked fiber were then measured as described previously (Lednev et al. 1982). After the addition of the relaxing solution (rigor solution plus 5 mmol/l MgATP) to the fiber a sharp rise of tension was observed in contrast to a tension drop in the control (unmodified) preparation. This effect is shown in Fig. 1*A*. No similar effect of the activating solution (rigor or Ca-activating solutions) on the fiber preparations, incubated with glutaraldehyde in relaxed state under identical conditions (Fig. 1*B*) was observed.

Fig. 2 illustrates tension changes induced by a quick stretch.

As seen from Figs. 2*B* and 2*C*, the immediate elastic tension response to the length change (rise time 0.5 ms) is followed by quick tension recovery, which is characteristic for the cycling cross-bridges (Ford et al. 1977). Thus the cross-bridges can cycle both in the presence and in the absence of Ca^{2+}-ions, in the latter case, however, under the conditions of the above modification only.

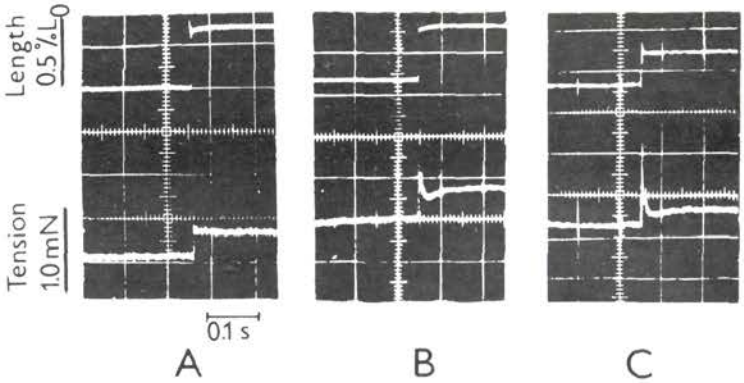

Fig. 2. Tension transients following quick changes in length: *A* — unmodified rigor fiber, *B* — rigor fiber crosslinked with 0.05 % glutaraldehyde after the addition of relaxing solution, *C* — relaxed fiber after Ca-activation.

It is believed that in vitro glutaraldehyde modifies in the first turn properties of thin filaments (Lehrer 1981). In particular, it crosslinks the tropomyosin molecules to actin. One may suggest that in rigor liber glutaraldehyde also crosslinks and fixes the tropomyosin molecule position (Weber and Murrey 1973). The addition of MgATP to specimens modified as above induces cycling of cross-bridges independently of the concentration of Ca^{2+}-ions. In the relaxed fiber the thin filaments are crosslinked by glutaraldehyde in the depressed ("off") state, so that subsequent addition of either rigor or Ca-activated solutions to the fiber does not induce contraction.

Our results show that it is possible to "freeze" the "on" and "off" states of thin filaments in skinned fibers in the absence of Ca-ions. Such a preparation

may be useful for the investigations of the contractile apparatus of the striated muscle.

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