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THE EFFECTS OF MONOVALENT AND DIVALENT CATIONS ON THE ATPase ACTIVITY OF MYOSIN

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SUMMARY

The activation of the ATPase activity of myosin by KCl follows sigmoidal kinetics owing to the simultaneous increase in concentration of two activating ions, K^+ and Cl^-. Activation by K^+ at a constant Cl^- concentration follows hyperbolic kinetics but becomes sigmoidal in the presence of a constant concentration of Na^+. There appears to be competition between Na^+ and K^+ for enzyme binding sites since increasing the concentration of K^+ overcomes the inhibitory effect of a given concentration of Na^+. Tryptic digestion of myosin to Subfragment-1 does not alter the sigmoidal activation by KCl nor that by K^+ in the presence of Na^+. The dependence of activity on the concentration of ATP follows Michaelis–Menten kinetics under all conditions tested.

In the presence of K^+ or NH_4^+, the ATPase activity of myosin is inhibited by divalent cations. Both the extent of the inhibition and the concentration of divalent cation required for half maximal inhibition vary with the nature of the divalent cation. The concentration of divalent cation required for half maximal inhibition appears to depend primarily on the ionic radius, the larger the ion the higher the concentration required to inhibit. The extent of inhibition of K^+-activated ATPase by divalent cations is inversely related to the extent of activation by divalent cations in the presence of Na^+, a non-activating cation. Divalent cations which activate in the presence of Na^+ produce only a partial inhibition in the presence of K^+. At high concentrations of divalent cations, ATPase activity is the same regardless of the monovalent cation present. These results suggest that the inhibition of K^+-activated ATPase by divalent cations involves the conversion of myosin from a monovalent cation-activated enzyme to a divalent cation-activated enzyme.

INTRODUCTION

The monovalent cations, K^+, NH_4^+, and Rb^+, activate the ATPase activity of myosin while Na^+ and Li^+ inhibit it[1–4]. The marked stimulation of K^+-activated ATPase by EDTA[5] has somewhat obscured the requirement for the monovalent cation, and no systematic study of the effects of K^+ on the ATPase activity of myosin has been made. It is well known that Mg^{2+} strongly inhibits the ATPase activity of myosin

and the activation of myosin by EDTA can be accounted for by chelation of traces of contaminating Mg^{2+} (refs. 6 and 7). It has also been reported that Ca^{2+}, which usually activates myosin, can under some conditions inhibit activity[8,9]. In view of these findings and of the fact that the enzymic properties of myosin differ markedly depending on whether Ca^{2+} or K^+ activates myosin[4,10-12], the present study of the effects of monovalent and divalent cations on the ATPase activity of myosin was undertaken.

The results suggest that myosin can function either as a monovalent cation-activated ATPase or as a divalent cation-activated ATPase. Divalent cations can be considered to convert myosin from a monovalent to a divalent cation-activated ATPase, and this conversion can involve either an increase or a decrease in activity depending on experimental conditions.

METHODS

Myosin was prepared essentially as described by KIELLEY AND BRADLEY[10] with EDTA included in all steps of the preparation[13]. Myosin was precipitated at 50 or 80% saturated $(NH_4)_2SO_4$ (pH 7) and stored at $-10°$ until used. Essentially all enzymic activity was retained up to 6 months under these conditions. $(NH_4)_2SO_4$ and EDTA were removed by dialysis against a solution containing 0.5 M KCl and 2 mM *N*-tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid at pH 7.0. Sub-fragment-1 and heavy meromyosin were prepared by tryptic digestion as described elsewhere[14].

ATPase activity was measured by determination of P_i released according to FISKE AND SUBBAROW[15] or by titration of protons released with the use of a Radiometer automatic titration apparatus. Contaminating metals were removed from ATP by passage through Dowex-50 (H^+), and the pH was adjusted to 7.0 with KOH or Tris. Since most of the experiments were carried out at high concentrations of monovalent salt (usually from 0.4 to 1 M), contributions to the ionic strength of divalent cations, ATP, and EDTA containing species can be neglected. The ionic strength can be considered to be equal to the total monovalent salt concentration as given in the legends.

The concentrations of ATP, free metals and metal–ATP complexes were calculated according to the program of PERRIN AND SAYCE[16] using an IBM 1800 computer. Stability constants for alkali metal–ATP complexes given by BOTTS *et al.*[17] and those for divalent cation–ATP complexes given by TAQUI KHAN AND MARTELL[18] were used in the computations.

RESULTS

The ATPase activity of myosin increases sigmoidally with increasing concentrations of KCl (Fig. 1). This sigmoidal increase is not observed if the total salt concentration is held constant with tetramethylammonium chloride as the K^+ concentration is varied. However, if the inhibitor Na^+ is added and if both the Na^+ and Cl^- concentrations are held constant, then activity increases in a sigmoidal manner with increasing K^+ concentration. At K^+ concentrations of 1 M, the inhibition by 0.05 M Na^+ is largely overcome. When the concentration of Na^+ is increased at a

constant K^+ concentration, activity decreases but with no sigmoidal shape to the curve (Fig. 2). The percentage of inhibition produced by a given Na^+ concentration decreases with increasing K^+ concentration. At low K^+ concentrations, Na^+ completely inhibits activity, while at high K^+ concentrations, Na^+ produces approx. 50% inhibition.

As shown in Fig. 3 the dependence of K^+-activated ATPase activity on ATP concentration follows Michaelis–Menten kinetics both in the absence and in the presence of Na^+. The double reciprocal plots remain linear on the addition of Na^+, but the slopes and intercepts are changed.

The effects of varying the concentrations of K^+ and Cl^- or K^+ alone on the ATPase activity of heavy meromyosin and Subfragment-1 were also studied, and the results were identical with those obtained with intact myosin. A sigmoidal curve

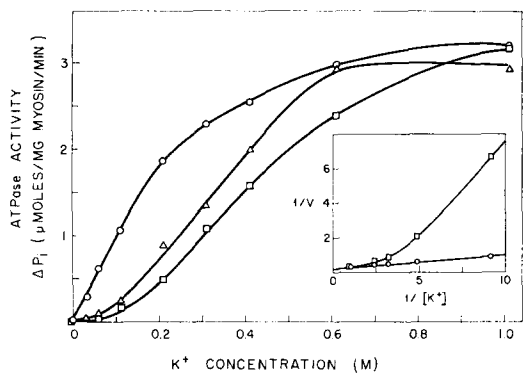

Fig. 1. Effect of K^+ and KCl concn. on K^+-activated ATPase activity. Activity was measured in the presence of 0.01 M imidazole, 5 mM EDTA, 5 mM ATP and 0.1 mg myosin per ml at pH 7.5, 25°. ATP and EDTA were present as Tris salts: △, [KCl] varied with no tetramethylammonium chloride present; ○, [KCl] varied with sufficient tetramethylammonium chloride present to maintain total monovalent salt concentration at 1 M; □, [KCl] varied with 0.05 M NaCl and sufficient tetramethylammonium chloride present to maintain total monovalent salt concentration at 1 M.

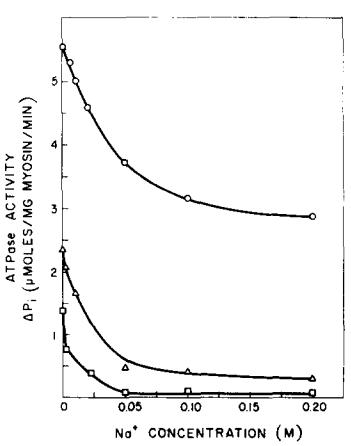

Fig. 2. Effect of Na^+ concentration on K^+-activated ATPase activity. Activity was measured in the presence of 0.05 M Tris, 5 mM EDTA, 5 mM ATP and 0.05–0.1 mg myosin per ml at pH 7.5 and 25°. ATP and EDTA were present as Tris salts. Tetramethylammonium chloride was added to make the total monovalent salt concentration 1 M in all cases: ○, 0.8 M KCl; △, 0.3 M KCl; □, 0.06 M KCl.

is observed when both K^+ and Cl^- are varied or when K^+ alone is varied in the presence of Na^+, and a hyperbolic curve is obtained when K^+ alone is varied in the absence of Na^+. Fig. 4 illustrates the data for Subfragment-1.

The effect of Ca^{2+} on ATPase activity in the presence of KCl, NaCl or NH_4Cl is shown in Fig. 5. Activity decreases as Ca^{2+} is added in the presence of K^+ or NH_4^+. In the presence of Na^+ no activity is observed without Ca^{2+} and an increase in activity is obtained with increasing Ca^{2+} concentrations. The limiting activity with increasing Ca^{2+} is the same whether K^+, NH_4^+ or Na^+ is present. The effects of Mn^{2+} are similar to those of Ca^{2+} in that Mn^{2+} partially inhibits K^+-activated ATPase and activates

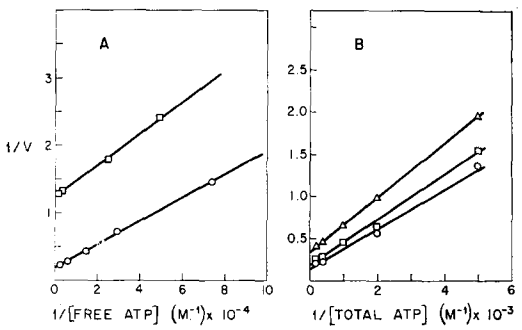

Fig. 3. Effect of ATP concn. on K^+-activated ATPase at high and low KCl concn. A. Activity at 0.1 M KCl was determined by measurement of the time course of P_i released by the method of FISKE AND SUBBAROW[15] in the Zeiss PMQ spectrophotometer using cuvettes having a 5-cm light path. Activity at 1 M KCl was followed in the pH stat. Assay conditions were: □, 0.1 M KCl, 0.05 M Tris, 5 mM EDTA, 0.025–0.05 mg myosin per ml and 0.02–1.0 mM total concentrations of ATP at pH 7.5 and 25°; ○, 1.0 M KCl, 5 mM EDTA, 0.03 mg myosin per ml and 0.2–5 mM total concns. of ATP at pH 7.5 and 25°. Free ATP concentrations were computed from stability constants of HATP, H_2ATP, KATP, K_2ATP and KHATP[17]. B. Activity was measured in the pH stat under conditions described for (A). The KCl concentration was 1 M: ○, no NaCl; □, 0.05 M NaCl; △, 0.2 M NaCl. Activities were expressed in μmoles P_i released per mg myosin per min.

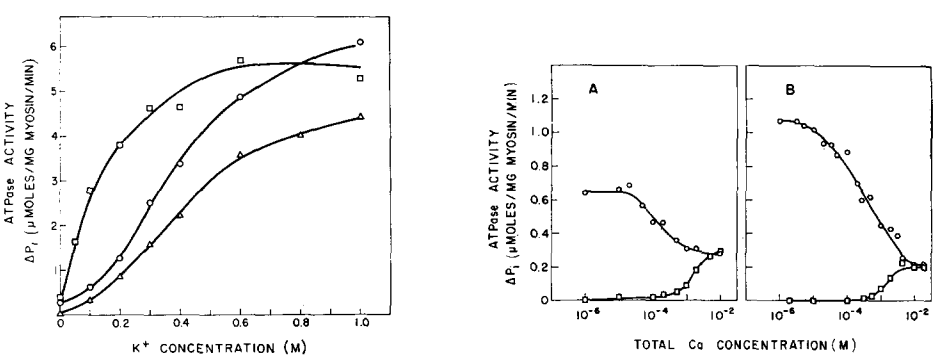

Fig. 4. Effect of K^+ and KCl concentration on K^+-activated ATPase of Subfragment-1. Conditions as described in Fig. 1: ○, [KCl] varied with no trimethylammonium chloride present; □, [KCl] varied with sufficient trimethylammonium chloride present to maintain total monovalent salt concentration at 1 M; △, [KCl] varied with 0.05 M NaCl and sufficient trimethylammonium chloride present to maintain total monovalent salt concentration at 1 M.

Fig. 5. Effect of Ca^{2+} on ATPase activity in the presence of KCl, NH_4Cl or NaCl. Activity was measured in the presence of 0.05 M Tris, 5 mM ATP, 0.4 M monovalent salt, and 0.2 mg myosin per ml at pH 7.5 and 25°. A. ○, KCl; □, NaCl. B. ○, NH_4Cl; □, NaCl.

in the presence of Na^+. The limiting activity with increasing Mn^{2+} is again the same with either Na^+ or K^+ present (Fig. 6). However, this value with Mn^{2+} is lower than that obtained with Ca^{2+}. Sr^{2+} also partially inhibits K^+-activated ATPase and activates in the presence of Na^+. At approx. 0.01 M Sr^{2+}, K^+-activated ATPase is reduced to 50 % of the initial activity, but at this Sr^{2+} concentration, activity in the presence of K^+ is still 2–3 times higher than activity in the presence of Na^+. Presumably the curve with Na^+ and that with K^+ would converge at higher Sr^{2+} concentrations, but higher concentrations of Sr^{2+} cause precipitation of Sr^{2+}–ATP and cannot be tested.

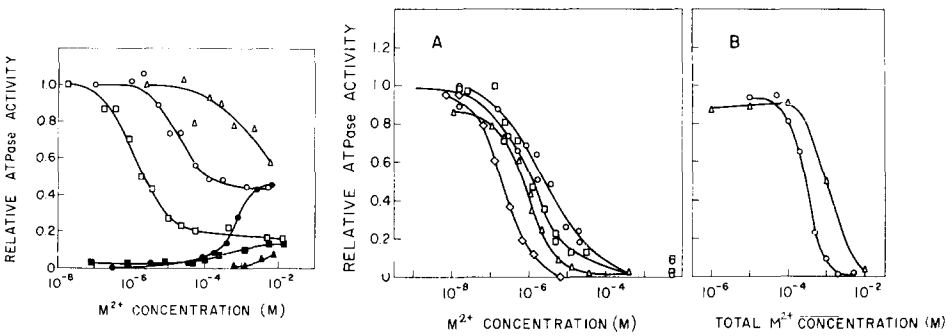

Fig. 6. Effect of Ca^{2+}, Mn^{2+} and Sr^{2+} on ATPase activity in the presence of KCl or NaCl. Activity was measured as described in legend to Fig. 5. Free metal concns. were computed using stability constants for the following complexes of ATP: $HATP^{3-}$, H_2ATP^{2-}, $MATP^{3-}$, M_2ATP^{2-}, $MHATP^{2-}$, $NATP^{2-}$, and $NHATP^-$, where M represents the monovalent metal and N the divalent metal. Solid symbols represent activities in 0.4 M NaCl and open symbols in 0.4 M KCl: ○, ●, $CaCl_2$; □, ■, $MnCl_2$; △, ▲, $SrCl_2$.

Fig. 7. Effect of divalent cations on K^+-activated ATPase. Activity measurements and computations are as described in the legends to Figs. 5 and 6. A. ◇, $MgCl_2$; △, $NiCl_2$; □, $CoCl_2$; ○, $ZnCl_2$. B. ○,$CuCl_2$; △, $CdCl_2$.

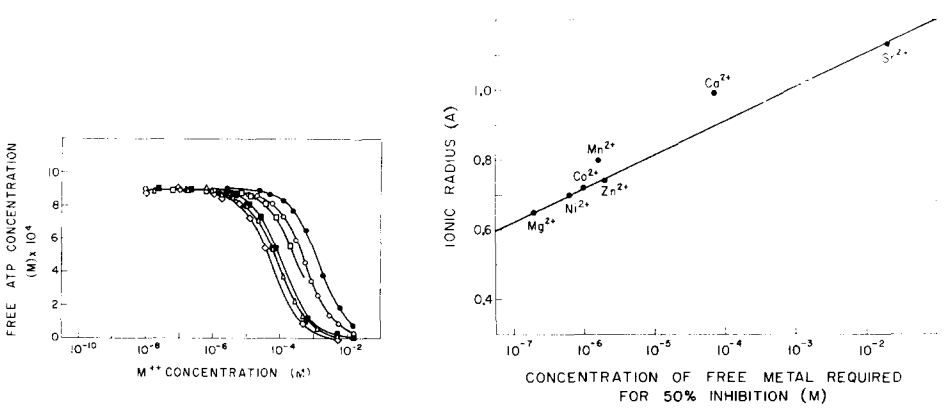

Fig. 8. Calculated concentrations of free ATP as function of free divalent metal concentrations. The concns. of free ATP and divalent metals were computed as described under METHODS. The stability constants for the following ATP complexes were used in the computation: $HATP^{3-}$, H_2ATP^{2-}, $KATP^{3-}$, K_2ATP^{2-}, $KHATP^{2-}$, $NATP^{2-}$ and $MHATP^-$, where N represents the divalent metal. The pH was 7.5; ○, Ca^{2+}; □, Mg^{2+}; △, Mn^{2+}; ◇, Ni^{2+}; ●, Sr^{2+}; ■, Co^{2+}; ▲, Zn^{2+}.

Fig. 9. Relationship between inhibitory effectiveness and ionic radius of divalent cation. The data from Figs. 6 and 7 were used to determine concentration required for 50 % inhibition of activity. Ionic radii were taken from PAULING[24].

Essentially complete inhibition of the K^+-activated ATPase is observed with other divalent cations, and little or no activation of ATPase in the presence of Na^+ is produced by these metals. The inhibition of K^+-activated ATPase by Mg^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} is shown in Fig. 7A. The results with Cu^{2+} and Cd^{2+} (Fig. 7B) are plotted against total divalent metal concentration since Cu^{2+} is essentially completely complexed by ATP and the stability constants of Cd^{2+}–ATP complexes have not been reported.

To determine whether the inhibition of activity by divalent cations is accompanied by a decrease in the concentration of free ATP, the dependence of the free ATP concentration on the concentration of divalent metals was computed from the stability constants of metal–ATP complexes (Fig. 8). Comparison of Figs. 7 and 8 shows that inhibition of K^+-activated ATPase by Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, or Zn^{2+} is essentially complete at metal concentrations which do not change the free ATP concentration. Ca^{2+} produces 50 % of the maximal inhibition with only a 10 % decrease in free ATP concentration. The concentrations of H^+ and K^+ complexes of ATP also remain unchanged at inhibitory concentrations of divalent cations.

The concentration of free divalent metal required to produce 50 % inhibition of K^+-activated ATPase shows a linear correlation with the ionic radius of the divalent cation (Fig. 9). The concentrations of Mn^{2+}, Ca^{2+} and Sr^{2+} required for activation of ATPase activity in the presence of Na^+ also parallel the ionic radii of these three cations. The approximate values of K_a determined from plots of $1/v$ against the reciprocal of the metal concentration are: 0.1 mM, 4 mM and $>$ 0.01 M for Mn^{2+}, Ca^{2+} and Sr^{2+}, respectively.

DISCUSSION

The sigmoidal dependence of the K^+-activated ATPase activity of myosin on KCl concentration is consistent with the finding that both K^+ and the anion contribute to the activation of ATPase activity by potassium salts[4]. Sigmoidal curves of activity against concentration are to be expected if the concentrations of two or more activators (in this case K^+ and Cl^-) are increased simultaneously[19]. When only the concentration of K^+ is varied a hyperbolic increase in activity is observed.

The reason for the sigmoidal activation by K^+ in the presence of Na^+ is less clear. It is unlikely that this effect involves interactions between myosin subunits since sigmoidal activation is observed with either myosin or with Subfragment-1, and it is generally agreed that Subfragment-1 consists of part of a single myosin subunit[14,20]. As is well known, myosin but not Subfragment-1 aggregates (precipitates) at low salt concentration, thus the identical results obtained with myosin and Subfragment-1 suggest that aggregation is not involved in these effects.

It is possible that Na^+ may bind with high affinity to a site other than the K^+ activating site, from which it can be displaced by K^+. In attempting to interpret these results it must be noted that in the presence of Na^+ and K^+ there are at least eight different metal or H^+ complexes of ATP present and the concentrations of all these forms of ATP change when the K^+ concentration is varied. The possiblity that changes in the concentration of one or more forms of ATP might contribute to the sigmoidal activation cannot be ruled out.

The dependence of ATPase activity on ATP concentration follows Michaelis–

Menten kinetics in all cases, indicating the absence of cooperative substrate binding over the range of ATP concentrations and ionic strengths studied. On varying the concentration of ATP, the concentration of all ATP complexes increases linearly with the total ATP concentration while the free K^+ concentration remains essentially unchanged, owing to the fact that the K^+ concentration is in large excess over that of ATP.

The studies of the inhibition of K^+-activated ATPase activity by divalent metals are complicated by the presence of Mg^{2+} contamination, chelation of which can account for the activation of ATPase activity by EDTA[6,7]. Even though myosin had been prepared in the presence of EDTA and ATP purified with Dowex-50 our assay system apparently still contained significant Mg^{2+} contamination since EDTA produced several-fold activation of K^+-activated ATPase activity. Since it is impractical if not impossible to remove this Mg^{2+} contamination, it is essential that the concentration of Mg^{2+} remains constant in studies of inhibition by other divalent cations. This can be achieved if changes in the total concentration of the metal being studied do not significantly change the free concentration of ligands which bind Mg^{2+}, a condition which is satisfied when the total concentrations of all metal binding ligands are in large excess over the total concentration of the metal being varied. If the free concentrations of ligands which bind Mg^{2+} and the total concentration of Mg^{2+} remain unchanged on varying the concentration of another divalent metal ion, then the concentration of Mg^{2+} also will be unchanged.

Because of the high affinity of EDTA for divalent metals, inhibition of ATPase activity by divalent metals in the presence of EDTA is observed only at total concentrations of divalent metal that are high enough to displace Mg^{2+} from its EDTA complexes. Therefore, studies of the inhibition by divalent metals have been carried out in the absence of EDTA even though activity is then partially inhibited by contaminating Mg^{2+}.

In the absence of EDTA the inhibition of K^+-activated ATPase by Ni^{2+}, Co^{2+}, Zn^{2+} and Mn^{2+} occurred at concentrations which did not change the concentration of free ATP or free Mg^{2+}.

The classical type of inhibition experiments in which the concentration of activator is varied at constant inhibitor concentrations has not been carried out, since at constant total ATP and divalent metal concentrations increasing the K^+ concentration would displace both the divalent cation being studied and the contaminating Mg^{2+} from their ATP complexes.

There is some uncertainty regarding the values of the stability constants of metal–ATP complexes. With the hope of minimizing the effect of this uncertainty in the calculation of the concentration of various ionic species, values of stability constants obtained in the same laboratory were used as far as possible. In the case of Mg^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} there is a difference of 2–4 orders of magnitude between the concentration of divalent metal required for inhibiton of activity and that required for changing the concentration of free ATP. This difference is more than could be attributed to uncertainty in the value of stability constants. Therefore, the inhibition of activity by divalent cations cannot be due to displacement of Mg^{2+} from its ATP complexes nor to changes in the concentrations of free ATP or K^+–ATP complexes. Displacement of Mg^{2+} from myosin binding sites by other divalent cations would be unlikely to produce an inhibition of activity since, with the possible exception of

Cu^{2+}, Mg^{2+} is the most effective inhibitor of the divalent cations tested. Thus it appears reasonable to conclude that inhibition by divalent cations can be attributed to the binding of the divalent cation or its ATP complex to the enzyme. The effectiveness of divalent cations in inhibiting K^+-activated ATPase activity closely parallels the ionic radius of the divalent cation. These results suggest that the binding of divalent cations to an inhibitory binding site of myosin depends primarily on the ionic radius, smaller ions having higher affinities.

Since these experiments were done at high ionic strengths (0.4) where myosin is not aggregated small changes in the ionic strength arising from changes in concentration of the various forms of ATP would not be expected to alter the results.

In the case of activation of ATPase activity by divalent metals in the presence of Na^+, the concentration of M^{2+} required for activation increases according to the series $Mn^{2+} < Ca^{2+} < Sr^{2+}$, also paralleling the ionic radius. The degree of activation by divalent metals agrees with earlier reports[21,22]; however, it should be noted that activation by Sr^{2+} is limited by the solubility of its ATP complex, which precipitates before maximal activity is obtained. The activation of ATPase by divalent cations is not determined solely by the ionic radius, since Cd^{2+} does not activate although it has the same ionic radius as Ca^{2+}. BLUM[23] has reported that Cd^{2+}, Cu^{2+} and Zn^{2+} have a diphasic effect on Ca^{2+}-activated ATPase similar to the effect of sulfhydryl reagents. Possibly the binding of Cd^{2+} to sulfhydryl groups may be related to the inability of Cd^{2+} to activate ATPase activity.

The present and earlier results[4] suggest that myosin acts either as a monovalent cation (M^+)-activated ATPase in the presence of K^+, Rb^+, or NH_4^+, or as a divalent cation (M^{2+})-activated ATPase in the presence of Mn^{2+}, Ca^{2+}, or Sr^{2+}. The M^+-activated ATPase is inhibited by all divalent cations and this effect is apparently the same as the previously reported inhibition of myosin ATPase activity by Ca^{2+} (refs. 8 and 9). In the case of Ca^{2+}, Mn^{2+} or Sr^{2+}, this inhibition is only partial and apparently involves a conversion from an M^+-activated ATPase to an M^{2+}-activated ATPase. At low concentrations of KCl the M^{2+}-activated ATPase has a higher activity than M^+-activated ATPase and under these conditions Ca^{2+} activates ATPase activity[8,12]. In the presence of activating concentrations of both monovalent and divalent cations myosin must function as an M^{2+}-activated ATPase, since Ca^{2+}-activated or Mn^{2+}-activated ATPase activity is the same regardless of the monovalent cation present.

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