6 V. KORMANČÍKOVÁ, L. KOVÁČ AND M. VIDOVÁ, *Biochim. Biophys. Acta*, 180 (1969) 9.
7 M. OGUR, R. ST. JOHN AND S. NAGAI, *Science*, 125 (1957) 928.
8 S. NAGAI, N. YANAGISHIMA AND H. NAGAI, *Bacteriol. Rev.*, 25 (1961) 44.
9 M. YČAS, *Exptl. Cell Res.*, 11 (1956) 1.
10 C. REILLY AND F. SHERMAN, *Biochim. Biophys. Acta*, 95 (1965) 640.
11 P. P. SLONIMSKI, *Formation des Enzymes Respiratoires chez la Levure*, Masson, Paris, 1953.
12 T. GALEOTTI, L. KOVÁČ AND B. HESS, *Nature*, 218 (1968) 194.
13 R. B. TOBIN AND E. C. SLATER, *Biochim. Biophys. Acta*, 105 (1965) 214.
14 K. UYEDA AND E. RACKER, *J. Biol. Chem.*, 240 (1965) 4682.
15 C. C. LINDEGREN AND S. HINO, *Exptl. Cell Res.*, 12 (1957) 163.
16 M. HARRIS, *J. Cellular Comp. Physiol.*, 48 (1956) 95.
17 T. NEGROTTI AND D. WILKIE, *Biochim. Biophys. Acta*, 153 (1968) 341.
18 C. J. E. A. BULDER, Thesis, *Institute of Technology*, Delft, 1963.
19 R. H. DEDEKEN, *J. Gen. Microbiol.*, 44 (1966) 157.

Received December 24th 1969

Biochim. Biophys. Acta, 205 (1970) 520–523

BBA 43264

The Ca^{2+}-dependent contraction and relaxation of actomyosin fibers

Since the discovery of native tropomyosin[1] or complex of tropomyosin and troponin[2,3], this protein system has been regarded as necessary for sensitizing actomyosin to Ca^{2+} (*cf.* ref. 4). Thus far, the Ca^{2+} sensitivity of actomyosin has been tested by the inhibition of superprecipitation or the inhibition of the Mg^{2+}-activated ATPase activity[2,5,6]. A partial reversibility of the contracted actomyosin suspension by the removal of Ca^{2+} has recently been reported[7]. However, a direct test of contraction or relaxation of actomyosin fibers controlled by Ca^{2+} has not yet been performed. The present study is concerned with the Ca^{2+}-dependent contraction and relaxation of actomyosin fibers and the requirement of the troponin–tropomyosin complex for the Ca^{2+} sensitivity.

Fibers were made from surface-spread actomyosin layers on a trough solution, containing 20 mM KCl, 1 mM $MgCl_2$ and 25 mM Tris–HCl buffer (pH 7.0) as described by HAYASHI[8], except that a loop of fiber was made[9]. Tension development was measured by a sensitive quartz lever as described previously[10].

Fibers made from natural actomyosin (myosin B) of rabbit skeletal muscle contracted maximally within one minute after addition of 5 mM ATP in a medium containing 0.08 M KCl, 8 mM $MgCl_2$, and 0.02 M Tris–histidine buffer (pH 7.0) (Fig. 1). When 3 mM (ethylene glycol bis-(β-amino ether)-*N,N'*-tetraacetic acid (EGTA)) was added to the contracted fiber to remove free Ca^{2+}, an immediate decrease in tension occurred. The extent of the drop in tension or the relaxability differed from preparation to preparation, ranging from 15 to 100%; the average of 12 runs was 50%. A typical result is seen in Fig. 1. On the other hand, when myosin B was washed 5 times by repeated suspension and sedimentation in a low ionic strength solution containing 1 mM $NaHCO_3$ and 2 mM Tris buffer (pH 7.6) to remove native tropomyosin[5], this washed myosin B became completely insensitive to Ca^{2+}; namely, it

Abbreviation: EGTA, ethylene glycol bis-(β-amino ether)-*N,N'*-tetraacetic acid.

did not relax upon the addition of EGTA at all. However, the Ca^{2+} sensitivity was completely restored by incubating the washed myosin B fibers in a solution of native tropomyosin prepared from rabbit muscle by the method of EBASHI *et al.*[3] (Fig. 1). Some 30–60 min of incubation was sufficient. The three preparations of fibers formed this way containing native tropomyosin up to 20–30 % by weight showed a relaxation of 34 % (average of 4 experiments). Fibers formed in a different way, by the addition of native tropomyosin to washed myosin B in 0.6 M KCl, were fragile and difficult to form, indicating a lack of structural continuity.

The dependence of contraction of actomyosin fibers on Ca^{2+} was examined in a different way: ATP was added in the presence of EGTA, followed by the addition of Ca^{2+}. Fig. 2 shows a typical result. The contraction of both fibers of intact myosin B and washed myosin B with native tropomyosin added was very poor in the presence of EGTA and a rapid contraction was induced by the addition of Ca^{2+}. The washed myosin B fiber without native tropomyosin was insensitive to Ca^{2+}.

Essentially, the same results as those shown in Figs. 1 and 2 were obtained with reconstituted actomyosin fibers. Fibers formed of purified actin and myosin recombined showed no EGTA sensitivity. Similarly, the same fibers incubated for several hours in a solution of tropomyosin alone or in troponin alone showed no relaxation when EGTA was added to the reaction vessel. However, the actomyosin fiber incubated in solutions of both tropomyosin and troponin relaxed and lost tension upon exposure to EGTA, and recontracted upon the addition of excess Ca^{2+}. Thus,

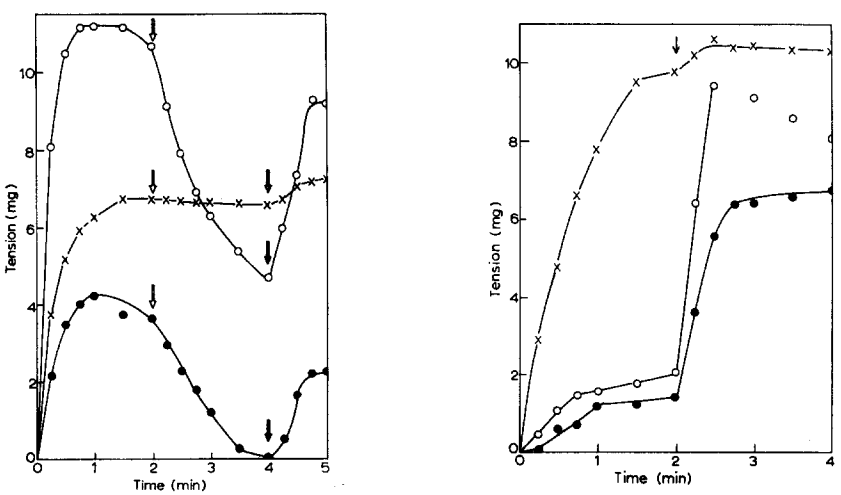

Fig. 1. Reversibility of contraction of actomyosin fibers as affected by Ca^{2+}. Fibers were made from approx. 12 mg of myosin B. When tested, washed myosin B fibers were incubated for 30 min at 5° in a solution containing 0.1 M KCl, 1 mM $NaCHO_3$ and native tropomyosin, 8 mg/ml. The reaction mixture consisted of 0.08 M KCl, 8 mM $MgCl_2$, 6 mM ATP and 0.02 M Tris–histidine buffer (pH 7.0). Total volume was 12 ml. The reaction was started by the addition of ATP at 25°. The open arrow shows the addition of 3 mM EGTA and the notched one shows the addition of 4 mM $CaCl_2$. ○, intact myosin B; ×, washed myosin B; ●, washed myosin B incubated with native tropomyosin.

Fig. 2. Reversibility of contraction of actomyosin fibers as affected by Ca^{2+}. Conditions and symbols are the same as in Fig. 1, except that 3 mM EGTA had been present before the addition of ATP and the arrow shows the addition of 4 mM $CaCl_2$.

both troponin[3] and tropomyosin are required for the Ca^{2+} sensitivity of tension loss; neither troponin nor tropomyosin alone was effective (*cf.* ref. 4).

It may be concluded from these experiments that the actin–myosin interaction that produces contraction requires the troponin–tropomyosin system not only to confer Ca^{2+} sensitivity but also to effect the mechanical changes that make possible the process of relaxation.

This work was supported by National Science Foundation Grant No. GB 72334 and National Institutes of Health Grant No. AM 12243.

Marine Biological Laboratory,
Woods Hole, Mass. and Department of Biology,
Illinois Institute of Technology,
Chicago, Ill. (U.S.A.)

C. WIDEMAN*
K. MARUYAMA**
T. HAYASHI

1 S. EBASHI AND F. EBASHI, *J. Biochem. Tokyo*, 55 (1964) 604.
2 S. EBASHI AND A. KODAMA, *J. Biochem. Tokyo*, 59 (1966) 425.
3 S. EBASHI, A. KODAMA AND F. EBASHI, *J. Biochem.*, 64 (1968) 465.
4 S. EBASHI AND M. ENDO, *Progr. Biophys. Mol. Biol.*, 18 (1968) 123.
5 S. V. PERRY, V. DAVIES AND D. HAYTER, *Biochem. J.*, 100 (1966) 289.
6 D. J. HARTSHORNE AND H. MUELLER, *Biochem. Biophys. Res. Commun.*, 31 (1968) 647.
7 K. MARUYAMA AND D. R. KOMINZ, *J. Biochem. Tokyo*, 65 (1969) 465.
8 T. HAYASHI, *J. Gen. Physiol.*, 36 (1952) 139.
9 W. D. COHEN, Ph.D. Thesis, 1966, Columbia University, New York.
10 T. HAYASHI, R. ROSENBLUTH, P. SATIR AND M. VOZICK, *Biochim. Biophys. Acta*, 28 (1958) 1.

Received February 16th, 1970

* In partial fulfillment of the research requirement in the Department of Biology at the Illinois Institute of Technology.
** Present address: Biological Institute, University of Tokyo, Meguro, Tokyo, Japan.