REQUIREMENT FOR CALCIUM IN THE SYNAERESIS OF MYOFIBRILS[1]

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Ebashi and Lipmann (personal communication; Ebashi, 1961) discovered that the particulate relaxing factor (Marsh-Bendall factor) in presence of magnesium and adenosinetriphosphate (ATP) binds calcium very strongly. This property of the relaxing factor could be linked directly to the mechanism of relaxation of actomyosin systems if it were shown that they require calcium for reactions involved in contraction.

It was recently demonstrated by Ebashi (1961) and by Weber and Winicur (1961 a and b) that superprecipitation and ATPase activity of natural and reconstituted actomyosin did in fact depend on Ca. During an earlier study of the Mg-activated ATPase of myofibrils, however, only indirect evidence indicated that Ca is involved in this reaction. We now are able to show directly that the synaeresis and maximal ATPase activity of myofibrils require Ca under conditions where the particulate relaxing factor, which sometimes contaminates even well-washed myofibrils (Nagai et al. 1960), is irreversibly inactivated.

Myofibrils, prepared as previously described (Weber 1959), were incubated with a saturated aqueous solution of thymol in order to denature any relaxing factor (Gergely et al. 1959), and then washed several times with 0.1 M KCl. The reaction mixture for ATPase activity (5 ml) and synaeresis (10 ml) con-

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tained 0.3 to 0.4 mg/ml and 0.55 mg/ml respectively of myofibrillar protein, 20 mM imidazole buffer pH 6.5, KCl to bring the ionic strength ($\Gamma/2$) to the desired value, and, unless otherwise indicated, 1.0 mM MgATP. ATPase was determined as previously described (Weber and Winicur, 1961b). The extent of synaeresis was determined by measuring the volume of a given amount of myofibrils after centrifugation in a clinical centrifuge for 100 seconds.

We found in 9 experiments with different myofibril preparations at $\Gamma/2$= 0.16 that addition of Ca caused an average increase in the ATPase activity of 2.5 times. Fig. 1 shows that the activating effect of Ca became much smaller with decreasing ionic strength. The rates of hydrolysis of ATP at various ionic strengths were identical in the absence of added Ca and in the presence of a mixture of EGTA[2] (ethylene glycol bis(β-aminoethylether)-N,N'-tetraacetic acid) and CaEGTA in a ratio of 3:2 which maintains at pH 6.5 the concentration of Ca^{++} at 0.0013 mM. The ATPase activity was considerably more reduced (to 5-10% of maximal) when 1.0 mM EGTA was added.

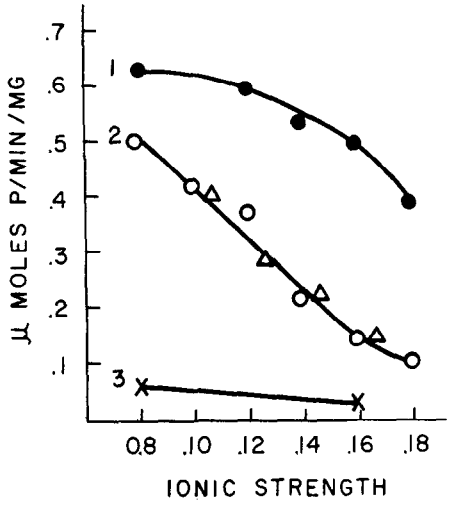

Fig. 1 ATPase with and without added Ca.

Curve 1: 0.1 mM $CaCl_2$; curve 2: ○, no Ca: △, 0.6 mM EGTA + 0.4 mM CaEGTA; curve 3: 1.0 mM EGTA.

[2]EGTA differs from EDTA in the low value of the stability constant for Mg (5.21) (Chaberek and Martell, 1959) and therefore is more useful than EDTA as a buffer for Ca ions when Mg is present. We are indebted to Geigy Chemical Corporation for their generous gift of this compound.

In the absence of Ca at $\Gamma/2$=0.16 synaeresis was incomplete and was decreased further after the addition of phosphoenolpyruvate (PEP) and pyruvate phosphokinase (PEPkinase) even though this enzyme system had no effect on the ATPase activity (Table I). PEP and PEPkinase maintained an ATP concentration of 0.1 to 0.4 mM (as calculated from the difference between the liberated phosphate and pyruvate) in the myofibril precipitate, whereas without the enzyme system all the ATP in the precipitate had been split at the end of the experiment. For the inhibition of synaeresis, however, the ATP concentration must be maintained above 0.05 mM, since at lower concentrations partial synaeresis occurred even in the presence of 1.0 mM EGTA. (In presence of 1.0 mM MgATP, EGTA always inhibits fully (Table I).)

TABLE I

Dependence of Synaeresis and ATPase Activity on Ca*

Salts added	0.1 mM $CaCl_2$		1.0 mM EGTA		none	
PEP, mM +(.01% PEPkinase)	0	2.7	0	2.7	0	2.7
Precipitate, ml	0.06 0.05(9)	0.06	0.22 0.17(8)	0.20	0.11 0.10(9)	0.18 0.15(3)
µmoles P/min/mg	0.40 0.48(3)	0.38 0.47(3)	0.03 0.05(1)	0.06(1)	0.15 0.23(3)	0.16 0.26(3)

*The values in the upper line of each horizontal column were obtained from the same myofibril preparation; those in the lower line are averages from different preparations (number indicated by figure in parentheses). ATPase activities are given only from those preparations which were tested with and without PEP and PEPkinase. $\Gamma/2$=0.16.

A reduction in the concentration of Mg to 0.001 mM resulted in complete synaeresis even in presence of 1.0 mM EGTA (Fig. 2).

Maximal synaeresis after addition of 0.01 mM Ca was partially reversed by 1.0 mM EGTA: the volume increased from 27% in presence of Ca to 75% of the original volume.

In view of our results it appears that at an ionic strength of 0.16 and 0.1 to 1.0 mM MgATP, Ca is required for both the ATPase activity and syn-

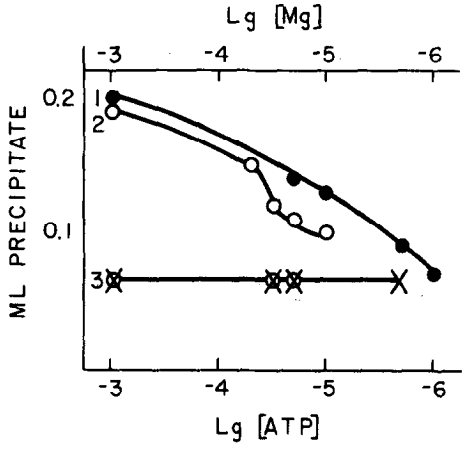

Fig. 2 Effect of lowering Mg or ATP on the inhibition of synaeresis by EGTA.

$\Gamma/2$=0.16. Curve 1: 1.0 mM EGTA, 10 mM ATP, Mg upper abscissa; curve 2: 1.0 mM EGTA, 10 mM Mg, ATP lower abscissa; curve 3 (controls): ○, 0.1 mM $CaCl_2$, 10 mM Mg Cl_2, ATP lower abscissa (the last point coincides with the last point of curve 2); ×, 0.01 mM $CaCl_2$, 10 mM ATP, Mg upper abscissa.

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aeresis of myofibrils. It is likely that the further reduction of ATPase activity to 5 to 10% of maximal and the absence of synaeresis in presence of EGTA are caused by a chelation of Ca contaminating our assays. In the experiment described by Fig. 1 a comparison of the ATPase activity at various ionic strengths before Ca addition and in presence of a Ca buffer (a mixture of EGTA and CaEGTA) of pCa 5.9 suggests a contamination with 0.0013 mM Ca. Ca was apparently largely removed in the experiments of K. Maruyama (personal communication) who found only 15% of the maximal ATPase activity of myofibrils after pretreatment of all solutions with the iminodiacetate resin Chelex 100 (a chelating resin for divalent ions).

We do not think that Ca is a reactant in the process of synaeresis because at very low concentrations of Mg synaeresis is complete even when 1.0 mM EGTA is present. This suggests that Ca prevents the inhibition of synaeresis by elevated Mg concentrations - possibly by competing with Mg for a site on actomyosin. We would like to speculate that this inhibitory Mg may

be bound as MgATP because inhibition of synaeresis becomes incomplete at very low ATP concentrations.

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