CONTRACTION OF GLYCERINATED MUSCLE FIBERS AND THE ROLE OF CALCIUM[1]

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Briggs and King (1962) recently reported that single glycerinated muscle fibers from rabbit psoas muscle contracted readily in solutions containing Mg and ATP which had been purified with Chelex 100 cation exchange resin and in which the free Ca^{++} concentration was calculated to be 6.9×10^{-10}M. However, the development of tension was strongly inhibited in the presence of EDTA, when the calculated Ca^{++} concentration was 1.4×10^{-8}M. As pointed out by Briggs and King, these results were at variance with the report of Weber and Winicur (1961) and that of Ebashi (1961) according to which half-maximal ATPase activity of actomyosin required 10^{-6}M Ca^{++} and 2.2×10^{-7}M Ca^{++}[3] respectively. More recently Weber and Herz (1963) reported that a Ca^{++} concentration of about 10^{-6}M was required for half maximal activation of myofibrillar ATPase activity in the presence of a Ca buffer system, EGTA (ethyleneglycol bis (β aminoethylether)-N, N'-tetraacetic acid)-CaEGTA.

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3. This value was calculated by Briggs and King (1962) from the data of Ebashi (1961). ATPase activities of myofibrils and actomyosin refer to activities in the presence of 1 to 5 mM Mg.

We have recently found that commercially available, crystalline ATP was the chief source of Ca contamination in the ATPase assay system, and that pretreatment of ATP with Chelex-100 or Dowex-50 resins removed this Ca (Seidel and Gergely, 1963,a,b). With the use of Ca-free ATP half maximal ATPase activity required the addition of 5 x 10^{-6}M $CaCl_2$ corresponding to about 10^{-6}M free Ca^{++}.

This report deals with ATP induced tension measurements on glycerinated single fibers with the use of Ca-free ATP (prepared by Dowex-50 treatment).

Rabbit psoas muscles were glycerinated for periods of 4 to 6 months and single fibers were prepared for tension measurements. Tension measurements were carried out by mounting the muscle fiber on a Sanborn TFA-1 differential type transducer and tension was recorded through a Sanborn preamplifier and a single channel recorder. The compliance of the transducer was 250 microns per gram load.

Table 1

Tension Developed by Single Fiber with Untreated and Dowex Treated ATP

Added $CaCl_2$ (M)	ATP	Tension (mg)	
		30 sec.	60 sec.
-	Untreated	10.6	14.4
-	Dowex treated	1.3	1.8
2 x 10^{-5}	Dowex treated	7.5	9.6

The test solutions contained 0.05M imidazole, pH 6.5, 0.13M KCl, 5 mM $MgCl_2$ and 3 mM ATP. Experiments were carried out on fibers first relaxed in a solution containing 0.05M imidazole, pH 6.5, 0.13M KCl, 5 mM $MgCl_2$, 5 mM ATP and 1 mM EGTA and then transferred to the test solution.

To insure relaxation the fiber was placed in a bath containing $MgCl_2$, ATP and EGTA. Tension developed rapidly when the bath was changed to one containing 5 mM $MgCl_2$ and 3 mM untreated ATP, however, very little tension developed if Dowex treated ATP was used (Table 1).

The tension developed did not become greater on repeated immersions in fresh solutions containing Dowex treated ATP. On the addition of Ca the rate of tension development increased and was half-maximal with 5 x 10^{-6}M to 10^{-5}M added $CaCl_2$ (Fig. 1).

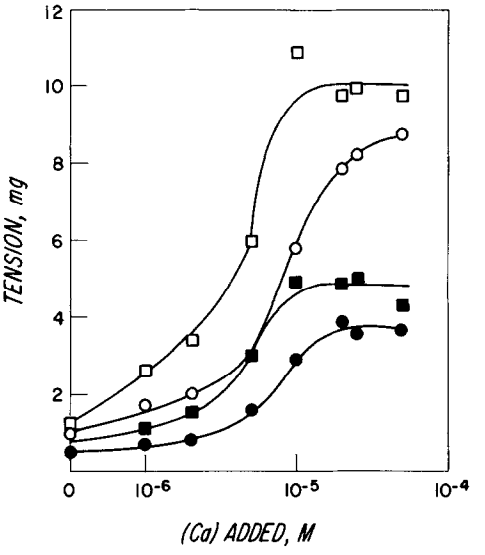

Fig. 1. A single muscle fiber was mounted and relaxed by immersion in a bath containing 0.13M KCl, 0.05M imidazole, 5 mM $MgCl_2$, 5 mM ATP and 1 mM EGTA at pH 6.5, then immersed in a solution containing 0.13M KCl, 0.05M imidazole, 5 mM $MgCl_2$ and 5 mM Ca-free ATP. The tension was recorded and the bath was replaced by the test solution. After each measurement the fiber was relaxed with EGTA. The test solutions contained 0.13M KCl, 0.05M imidazole, 5 mM $MgCl_2$ and 5 mM ATP, pH 6.5, and $CaCl_2$ as indicated on the abscissa. Key: ○, □, untreated fiber; ●, ■, desoxycholate treated fiber; ○, ●, tension developed in 30 sec.; □, ■, tension developed in 60 seconds.

Desoxycholate (DOC) treatment of the fiber with 1 mM DOC for 5 minutes at room temperature (Briggs and King, 1962) did not affect the concentration of Ca required for maximal

rate of contraction. Thus the effect of Ca cannot be attributed to its action on fragments of the sarcoplasmic reticulum present in the fiber since DOC is known to destroy the relaxing effects (Ebashi, 1958; Lorand et al., 1958) and Ca binding (Ebashi and Lipmann, 1962) of these fragments. The decreased absolute value of tension after DOC treatment suggests that the contractile proteins were affected by the treatment.

It appeared possible that the differences in the medium used by Briggs and King (30 mM KCl, 10 mM phosphate, 10 mM histidine, 5 mM potassium oxalate, 5 mM $MgCl_2$ and 5 mM ATP, pH 6.7) and that used routinely by us might account for the difference in results. As shown in Table II, when ATP purified with Dowex 50 was used in the medium used by Briggs and King the same Ca^{++} requirement was observed, although somewhat more tension developed in the system of Briggs and King which may have been the result of a lower ionic strength.

Table II

Effect of Ca on Tension Developed in the System Used by Briggs and King

Added $CaCl_2$ (M)	Tension (mg)	
	30 sec.	60 sec.
0	2.2	3.7
5×10^{-6}	7.2	11.0
2×10^{-5}	12.5	15.0
10^{-4}	13.2	16.5

The test solutions contained 0.03M KCl, 0.01M histidine, 0.01M phosphate, 5 mM potassium oxalate, 5 mM $MgCl_2$ and 5 mM ATP, pH 6.7. Experiments were carried out as described in the legend to Figure 1.

No explanation is apparent for the discrepancy between the present results and those of Briggs and King (1962). However, in view of the agreement between the level of Ca (5×10^{-6}M) required for half-maximal rate of contraction and for half-maximal ATPase activity and syneresis of myofibrils and actomyosin, the lack of an inhibitory effect of Chelex treatment in the work of Briggs and King suggests the presence of Ca contaminations of the order of 10^{-5}M or higher in their experiments.

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