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THE KINETICS OF ACTIVATION OF MYOSIN ATPase BY MONOVALENT CATIONS

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SUMMARY

The dependence of the kinetic constants K_m, v_{max} and k_1 of myosin ATPase on the species and concentration of monovalent cations and on pH was analyzed and results were compared with similar data obtained on Mg^{2+} mediated myosin ATPase.

A linear relationship between K_m and v_{max} was observed when myosin ATPase was activated by equal concentration of different alkali cations. This observation was utilized for the calculation of the dissociation constant K_s, the formation rate constant k_1 and the decomposition rate constant k_{-1} of the enzyme–substrate complex.

The increase in KCl or NH_4Cl concentration causes the measured values of K_m and v_{max} to increase and that of k_1 to decrease in the monovalent cation activated ATPase.

K_m and v_{max} vary with pH between 6 and 9 in the same way. The value of k_1 is slightly affected by the change in pH.

In the Mg^{2+} mediated myosin ATPase K_m and v_{max} decrease while k_1 increases with increasing Mg^{2+} concentrations.

The observations indicate that the monovalent cations enhance the rate of the steady state hydrolysis of ATP and reduce the stability of the myosin–ATP complex while Mg^{2+} inhibits the hydrolysis and improves the stability of this complex. This strongly supports the view that the activation of myosin ATPase with monovalent cations, including the low degree activation with Na^+ and Li^+, is essentially different from that with Mg^{2+}.

INTRODUCTION

It has been shown by several authors that the mechanism of myosin ATPase is substantially different in the presence of divalent cations from that in their absence[1–4]. In the absence of divalent cations the ATPase activity of myosin depends on the species and the concentration of the monovalent cation present in the solution[1–6]. The presence of Mg^{2+} at even such low a concentration as $1 \cdot 10^{-7}$ M, inhibits the myosin ATPase activity and changes its mechanism so that the proper value of the

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monovalent cation activated ATPase can be measured only in the presence of EDTA or other chelating agents[7-9].

The kinetics of divalent cation activated myosin ATPase has already been thoroughly studied[2,4,7,10-17]. Far less data are available on the kinetic constants of the myosin ATPase activated by monovalent cations[2,4,12,15]. In the present paper the results of a study of the variation of the kinetic constants K_m and v_{max} of myosin ATPase with the species and the concentration of the activating monovalent cations and with the pH are reported.

MATERIALS AND METHODS

Myosin was prepared essentially as described by PORTZEHL *et al.*[18], and purified by ultracentrifugation at 105 000 × *g* in a Spinco L-50 preparative centrifuge. Only fresh myosin, not older than 3 days, and reagent grade chemicals were used in the experiments. The ATP was a Sigma or Reanal (Budapest) product.

ATPase activity was measured essentially by the method of BÁRÁNY *et al.*[12] The test solution contained 8–15 μg of myosin per ml, 9 mM EDTA, 40 mM Tris–maleate buffer (pH 8) and 0.1–1.0 M chlorides of monovalent cations, if not stated otherwise. The pH was carefully controlled especially at high salt concentrations. The ATP concentration varied between 0.1 and 1.0 mM for the values of K_m higher than $4 \cdot 10^{-5}$ M, and between 0.01 and 0.1 mM for K_m lower than $4 \cdot 10^{-5}$ M. The measurements were carried out at 20° using samples of 8 ml for K_m higher than $4 \cdot 10^{-5}$ M and samples of 60 ml below this value. (In the latter case it was necessary to use large volume cells with 10-cm light path for determination of P_i). Incubation times (1–60 min) were chosen so as to obtain a decomposition of ATP of less than 20%. Incubation was terminated by the addition of 1/13 vol. of 0.88 M sulfuric acid. Inorganic phosphate P_i was measured by the method of FISKE AND SUBBAROW[19]. ATPase activities were evaluated from the averages of three parallel measurements (moles P_i liberated per mole of myosin per sec). The molecular weight of myosin was taken to be $5 \cdot 10^5$ daltons.

The very low Na^+ activated myosin ATPase ($3.75 \cdot 10^{-3}$ mole P_i per mole myosin per sec) was measured on samples of 70 ml containing 0.32 mg of myosin per ml and the incubation was terminated by the addition of 4.2 ml of 100% trichloroacetic acid to remove the protein which would disturb the measurement of P_i at this relatively high myosin concentration.

The Mg^{2+} mediated ATPase activity was measured in the presence of Mg^{2+}–EDTA buffer to abolish any effect of unknown Mg^{2+} contaminations. The association constants used for the calculation of the concentrations of ionic species were the following: K_{ass} Mg^{2+}–EDTA (pH 8) = $4.65 \cdot 10^6$ and K_{ass} Mg^{2+}–ATP (pH 8) = $2.27 \cdot 10^4$ (ref. 20). The free EDTA concentration was kept constant at 1 mM in all samples.

The measurements in the presence of tetramethyl ammonium chloride, which also disturbs the determination of P_i, were performed on 6-ml samples, incubation was terminated by the addition of 6 ml of a solution containing 10% trichloroacetic acid and 40% sodium perchlorate (the latter for removal of tetramethyl ammonium[3]). The values of K_m and v_{max} were determined from Lineweaver–Burk double reciprocal plots. Each plot was made from data obtained for five substrate concentra-

TABLE I

K_m AND v_{max} AS MEASURED ON MYOSIN ATPase ACTIVATED BY DIFFERENT MONOVALENT CATIONS
ATPase activity was measured in the presence of 9 mM EDTA and 0.49 M of the chloride of each cation at pH 8. For other details of the ATPase measurement and the evaluation of K_m and v_{max} see METHODS.

Monovalent cation	K_m	v_{max}
NH_4^+	$6.4 \cdot 10^{-4}$	59.5
K^+	$2.13 \cdot 10^{-4}$	9.1
Rb^+	$1.13 \cdot 10^{-4}$	4.3
Li^+	$9.0 \cdot 10^{-6}$	0.122
Na^+	$8.06 \cdot 10^{-6}$	0.00375

tions; all the values of K_m and v_{max} are averages taken over the data from three runs with different myosin preparations.

The protein content was measured by the biuret method of GORNALL *et al.*[21].

RESULTS

In the absence of divalent cations the activity of myosin ATPase activated by different monovalent cations is known to vary with the species in the order $NH_4^+ > K^+ > Rb^+ > Li^+ > Na^+$ (refs. 4-6). The values of the kinetic constants K_m and v_{max} obtained in the present experiment are listed in Table I. It is apparent that K_m increases with the activating species in the same order as v_{max}. Considering only the values obtained in the presence of alkali cations (Fig. 1) a linear relationship is seen to exist between K_m and v_{max}. It was shown by SLATER AND BONNER[22] that in the case of a linear dependence the plot of K_m against v_{max} permits to calculate the rate constants of the enzymic reaction and the dissociation constant of the enzyme-substrate complex if the formation rate constant k_1 and the decomposition rate constant k_{-1} of the enzyme substrate complex are assumed to be insensitive to the spe-

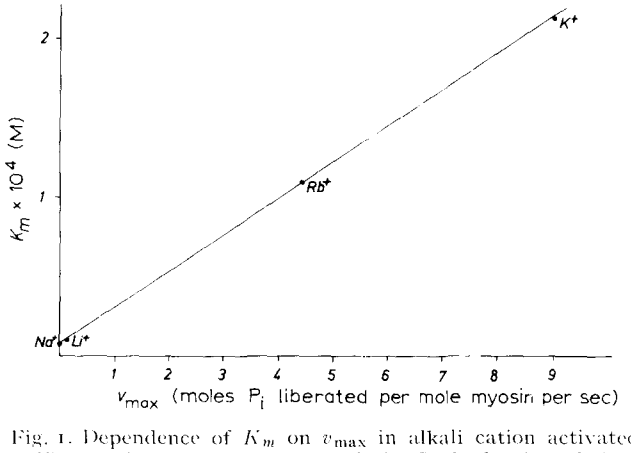


Fig. 1. Dependence of K_m on v_{max} in alkali cation activated myosin ATPases. Conditions of ATPase activity measurement see Table I. Evaluation of K_m and v_{max} see METHODS.

cies of the activator. Using the enzyme reaction expressed in the general form

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (1)$$

from which the dissociation constant K_s of the enzyme substrate complex is given by

$$K_s = \frac{k_{-1}}{k_1}$$

the values of K_s and $-k_1 E_t$ (E_t being the concentration of the enzyme) are determined by the intercepts of the K_m *versus* v_{max} curve on the ordinate and the abscissa, respectively, and $1/k_1 \cdot E_t$ is given by the slope of the curve. $k_2 = v_{max}/E_t$ and if v_{max} is calculated per mole enzyme as in our case (see METHODS) k_2 is numerically equal to v_{max}. The plot of the experimental data shown in Fig. 1 gives $K_s = 8 \cdot 10^{-6}$ M; $k_1 = 4.37 \cdot 10^4$ $M^{-1}sec^{-1}$ and $k_{-1} = 0.35$ sec^{-1}. The value of k_1 obtained by this method agrees well with that reported by LYMN AND TAYLOR[4], as estimated from the kinetics of K^+-activated ATPase in the transient state.

It follows from (Eqn. 1) that $K_m = (k_{-1} + k_2)/k_1$ and since $K_s = k_{-1}/k_1$, if we have for $k_{-1} \gg k_2$, as observed in the Na^+-activated ATPase, $K_m = K_s$. If $k_2 \gg k_{-1}$, as in the case of Rb^+- or K^+-activated ATPase, we find that $K_m = k_2/k_1$, as it was observed on the K^+-activated myosin ATPase also by LYMN AND TAYLOR[4]. It is very probable that $K_m = k_2/k_1$ in the NH_4^+-activated ATPase, too, since the highest value of k_2 (59.5 sec^{-1}, see Table I) was obtained in this case.

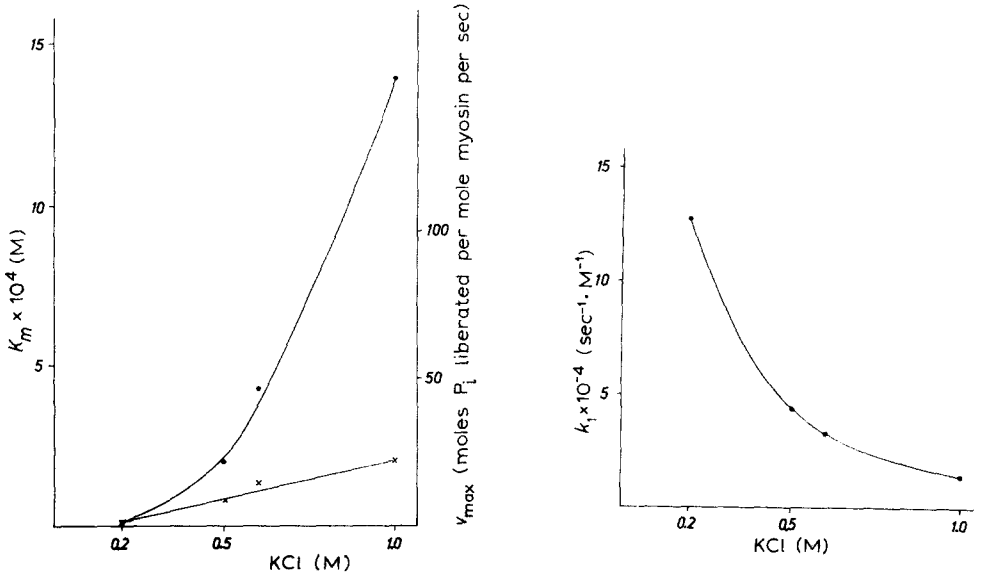


Fig. 2. Variation of K_m and v_{max} with KCl concentration in K^+-activated myosin ATPase. ATPase activity was measured in the presence of 9 mM EDTA and KCl concentrations specified on the abscissa at pH 8. For details of the ATPase measurement and evaluation of K_m and v_{max} see METHODS. ●, K_m; ×, v_{max}.

Fig. 3. Dependence of k_1 in K^+ activated myosin ATPase on KCl concentration. ATPase activity measurement see Fig. 2.

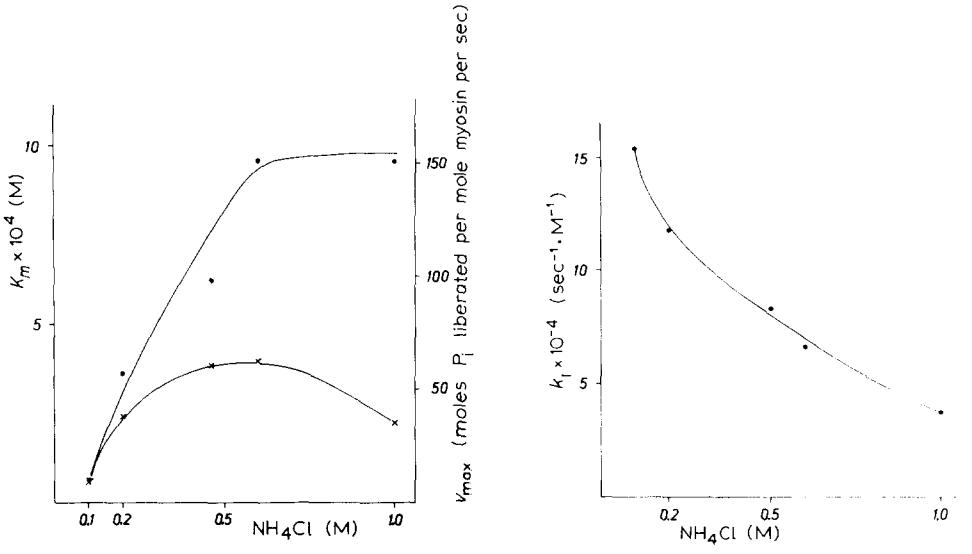


Fig. 4. Dependence of K_m and v_{max} in NH_4^+-activated myosin ATPase on NH_4Cl concentration. ATPase activity was measured in the presence of 9 mM EDTA and NH_4Cl concentrations specified on the abscissa at pH 8. For details of the ATPase measurement and evaluation of K_m and v_{max} see METHODS. ●, K_m; ×, v_{max}.

Fig. 5. Dependence of k_1 in NH_4^+-activated myosin ATPase on NH_4Cl concentration. ATPase activity measurement see Fig. 4. k_1 was evaluated as described in the paper for K^+-activated myosin ATPase, see text.

The observed variations of K_m and v_{max} with the KCl concentration are shown in Fig. 2. The values of both parameters increase with increasing KCl concentrations but the increase in K_m is much higher than that in v_{max}. Since $k_2 = v_{max}/E_t$ and for K^+-activated ATPase $K_m = k_2/k_1$, the value of k_1 is given by the formula $k_1 = v_{max}/K_m \cdot E_t$. If the value of k_2 does not exceed by at least an order of magnitude that of k_{-1}, as, *e.g.* in the presence of 0.2 M KCl, one has to take into account also the value of k_{-1}, and the formula becomes $k_1 = (v_{max} + k_{-1})/K_m \cdot E_t$. In our calculations of k_1 the value of $k_{-1} = 0.35$ sec^{-1}, obtained from Fig. 1, was used throughout. As shown in Fig. 3, k_1 was found to decrease as a function of KCl concentration.

TABLE II

EFFECT OF TETRAMETHYL AMMONIUM CHLORIDE ON THE VALUES OF K_m, v_{max} AND k_1 OF NH_4^+-ACTIVATED MYOSIN ATPase

ATPase activity was measured in the presence of 9 mM EDTA and 0.2 M NH_4Cl. For the details of the ATPase measurement and the evaluation of K_m and v_{max} see METHODS.

Tetramethyl ammonium chloride (M)	K_m	v_{max}	$\frac{v_{max}}{K_m \cdot E_t} = k_1$
—	$1.74 \cdot 10^{-4}$	36.3	$2.08 \cdot 10^5$
0.3	$2.78 \cdot 10^{-4}$	37.5	$1.35 \cdot 10^5$
0.4	$3.3 \cdot 10^{-4}$	41.5	$1.26 \cdot 10^5$
0.7	$3.64 \cdot 10^{-4}$	37.0	$1.02 \cdot 10^5$

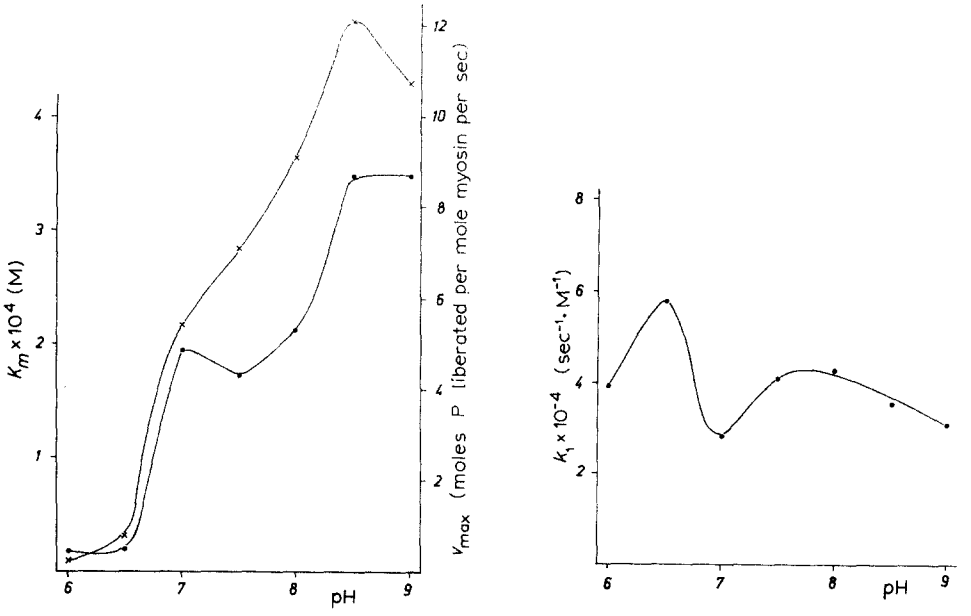


Fig. 6. Effect of pH on K_m and v_{max} in K^+-activated myosin ATPase. ATPase activity was measured in the presence of 9 mM EDTA and 0.5 M KCl at pH specified on the abscissa. 40 mM Tris–maleate buffer was used between pH 6–7.5 and 40 mM Tris–HCl buffer was used between pH 7.5–9. ●, K_m; ×, v_{max}.

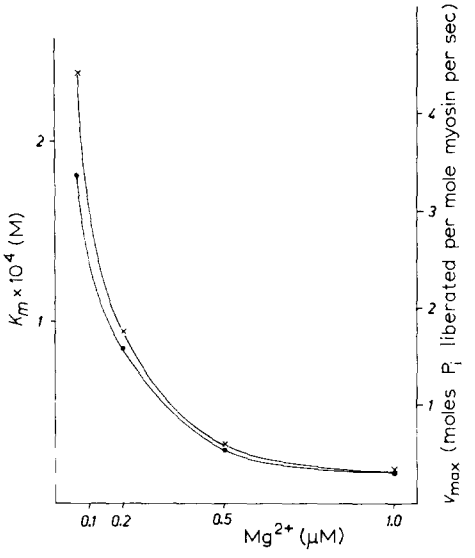
Fig. 7. pH dependence of k_1 in K^+-activated myosin ATPase. ATPase activity measurement see Fig. 6.

The effect of NH_4Cl on the kinetic constants of myosin ATPase, shown in Fig. 4, is similar to that of KCl. K_m increases up to 0.5 M NH_4Cl and shows a plateau above this concentration. v_{max} gives a curve with a maximum between 0.5 and 0.6 M NH_4Cl. k_1 decreases with increasing NH_4Cl concentration (Fig. 5), but the decrease is smaller than that observed for KCl.

Since it was shown by SEIDEL[3] that the tetramethyl ammonium cation does not induce any change in the myosin ATPase activity, to see which component of the salt (the cation or the anion) is mainly responsible for the observed effect of NH_4Cl and KCl, tetramethyl ammonium chloride was used to study the influence of Cl^- and of the ionic strength. The results of this measurement are shown in Table II. v_{max} did not change appreciably and K_m and k_1 changed but slightly with the tetramethyl ammonium chloride concentration. The observed insensitivity of the kinetic constants to the increase of tetramethyl ammonium chloride concentration suggest that the changes observed in the kinetic constants under the action of KCl or NH_4Cl are brought about mainly by the cations.

The effect of pH on the kinetic constants of K^+-activated ATPase can be seen from Figs. 6 and 7. Both v_{max} and K_m show a maximum at pH 8.5, but that is less pronounced for the latter. The curve for k_1 has two maxima, at pH 6.5 and pH 7.5, neither seems to be large enough to indicate a significant effect of pH on k_1.

The effects of the monovalent and the divalent cations on the kinetic constants were compared by measuring the myosin ATPase in the presence of 0.6 M KCl with



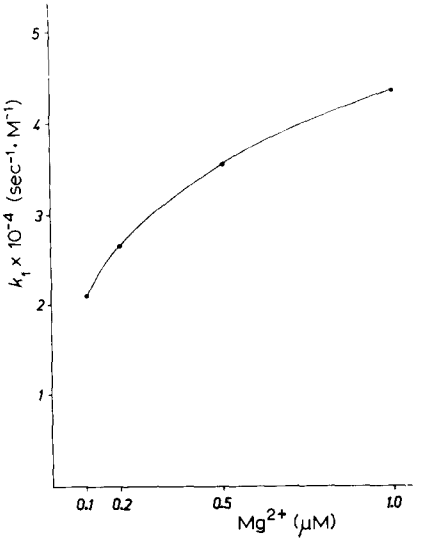


Fig. 8. Dependence of K_m and v_{max} in Mg^{2+}-mediated myosin ATPase on Mg^{2+} concentration. ATPase activity was measured at pH 8 in the presence of Mg^{2+}–EDTA buffer, 1 mM free EDTA and free Mg^{2+} concentration specified on the abscissa. For details of the ATPase measurement and evaluation of K_m and v_{max} see METHODS. ●, K_m; ×, v_{max}.

Fig. 9. Dependence of k_1 in Mg^{2+}-mediated myosin ATPase on Mg^{2+} concentration. ATPase activity measurement see Fig. 8. k_1 was evaluated using the formula $k_1 = (v_{max} \cdot k_{-1})/K_m \cdot E_t \cdot k_{-1}$ was taken to be 0.35 sec^{-1} as evaluated from Fig. 1.

upon the addition of Mg^{2+} at different concentrations. As apparent from Figs. 8 and 9, the values of K_m and v_{max} show a sharp decrease while that of k_1 a slight but constant increase as the Mg^{2+} concentration increases.

DISCUSSION

The K_m and v_{max} values of monovalent cation activated myosin ATPase presented in this paper are in agreement with the relatively few data published already in the literature mainly about K^+ (refs. 4, 12 and 15) and NH_4^+ (ref. 2) activated myosin ATPase.

The plot of SLATER AND BONNER[22] was used for the calculation of the individual rate constants and dissociation constant of myosin ATPase activated by different species of alkali cations. The condition of the application of this plot is that k_1 and k_{-1} has to be insensitive to the species of activator. This seems to be valid in the experiment presented in Fig. 1 as linear relationship between K_m and v_{max} would be hardly probable in any other case and our k_1 value agrees well with that of LYMN AND TAYLOR[4] obtained by an essential different method. MORITA[14] measured the rate constants of the Mg^{2+}-mediated ATPase of H-meromyosin using the ATP induced difference spectrum for the measurement. The thus obtained k_1 is higher than the present value, but k_{-1} is practically the same as obtained here. This indicates a practical insensitivity of k_{-1} to the conditions of the measurement of the monovalent cation or Mg^{2+}-activated ATPases.

An interesting observation was the opposite effect of K^+ or NH_4^+ on the rate constants k_2 and k_1. The former increases, the latter decreases with increasing salt concentrations, the decrease being particularly striking in the case of K^+. The increase in k_2 has been already observed[2,5,6,23] but no data have been reported on the decrease in k_1. Considering the above mentioned insensitivity of k_{-1} to the experimental conditions, it seems reasonable to assume that k_{-1} does not change appreciably with increasing monovalent cation concentrations and in this case the decrease in k_1 means the decrease in the stability of the enzyme–substrate complex. Mg^{2+}, on the other hand, causes k_2 to decrease and k_1 to increase with increasing Mg^{2+} concentrations. This increase in k_1 with increasing Mg^{2+} concentration was observed by MORITA[14], too. It follows from the conclusion made in the case of K^+ and NH_4^+ that the presence of Mg^{2+} stabilizes the enzyme–substrate complex. This stabilizing effect of Mg^{2+} on the myosin–ATP complex may have some functional role in the molecular mechanism of muscular contraction.

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