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Effect of magnesium and calcium on the ATPase activity of actomyosin at low ionic strength

Since the pioneering work of BANGA AND SZENT-GYÖRGYI[1] and the subsequent detailed work of HASSELBACH[2], it has been well known that Mg^{2+} activates the ATPase activity of actomyosin (EC 3.6.1.3) at low ionic strength, where actomyosin is superprecipitated with ATP. However, exact data of the dependence of the ATPase activity upon the ionic strength have been lacking. In our previous report[3], it appeared that the Mg^{2+}-activated enzyme action is increased progressively as the ionic strength is decreased down to about 0.04. We have further attempted to investigate the ATPase activity at lower ionic strength with special reference to the activating effect of Mg^{2+} and Ca^{2+}. The experimental technique is referred to in the paper cited above[3].

As seen in Fig. 1, the ATPase activity of natural actomyosin (myosin B) is increased up to $I = 0.035$ and then at lower ionic strength decreased in presence of 1 mM $MgCl_2$ at pH 8.0 and 20°. Superprecipitation took place rather incompletely at very low ionic strength ($I < 0.03$). A notable decrease in the ATPase activity around $I = 0.08$ is due to the clearing response of actomyosin where actomyosin is dissociated[4]. It is of significance that the maximal ATPase activity in presence of 1 mM $MgCl_2$ ($I = 0.035$) is higher than that in presence of 1 mM $CaCl_2$ at its optimal ionic strength ($I \sim 0.15$), although the ATPase activity in presence of optimal concentration of Ca^{2+} ($\sim 10^{-2}$ M) at $I \sim 0.15$ is comparable with the maximal activity in presence of Mg^{2+}. At very low ionic strength ($I \sim 0.03$) Mg was the most

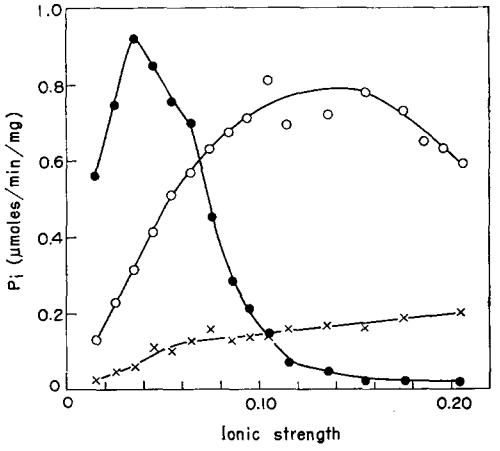

Fig. 1. Effect of ionic strength on the actomyosin ATPase activity in presence of Ca^{2+} or Mg^{2+}. Conditions: 1 mM ATP, and 7 mM Tris buffer (pH 8.0); 20°. 1 mM metals, when added. KCl was added to adjust ionic strength. ●—●, Mg^{2+}; ○—○, Ca^{2+}; ×—×, control.

effective activating metal for the actomyosin ATPase activity. Mn enhanced the activity to the same extent as Ca did. Sr, Co and Ni affected only slightly.

The enhancement of the ATPase activity by Ca^{2+} was moderate at low ionic strength and the Ca^{2+}-activated activity was roughly increased proportionally to the increase in the KCl concentration up to about 0.1 M. A broad optimum between $I = 0.10$ and 0.15 was observed and a slight decrease took place at the higher ionic strength[4,5]. At the ionic strength of 0.07, the ATPase activity was equal each other in presence of Mg^{2+} and Ca^{2+}.

In the present experiments K^+ was added as monovalent cation to increase the ionic strength. When the ionic strength was adjusted by NaCl, essentially similar dependence of the ATPase activity upon the ionic strength was observed, except that the Ca-enhanced activity in presence of Na^+ was considerably lower than that in presence of K^+ (see Ref. 6 for the effect of the kind of salt).

At $I = 0.025$, $3 \cdot 10^{-4}$–$5 \cdot 10^{-4}$ M Mg^{2+} increased the ATPase activity maximally (10 times that of the control), half maximal activation was observed at 10^{-4} M Mg^{2+}

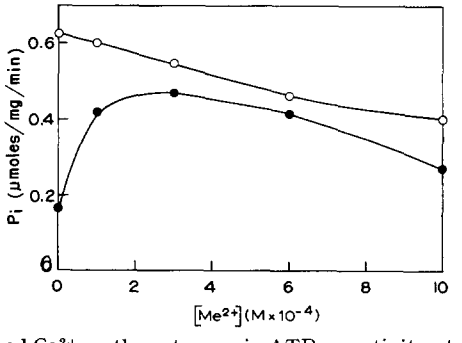

Fig. 2. Influence of Mg^{2+} and Ca^{2+} on the actomyosin ATPase activity at $I = 0.025$. ○—○, 1 mM $MgCl_2$ plus varied concentration of $CaCl_2$; ●—●, 1 mM $CaCl_2$ plus varied concentration of $MgCl_2$.

and a slight decrease occurred at 10^{-3} M Mg^{2+}. On the other hand, Ca^{2+} did not affect ATPase activity at all up to 10^{-4} M, and 10^{-3} M Ca^{2+} doubled the activity. The maximal activity in presence of $3 \cdot 10^{-3}$–$10 \cdot 10^{-3}$ M $CaCl_2$ was less than half of that in presence of $3 \cdot 10^{-4}$–$10 \cdot 10^{-4}$ M $MgCl_2$. When an increasing concentration of $MgCl_2$ was added in presence of 1 mM $CaCl_2$, an enhancement in the ATPase activity was noticed to take place (Fig. 2) and on the other hand, when 1 mM $MgCl_2$ was present, increasing amounts of $CaCl_2$ inhibited the enzyme activity to some extent. This situation is the same as in the apyrase activity of a contractile protein from sea-anemone[7].

The effect of Mg^{2+} and Ca^{2+} on myosin ATPase activity is completely different under the similar conditions, as indicated in Fig. 3. The myosin ATPase activity is inhibited by Mg^{2+} at all the ionic strength tested and greatly activated by Ca^{2+}, which was not appreciably dependent upon the KCl concentration. Interestingly when polyethylene sulphonate, a relaxing agent (interaction inhibitor) of the acto-myosin–ATP system[8], was present ,the actomyosin ATPase changed into the myosin ATPase type, as clearly shown in Fig. 3. This fact is also in favor of the view[8] that polyethylene sulphonate inhibits the interaction between F-actin and myosin.

At very low ionic strength, in presence of Mg^{2+}, the high ATPase activity is greatly inhibited by 1 mM EDTA or ethylene glycol-bis-(β-aminoethyl ether)-*N*,*N*-

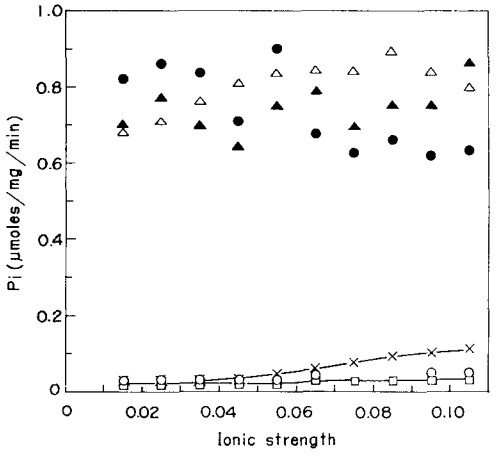

Fig. 3. Conversion of actomyosin ATPase to myosin ATPase by polyethylenesulphonate at low ionic strength. Polyethylenesulphonate, when added, 10^{-5} M. $MgCl_2$ or $CaCl_2$, when added, 1 mM. ●, actomyosin + Ca^{2+} + polyethylenesulphonate; ○, actomyosin + Mg^{2+} + polyethylene-sulphonate; ▲, myosin + Ca^{2+} + polyethylenesulphonate; △, myosin + Ca^{2+}; □, myosin + Mg^{2+}; ×, myosin.

tetraacetic acid and further addition of 0.5–1 mM $CaCl_2$ restored the original level of the activity, as already demonstrated at somewhat higher ionic strength[9].

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The product inhibition of citrate–oxaloacetate lyase from *A. aerogenes*

Citrate–oxaloacetate lyase (EC 4.1.3.6) has also been known as citrase, citratase, citrate aldolase or citridesmolase. It cleaves citrate to oxaloacetate and acetate and requires divalent metal ions such as Mg^{2+} or Mn^{2+}. It has been obtained in highly purified condition from *Escherichia coli* by BOWEN AND SIVA RAMAN[1], from *Aerobacter aerogenes* by SIVA RAMAN[2] and from *Streptococcus diacetilactis* by HARVEY AND COLLINS[3]. Using partially purified preparations DAGLEY AND DAWES[4] concluded that during the course of the reaction the enzyme became progressively inhibited by accumulation of oxaloacetate in the medium. Such product inhibition is well known and many examples are listed by FRIEDEN AND WALTER[5,6]. The inhibition may be reversible or apparently irreversible; of these the latter case may be of greater quantitative significance[5,6].

The citrate lyase used in the present studies was purified from *A. aerogenes* as described by BOWEN AND ROGERS[7] and used to study the equilibrium constant of the reaction

$$\text{Citrate} \overset{Mg^{2+}}{\rightleftharpoons} \text{Oxaloacetate} + \text{Acetate}$$

HARVEY AND COLLINS[3] have quoted a value for the equilibrium constant for the enzyme from *S. diacetilactis* which differs from that of SMITH *et al.*[8] using the enzyme from *Streptococcus faecalis*. They explain this difference on the basis that the keto form of oxaloacetate is the reaction product and not the enol form, a distinction not made in the earlier work. They also drew attention to inhibition caused by high levels of magnesium ions but made no mention of inhibition caused by the oxaloacetate produced. In the present work on the enzyme from *A. aerogenes* the inhibitory effect of excess Mg^{2+} was noted but the inhibitory effect of oxaloacetate was the major effect. The present communication seeks to show that for this enzyme the inhibition by oxaloacetate is so powerful and irreversible as to make it impossible to calculate an equilibrium constant for the reaction.

The substrate used 20 g/l sodium citrate dihydrate in 0.03 M KH_2PO_4 (pH 7.4), is called the citrate test medium, *i.e.* 2% citrate test medium for the medium described. All solutions contained 1.6 mM $MgSO_4$ since Mg^{2+} was the most efficient