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Effect of Chloromercuribenzoate on the Mg-Enhanced ATPase Activity of Actomyosin*

With 4 Text.figures

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(Communicated by J. ISHIDA)

Since the pioneering work of Kielley and Bradley (1956), the role of SH groups of myosin in the function of myosin has been investigated in detail. The ATPase activity of myosin or actomyosin is enhanced when about half of the total SH groups are titrated by SH-blocking agents such as chloromercuribenzoate (CMB) and it is completely abolished when all the SH groups are blocked (about 7 per 10^5 g of myosin) (cf. Blum, 1962).

Tonomura and Yoshimura (1960) first discovered that treatment with a small amount of CMB or salyrgan results in the onset of superprecipitation of actomyosin with high ATPase activity in the presence of 0.075 M KCl, whereas in the absence of SH-blocking agents clearing response occurred with low ATPase activity. We have studied this superprecipitation-enhancing effect of CMB or salyrgan in greater detail at higher KCl concentrations (Maruyama and Gergely, 1962b).

In a series of papers we have shown that the Mg-enhanced ATPase activity depends upon the KCl concentration, which determines the onset of superprecipitation or clearing of actomyosin upon addition of ATP (Maruyama and Ishikawa, 1963, 1964a, b). The present study is concerned with the change in ATPase activity dependence on KCl concentration upon treatment with CMB. The effect of some relaxing agents will be also reported.

MATERIAL AND METHODS

Natural actomyosin or myosin B was purified from 24 hour extract of rabbit skeletal muscle (Maruyama and Ishikawa, 1964a).

The ATPase activity was assayed as described before (Maruyama and Ishikawa, 1964a) The reaction mixture consisted of 0.01 M Tris buffer, pH 8.0, 1 mM ATP, 1 mM $MgCl_2$ and varied concentrations of KCl, as specified in each experiment. Myosin B concentration was

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usually 0.26 mg/ml. The total volume was 3 ml. Temperature was 25°. The details of the use of relaxing agents are referred to in a previous article (Maruyama and Ishikawa, 1964b).

It is known that the length of CMB treatment of actomyosin appreciably affects the CMB action thereafter (cf. Blum, 1962). In the present study, actomyosin suspension was pretreated with CMB for 5 minutes at 25° before the addition of ATP.

Results

Effect of CMB on the actomyosin ATPase activity at varied KCl concentrations

As reported by Blum (1962), 0.1 to 1 moles of CMB per 10^5 g of actomyosin enhanced the Mg-enhanced ATPase activity of actomyosin in the presence of 0.1 M KCl. It was found that the action of CMB on actomyosin greatly depended upon ionic strength. Figure 1 shows the effect of increasing concentrations of

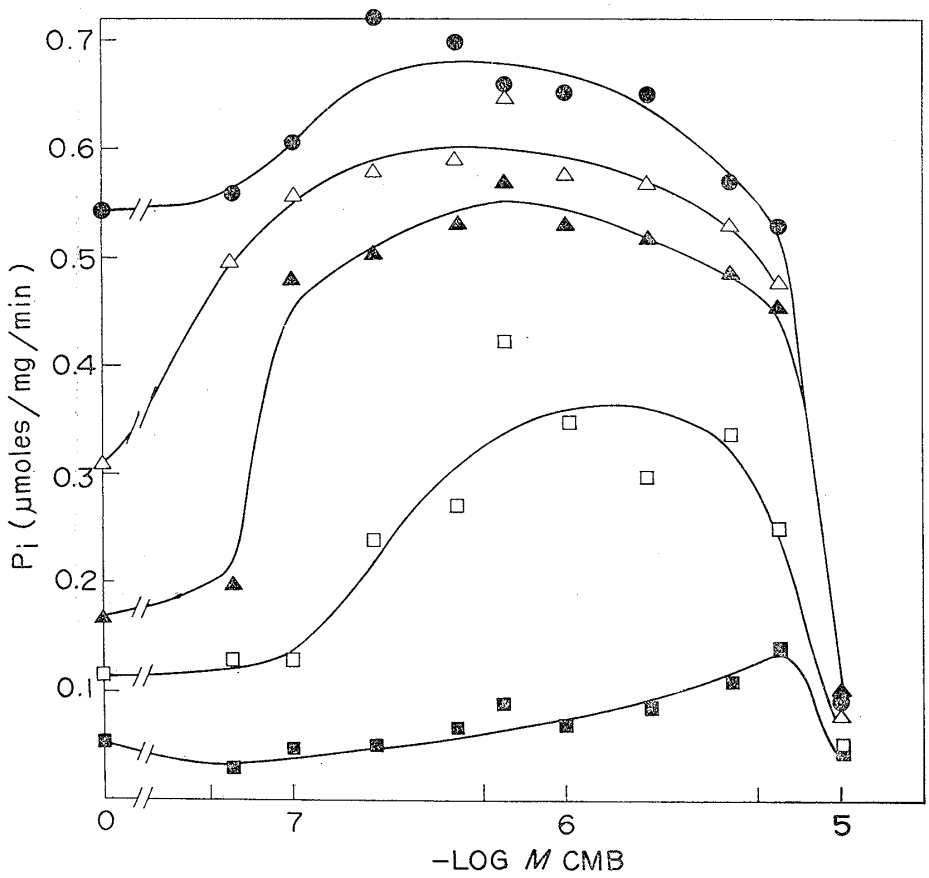

Fig. 1. Effect of varied concentrations of chloromercuribenzoate on the ATPase activity of actomyosin. KCl concentrations were 0.05 M (●), 0.07 M (△), 0.08 M (▲), 0.10 M (□) and 0.12 M (■).

CMB at several KCl concentrations. The activation by CMB was most remarkable in the presence of 0.07–0.10 M KCl. Hence, three to four fold increase in the enzyme action was observed to take place at suitable CMB concentrations. However, at KCl concentrations lower than 0.05 M or higher than 0.12 M, the activation was very small (Fig. 1).

It should be mentioned that the KCl dependency of the CMB-treated actomyosin was somewhat different at different protein concentrations. Figure 1 shows the results with 0.8 mg of actomyosin. Similar experiments with 0.6 mg of actomyosin showed smaller activation at 0.10 M KCl and those with 1.2 mg of actomyosin revealed greater activation at 0.12 M KCl.

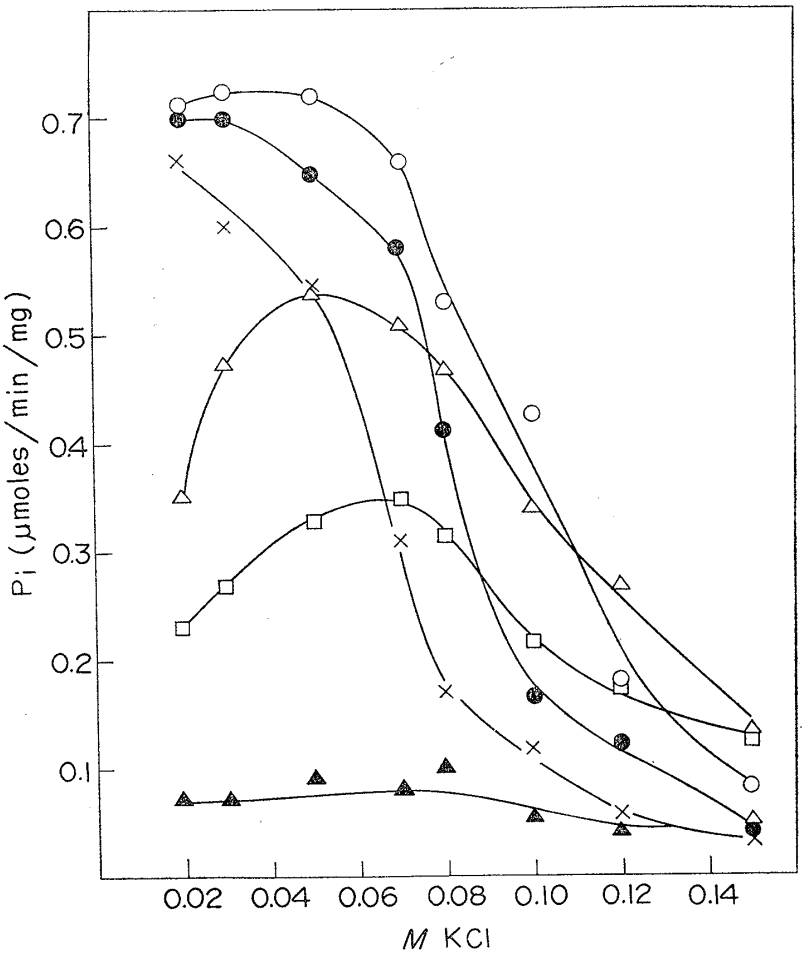

Fig. 2. KCl concentration dependence of the ATPase activity of CMB-treated actomyosin. CMB concentrations: 0 (×), 1-2 (○), 4 (●), 6 (△), 8 (□), and 10×10^{-6} M (▲).

The KCl dependency of the CMB effect is presented in Figure 2 in more detail. In the presence of 0.3-1.5 moles of CMB per 10^5 g of actomyosin (1-4 $\times10^{-6}$ M), the activation was observed in all the KCl concentrations tested, although negligible at 0.02 M and 0.15 M KCl, respectively. 2-3 moles of CMB per 10^5 g actomyosin (6-8$\times10^{-6}$ M CMB) rather inhibited the enzyme activity below 0.05 M KCl, and activated at higher KCl concentrations. Approximately 4 moles of CMB per 10^5 g actomyosin depressed the ATPase activity.

Effect of some relaxing agents on the ATPase activity of CMB-treated actomyosin

Tonomura and his collaborators (1960, 1963) have shown that CMB treatment

results in the disappearance of clearing response of actomyosin at 0.075 M KCl, and EDTA becomes ineffective in inhibiting the ATPase activity. This finding was confirmed by the present study. As is presented in Figure 3, EDTA

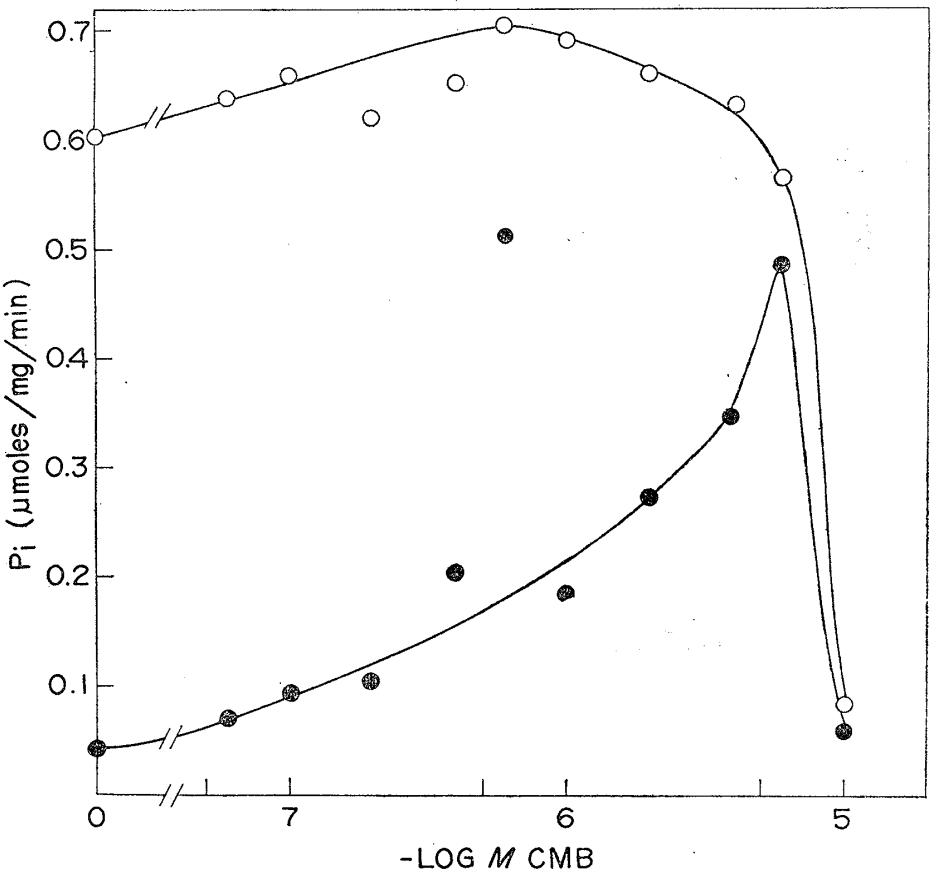

Fig. 3. Effect of EDTA on the ATPase activity of CMB-treated actomyosin. 0.03 M KCl. Control (○); 1 mM EDTA (●).

inhibition was completely abolished by 6×10^{-6} M CMB (3 moles/10^5 g) in the presence of 0.03 M KCl. Thus, EDTA or EGTA (ethylene glycol-bis (amino-ethylether) N, N-tetracetic acid) became ineffective as relaxing agents at low KCl concentrations, but at KCl concentrations higher than 0.10 M, they induced clearing response of CMB-treated actomyosin with low ATPase activity (Fig. 4).

It is of some interest to investigate whether or not other relaxing agents act on CMB-treated actomyosin. Figure 4 summarizes the results with an actomyosin (1.8 moles CMB per 10^5 g): the action of polyethylenesulphonate (PES), urea and formamide was less remarkable than on intact actomyosin (cf. Maruyama and Ishikawa, 1964b). Clearing response was observed to occur in the presence of urea and PES. With formamide clearing was not observed, although the ATPase activity was not much different from the case of urea.

The similar effects of those relaxing agents were observed with actomyosin (0.37 moles CMB per 10^5 g) except that Ca-chelating agents caused clearing

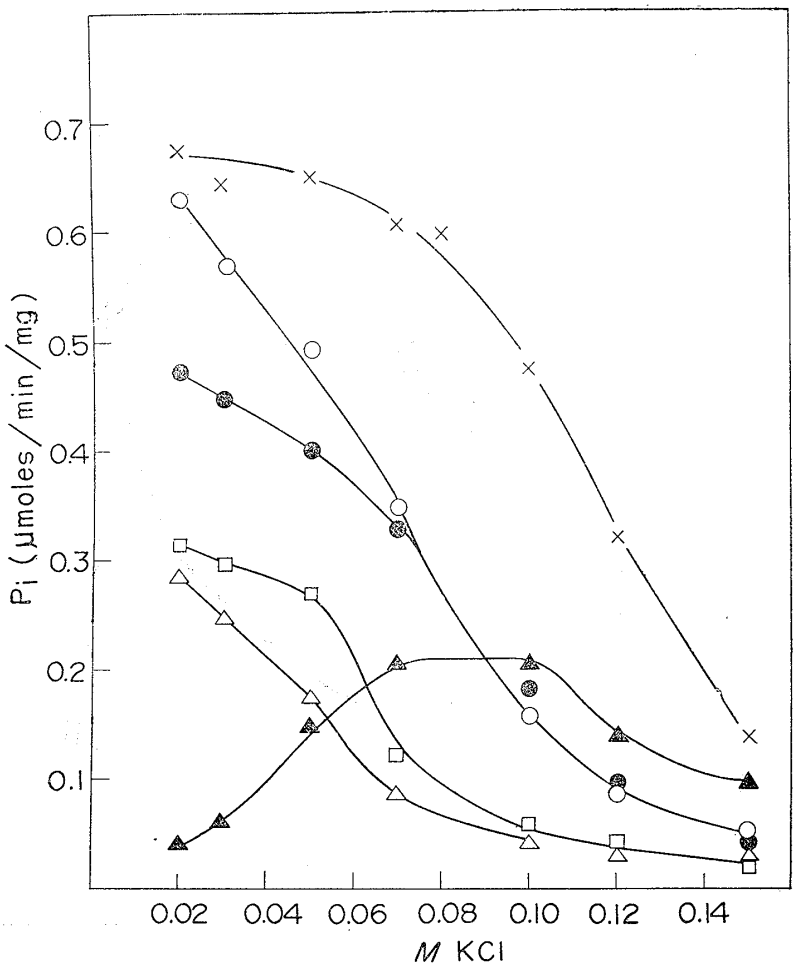

Fig. 4. Effect of some relaxing agents on the ATPase activity of CMB-treated actomyosin. 5×10^{-6} M CMB. Control (×), 1 mM EGTA (○), 1 mM EDTA (●), 6.6% formamide (□), 10^{-5} M PES (▲), and 1 M urea (△).

response even at low KCl concentrations (cf. Fig. 3).

DISCUSSION

It is generally accepted that there are at least two categories of SH groups in myosin: one group is essential for ATPase activity of myosin and the other somehow hinders ATPase activity. Therefore, when the latter SH groups are blocked, the ATPase activity is enhanced (cf. Blum, 1962).

In the present study it was shown that the situation was true for Mg-enhanced ATPase activity of actomyosin in the presence of varied KCl concentrations.

However, we should be aware of the special situation in the present study: the apparent activation by CMB at 0.05–0.12 M KCl was not largely due to the activation of the enzyme in true sense. This is ascribed to the physical change from clearing to superprecipitation (Tonomura and Yoshimura, 1960; Maruyama and Gergely, 1962a, b). Needless to say, superprecipitated actomyosin exhibits

much higher ATPase activity than the cleared one. As seen in Figures 1–4, the ATPase activity is only slightly elevated by CMB at low KCl concentrations (0.03 M KCl), where superprecipitation set in immediately after the addition of ATP, even in the absence of CMB.

It is clear that CMB, 0.2–2 moles per 10^5 g of actomyosin, enhances the onset of superecipitation at moderate KCl concentrations. Tonomura and his collaborators (1961) have shown that CMB treatment results in the liberation of the tightly bound calcium of actomyosin. They ascribed this loss of Ca to the onset of superprecipitation of actomyosin instead of clearing. However, it is to be noted that when the KCl concentration increased to 0.15 M, the CMB effect became negligible. Although the KCl concentration dependency of the ATPase activity is somewhat different between natural actomyosin (myosin B) and reconstituted actomyosin (cf. Maruyama and Gergely, 1962b), the CMB treatment leads to the shift of the KCl concentration of superprecipitation to higher concentration. The action of the SH-blocking agent on actomysin may be related to the fact that myosin aggregates at low KCl concentrations are further aggregated by CMB (Maruyama, unpublished experiments, 1961). The exact mechanism remains to be elucidated.

It is noteworthy that EDTA or EGTA became ineffective as the relaxing agent on CMB-treated actomyosin at low KCl concentrations. This is to confirm Tonomura's finding at a fixed KCl concentration (Tonomura and Yoshimura, 1960; Tonomura *et al.*, 1963). Again, at somewhat higher KCl concentrations, these Ca-chelating agents inhibited the ATPase activity of CMB-treated actomyosin (cf. Fig. 4). On the other hand, urea or PES lead to clearing response of CMB-treated actomyosin at low KCl concentrations, although the inhibition of the ATPase activity became less remarkable than the intact one (cf. Maruyama and Ishikawa, 1964b). The action of formamide also became less effective, and the onset of superprecipitation was observed, in spite of lowered ATPase activity. The altered KCl concentration dependency of the ATPase activity in the presence of PES, shown in Figure 4, is of some interest, since the curve became similar to the hydrolysis of nucleoside triphosphate other than ATP in the presence of PES (cf. Maruyama and Ishikawa, 1964b).

Summary

The KCl dependency of the ATPase activity of actomyosin, whose SH groups were partially blocked by chloromercuribenzoate, was studied in detail. At moderate KCl concentrations (0.05–0.10 M KCl), CMB treatment results in the transition of actomyosin from clearing to superprecipitation, and the corresponding increase in the Mg-enhanced ATPase activity was observed to take place.

The action of EDTA or of ethylene glycol-bis (aminoethylether) N, N-tetracetic acid as the relaxing agent became ineffective on the CMB-treated actomyosin at low KCl concentrations. This was also observed with formamide. On the other hand, the action of polyethylenesulphonate or urea remained effective.

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