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MECHANISM OF MUSCULAR CONTRACTION#

II. KINETIC STUDIES ON MUSCLE ATP-ASE##

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Since the discovery of the splitting of ATP in the presence of actomyosin, and of the deformation of actomyosin particles by the addition of ATP, various attempts have been made to explain the muscular contraction in terms of the interaction of actomyosin with ATP.

In the previous paper (*1*), it was shown that the change in light-scattering of actomyosin solution caused by addition of ATP can be divided into three steps as follows:

First step: $AM+S \rightleftarrows AMS \longrightarrow AM^*S$###

Second Step: $AM^*S \longrightarrow AM^*+P$

$AM^*+S \rightleftarrows AM^*S$

Third step: $AM^* \longrightarrow AM$.

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In this paper, the following abbreviations are used: ATP=adenosine triphosphate, ADP=adenosine diphosphate, M=myosin, AM=actomyosin, P=inorganic ortho-phosphate (or plus ADP).

In the previous paper (*1*), the following mechanism was proposed:

$$AM+S \longrightarrow AMS \longrightarrow AM^*S$$
$$\searrow AM+P$$

The modification adopted in this paper is believed to be better based on the fact that the speed of the first step is not influenced by the addition of calcium ions (*cf.* (*1*) Fig. 13a.). Corresponding to this change of our picture, parts of Table VI in the previous paper must be corrected as follows:

Reaction	Velocity Constant	
$AM+S \longrightarrow AMS$	${}_kk={}_{Ca}k={}_{Mg}k=$	10×10^4 (*lit./mole sec.*)
$AMS \longrightarrow AM+S$	${}_kk={}_{Ca}k=2$	(*1/sec.*)

That is, the binding reaction of actomyosin with ATP is not affected by calcium and magnesium ions.

In this scheme, AM is a component unit of actomyosin which contains 140,000 gm. of myosin and its affix * represents the deformed state: then S and P stand for the substrate of the ATPase, *i.e.*, ATP plus H_2O and the products of enzymatic hydrolysis of ATP, *i.e.*, ADP plus inorganic phosphate, respectively. Further, our kinetic study of ATPase action (Second step) in the presence of ca. 0.2 *M* KCl revealed that the action is inhibited by Mg^{2+} and is activated by Ca^{2+} and that the relation of the ATP concentration to the reaction rate obeys the well known Michaelis-Menten's formula.

In this paper are presented further experiments on the kinetics of myosin-ATPase and natural actomyosin (myosin B)-ATPase.

EXPERIMENTAL PROCEDURES

Preparation of Materials—ATP and myosin B (actomyosin) were prepared as previously described (*1*). Purified myosin was prepared according to Szent-Gyorgyi's method (*2*).

Determination of ATPase Activity—The enzyme reaction was started by adding 0.5 ml. of K-ATP solution to 2.5 ml. of the enzyme solution containing salts and buffers as to give the final concentration of salts and pH value desired (the concentration of the enzyme in the reaction mixture was about 0.1∼0.3 mg. protein per ml.). At certain intervals, the reaction was stopped by adding 1.0 ml. of 10 per cent trichloracetic acid. Other procedures were identical with those described in the previous paper (*1*).

EFFECTS OF MAGNESIUM AND CALCIUM IONS ON ATP-ASE ACTION

It is well known that the effects of divalent ions, especially that of Mg^{2+}, on the muscle ATPase are greatly modified by the KCl concentration (3). In the present work, the effects of Mg^{2+} and Ca^{2+} on actomyosin-ATPase were investigated its relation to the level of KCl concentration in the reaction medium and also the antagonism between Mg^{2+} and Ca^{2+} was studied. The concentrations of ATP used in these experiments were about 10^{-3} *M*, taking acount on the situation that the activity of ATPase was approximately independent of the ATP concentration at about 10^{-3} *M*.

Effects of Calcium Ion—As can be seen in Fig. 1 a-b, Ca^{2+} strongly enhances the ATPase actions of both actomyosin and myosin in the presence of all concentrations of KCl investigated. The ratio of the velocity of ATPase action in the presence of less than 10^{-2} *M* Ca^{2+} (v_{Ca}) to that of the control (in the absence of Ca^{2+}) (v_K) is given by the

equation:

$$\frac{v_{Ca}}{v_K}=1-\frac{1-\Delta_{Ca}}{1+\frac{K_{Ca}}{[Ca]}}.$$

This means that the ATPase activity of actomyosin (or myosin) unit increases to Δ_{Ca} times that of the control on binding with one mole of Ca^{2+} and the dissociation constant for this binding reaction is K_{Ca}. For example, Δ_{Ca} and K_{Ca} of actomyosin (myosin B)-ATPase at pH 9.2, 10°, in the presence of 0.15 *M* ($K^+ + Na^+$), are 13 and $10^{-2.4}$ *M* respectively (Fig. 1a). Hereafter, the binding point of myosin and actomyosin responsible for the combination with Ca^{2+} in question ($pK=2.4$) will be termed " the first ion-binding point " and the resulting Ca-complexes will be denoted by Ca(I)-M and Ca(I)-AM*. A relationship of the ATPase activity of Ca(I)-AM* to the KCl concentration is roughly shown in Fig. 2.

In the presence of Ca^{2+} in concentrations higher than about 5×10^{-2} *M*, the ATPase activity decreases with the increase of the Ca^{2+} concentration. This inhibitory effect is not discussed in this paper.

Effects of Magnesium Ion and Antagonism between Magnesium and Calcium Ions at Higher K-concentrations—As already reported by Banga *et al.* (*3*),

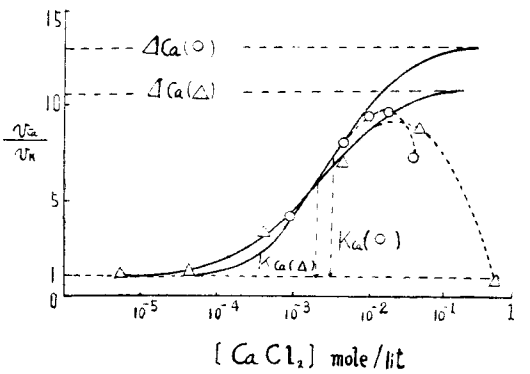


FIG. 1a. Effect of calcium ion on actomyosin-ATPase
(○) pH 9.2 (glycine), 10°, $[K^+]=0.15$ *mole/lit.*,
$[ATP]=1\times10^{-3}$ *mole/lit.*
(▲) pH 6.5 (veronal-acetate), 22°,
$[K^+ + Na^+]=0.08$ *mole/lit.*,
$[ATP]=1.17\times10^{-3}$ *mole/lit.*.

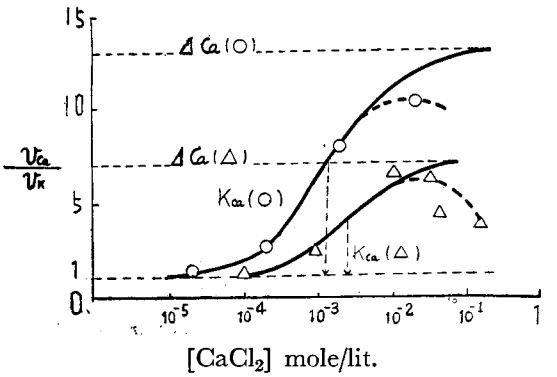


FIG. 1b. Effect of calcium ion on myosin-ATPase
(○) pH 6.6 (veronal-acetate), 20.5°
$[K^+ + Na^+] = 0.073$ *mole/lit.*
$[ATP] = 1.67 \times 10^{-3}$ *mole/lit.*
(△) pH 7.0 (veronal-acetate), 20°
$[K^+ + Na^+] = 0.077$ *mole/lit.*
$[ATP] = 1.69 \times 10^{-3}$ *mole/lit.*.

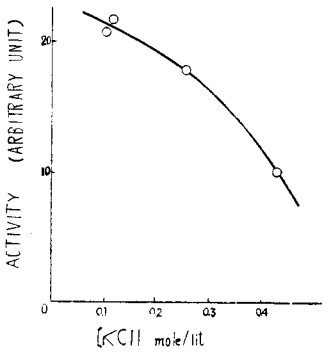


FIG. 2. Effect of the KCl concentration on actomyosin-ATPase in the presence of calcium ion. pH 6.5 (veronal-acetate), 18.7°

the effects of Mg^{2+} on actomyosin-ATPase are strikingly influenced by the concentration of KCl. In the presence of KCl over 0.1 *M*, the ATPase action of actomyosin is inhibited by Mg^{2+} and, as will be seen in Fig. 3a, the ratio of the velocity of its ATPase action in the presence of varied $MgCl_2$ concentrations less than 10^{-3} *M* (v_{Mg}) to that of the control (v_K) is approximately given by:

$$\frac{v_{Mg}}{v_K}=1-\frac{1-\Delta_{Mg}}{1+\frac{K_{Mg}}{[Mg]}}$$

where Δ_{Mg}, unlike Δ_{Ca} described above, is very much smaller than unity. That is, the ATPase activity of an actomyosin unit is depressed to Δ_{Mg} times that of the control on binding with one mole of Mg^{2+} and the dissociation constant for the binding of actomyosin (or myosin) with Mg^{2+} is K_{Mg}. For example, Δ_{Mg} and K_{Mg} at pH 9.2, 10°, in the presence of 0.15 M ($K^+ + Na^+$) are 0.2 and $10^{-4.4}$ M, respectively (Fig. 3a).

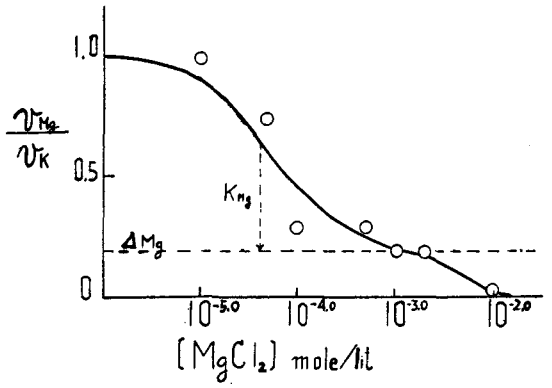


FIG. 3a. Effect of magnesium ion on actomyosin-ATPase (I).
pH 9.2 (glycine), 10°, $[K^+]=0.15$ *mole/lit.*
$[ATP]=1\times10^{-3}$ *mole/lit.*.

The fact that 10^{-2} *mole/lit.* Mg^{2+} caused stronger inhibition than 10^{-3} *mole/lit.* Mg^{2+} may be supposed to be due to the formation of Mg (I)Mg(II)-AM.* (*cf.* Page 393)

In the presence of a certain concentration of Ca^{2+} ($[Ca^{2+}]\gg K_{Ca}$), addition of Mg^{2+} results in an inhibition of ATPase activity. This inhibition was investigated in detail in the foregoing work (*1*) and found to be competetitive with regard to Ca^{2+} and Mg^{2+}. In this case (see (*1*) Fig. 5, 6), a similar equation to that described above may be formulated approximately:

$$\frac{v_{Mg,Ca}}{v_{Ca}}=1-\frac{1-\Delta_{Mg,\,Ca}}{1+\frac{K_{Mg,\,Ca}[Ca]}{[Mg]}}$$

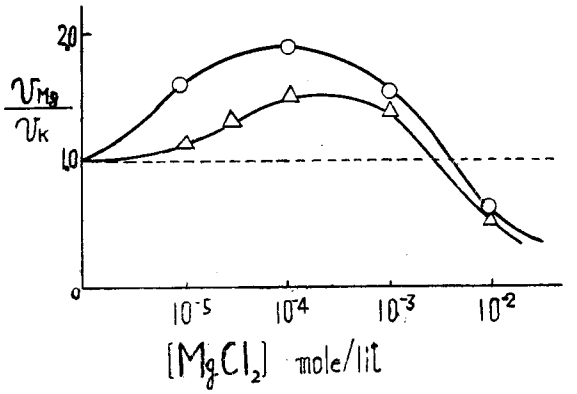


FIG. 3b. Effect of magnecium ion on actomyosin-ATPase (II)
pH 6.5 (veronal-acetate)
$[K^+ + Na^+] = 0.08$ *mole/lit.*
$[ATP] = 1.17 \times 10^{-3}$ *mole/lit.*
24° (○) and 20° (△).

Here, $\Delta_{Mg, Ca}$ in the presence KCl over 0.1 *M* is very much smaller than 1 and $K_{Mg, Ca}$ at pH 9.2, 10° in the presence of 0.15 *M* ($K^+ + Na^+$) is 10^{-2} *M* which is equal to the ratio of K_{Mg} ($10^{-4.4}$) to K_{Ca} ($10^{-2.4}$) under the identical experimental conditions as derived from the experiments on the Mg-inhibition (Fig. 3a) and Ca-activation (Fig. 1a). The latter fact suggests that the Mg^{2+}-binding point of actomyosin with $pK=4.4$ is identical with the first Ca^{2+} -binding point with $pK=2.4$ mentioned above.

Effect of Magnesium Ion and Antagonism between Magnesium and Calcium Ions at Lower KCl-Concentrations—As shown in Fig. 3b, in the presence of KCl less than 0.1 *M*, addition of Mg^{2+} ($10^{-5} \sim 10^{-3}$ *M*) stimulates the ATPase action of actomyosin‡ but an inhibitory effect is observed when the concentration of Mg^{2+} is increased to 10^{-2} *M*. These facts are probably due to the situation that, in these concentrations of KCl, the enzymatic activity of Mg(I)-actomyosinate is stronger than that of K-actomyosinate (about 1.5~2 times) and, when another mole of Mg^{2+} combines with Mg(I)-actomyosinate (the dissociation constant of this binding is about $10^{-2.5}$), the enzymatic activity of Mg-acto-

‡ The magnitude of this activation depends not only upon the KCl concentration but also on the preparative conditions of enzyme which are uncontrollable for us.

myosinate becomes less than that of K-actomyosinate (about one half that of K-actomyosinate). This Mg^{2+}-binding point of actomyosin with $pK=2.5$ will be termed " the second ion-binding point." As can be seen in Fig. 4, the pH-activity curve of Mg(I)-actomyosinate has one maximum at about pH 7.5 whereas that of Ca(I)-actomyosinate has been shown to have two maxima at pH 6.3 and 9.7 (see (*I*) Fig. 4).

Now, it is very interesting that, in the presence of 10^{-2} *M* Mg^{2+}, a concentration enough to set all the AM molecules in combination with Mg^{2+} (Mg(I)Mg(II)-AM*), the addition of a small amount of Ca^{2+} (10^{-3} *M*) causes the increase of the enzymatic activity of actomyosin as shown in Fig. 5.# It is unlikely that this effect is caused by the partial substitution of Mg^{2+} in the first ion-binding point by Ca^{2+} because the ratio of $[Ca^{2+}]$ to $[Mg^{2+}]$ (1/10 in this experiment) is as low as about one-thousandth of the ratio of the dissociation constants for the Ca^{2+} binding to that for the Mg^{2+} binding with the first ion-binding point (60$\sim$180, see Figs 1a, 3a). Further, as shown in Fig. 5, the ATPase activity is unchanged over the wide rage of Ca^{2+} concentrations added ($10^{-3}\sim3\times10^{-2}$ *M*) and the activity begins to rise when the Ca^{2+} concentration increases to such height (more than 10^{-1} *M*) that the substitution at the first ion-binding point is expected to take place. Hence, the above effect is probably derived from the conversion of Mg(I)Mg(II)-actomyosinate## into Mg(I)Ca(II)-actomyosinate as the affinity of Ca^{2+} to the second ion-binding point (in contrast with that to the first ion-binding point) is much stronger than that of Mg^{2+}.### Similar effects are observed also in the presence of 0.15 *M* KCl and the activity of Mg(I)Ca(II)-AM* is, in this case, about seven times stronger than that of Mg(I)Mg(II)-AM.*####

This activation is markedly dependent upon uncontrollable conditions of the enzyme preparations. The slight inhibition is occasionally observed. The investigation of this point must be the subject of the future research.

Mg(I)Mg(II)-actomyosinate represents the actomyosin combined with Mg^{2+} in both first and second ion-binding points. If the similar expression is to be applied to the already mentioned Ca-actomyosinate or Ca(I)-actomyosinate, it should be expressed as Ca(I)Ca(II)-actomyosinate.

Since the addition of 10^{-3} *M* Ca^{2+} in the presence of 10^{-2} *M* Mg^{2+} causes the complete substitution of Mg^{2+} in the second ion-binding point by Ca^{2+}, it follows that the dissociation constant for the binding of Ca^{2+} with the second ion-binding point is sufficiently smaller than ($10^{-2.5}\times10^{-3}/10^{-2}=$) $10^{-3.5}$.

In this case, the activity of Mg(I)Ca(II)-AM* is high as compared with that of Mg(I)Mg(II)-AM* but is only about one-fifth of that of K-AM*.

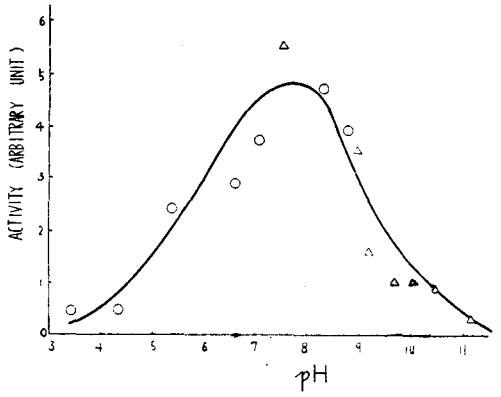


FIG. 4. pH-activity curve of Mg^{2+}-activated actomyosin-ATPase.
24°, $[K^+ + Na^+] = 0.07$ *mole/lit.*
$[ATP] = 1.17 \times 10^{-3}$ *mole/lit.*
$[MgCl_2] = 1.1 \times 10^{-4}$ *mole/lit.*
(○): veronal-acetate buffer (△): glycine buffer

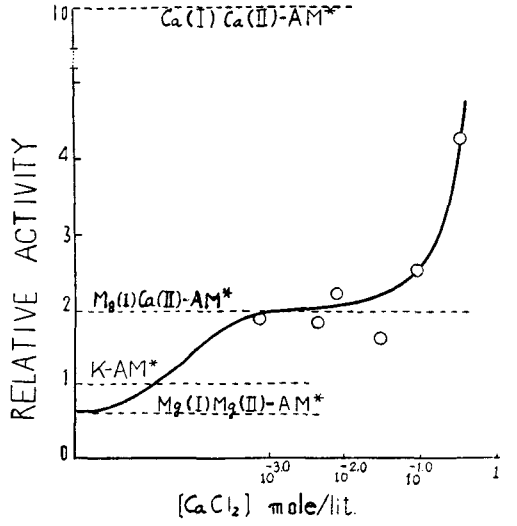


FIG. 5. Activation of actomyosin-ATPase by the addition of Ca^{2+} ion in the presence of Mg^{2+} ion.
pH 6.5 (veronal-acetate), 22°
$[K^+ + Na^+] = 0.09 \sim 0.1$ *mole/lit.*
$[MgCl_2] = 1 \times 10^{-2.0}$ *mole/lit.*

Whatever the mechanism of the above phenomena may be, this activating effect of a small amount of Ca^{2+} on the ATPase action in the presence of Mg^{2+} and K^+ in the physiological concentrations (10^{-2} *M* and 0.1~0.15 *M* respectively) seems to offer an important key to the understanding of the role of these ions in the muscular contraction. This will be discussed later in this paper.

Some properties of various Mg^{2+}-and Ca^{2+}-actomyosinates in question thus far derived from our experimental results are summarized in Table I.

TABLE I

Complex	pK#	Optimum pH	Relative Activity	
			$[K^+ + Na^+] = 0.09 - 0.1M$	$0.15M$
K-AM*	–		1	1
Mg(I)-AM*	p$K_{Mg(I)} \fallingdotseq 4.5$	7.5	2	0.2
Mg(I)Mg(II)-AM*	p$K_{Mg(II)} \fallingdotseq 2.5$		0.6	0.03
Mg(I)Ca(II)-AM*	p$K_{Ca(II)} > 3.5$		2	0.2
Ca(I)Ca(II)-AM*	p$K_{Ca(I)} \fallingdotseq 2.5$	6.4, 9.7	10	13

p$K_{Mg(I)}$ represents the logarithm of the reciprocal of the dissociation constant for the Mg^{2+} binding at the first ion-binding point and so on.

THE RELATIONSHIP BETWEEN THE CONCENTRATION OF ATP AND THE VELOCITY OF ATP-ASE ACTION

Ouellet *et al.* (*4*) and the present authors (*1*) have already shown that the relationship of the concentration of ATP (less than 10^{-3} *M*) to the ATPase activity (v) of actomyosin in the presence of Ca^{2+} is given by the so-called Michaelis-Menten's formula:

$$v = \frac{V_{max}}{1 + \frac{K_m}{[S]}}$$

Either groups of investigators accord in recognizing that the addition of Mg^{2+} thereto (in the presence of $Ca^{2+} + Mg^{2+}$) leaves Km unchanged. This fact means that these ions have no effect on the binding reaction between actomyosin and ATP. The present authors have reinvestigated the relation further in detail and obtained the following results.

Effect of Calcium Ion on Michelis Constant—As can be seen in Fig. 6a, Km of actomyosin-ATPase action is nearly doubled by the addition of

Ca^{2+}: Km of K-AM* at pH 6.5 (veronal-acetate), 25°, in the presence of 0.15 M K^+ plus Na^+ is 1.5×10^{-4} *mole/lit.* and that of Ca-AM* is 3.6×10^{-4} and 3.3×10^{-4} *mole/lit.* (in the absence and presence of glycine, respectively).#

It is well known that the Michaelis constant Km is equal to $(k_1{*}'+k_2{*})/k_1{*}$ when the mechanism of ATPase action is as follows:

$$\mathrm{AM{*}+S}\underset{k_1{*}'}{\overset{k_1{*}}{\rightleftarrows}}\mathrm{AM{*}S}$$

$$\mathrm{AM{*}S}\xrightarrow{k_2{*}}\mathrm{AM{*}+P}$$

Since Ca^{2+} has no effect on the binding reaction between actomyosin and ATP (for the light-scattering experiment, see (*1*) and p. 387 in this report), the increase of Km upon the addition of Ca^{2+} is probably due to the increase of $k_2{*}$. From the values of Vmax, $k_2{*}$ of K-AM* is 0.4 1/*sec.* and $k_2{*}$ of Ca-AM* is 3 1/*sec.* By substituting these $k_2{*}$ values into the above Km, it is calculated that $k_1{*}$ and $k_1{*}'$ are 1.3×10^4 *lit./mole. sec.* and 1.5 1/*sec.* respectively; these values show good agreement with those of the undeformed actomyosin which were obtained from the light-scattering study ($k_1=10\times10^4$ *lit./mole. sec.* and $k_1'=2$ 1/*sec.*, see (*1*) Table VI). Some differences between these values seem to be due to the situation that the light-scattering experiment was conducted in the presence of 0.5 M KCl and, on the other hand, the ATPase activity was measured in the presence of 0.15 M $[Na^+ + K^+]$. Ouellet *et al.* (*4*) showed that $k_1'{*}/k_1{*}=1.3\times10^{-5}$ *mole/lit.* in the presence of 0.6 M KCl. This value is in very good agreement with $k_1'/k_1=2\times10^{-5}$ *mole/lit.* in our light-scattering experiment.

As can be seen in Fig. 6a–b, *Km* of Ca-actomyosinate decreases with the decrease of temperature.##

Effect of higher concentrations of ATP—As shown in Fig. 7, the ATPase activity decreases at ATP concentrations higher than 10^{-3} *mole/lit.*###

Due to the difficulty of estimation of ATPase activity, too much reliance is not allowed as to the accuracy of Km values obtained.

This is incompatible with the result of Ouellet *et al.* (*4*). Their result shows that *Km* increases with the decreases of temperature. This is probably due to the difference of the Ca^{2+}- and K^+-concentrations applied.

H. H. Weber (*5*) described the similar effect of the ATP concentration on the extracted muscle fiber-ATPase.

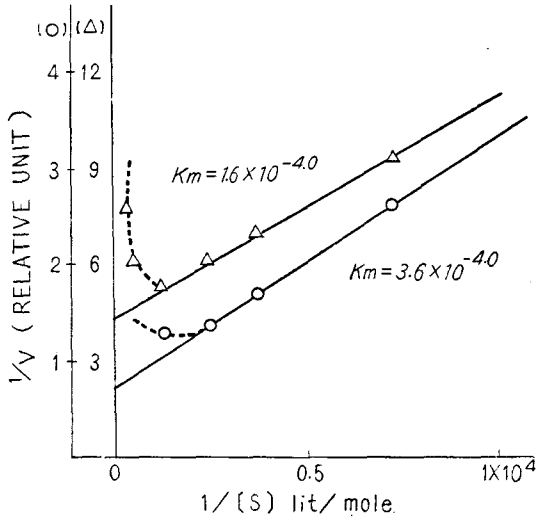


FIG. 6a. Estimation of the Michaelis constant of actomyosin-ATPase (I).
pH 6.5 (veronal-acetate), 25°,
$[K^+ + Na^+] = 0.15$ *mole/lit.* (△)
$[K^+ + Na^+] = 0.15$ *mole/lit.* + $[CaClc_2] = 5 \times 10^{-2}$ *mole/lit.* (○).

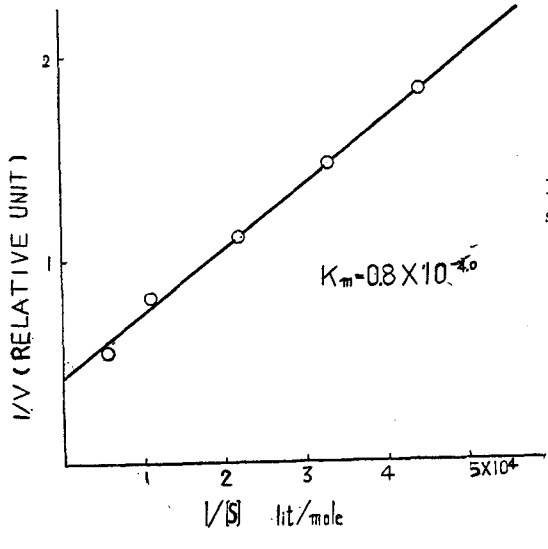


FIG. 6b. Estimation of the Michaelis constant of actomyosin-ATPase (II).
pH 6.3 (veronal-acetate). 6°,
$[K^+ + Na^+] = 1.9 \times 10^{-1}$ *mole/lit.*
$[CaCl_2] = 1.6 \times 10^{-2.0}$ *mole/lit.*
[AM] = 0.35 mg. protein/ml.

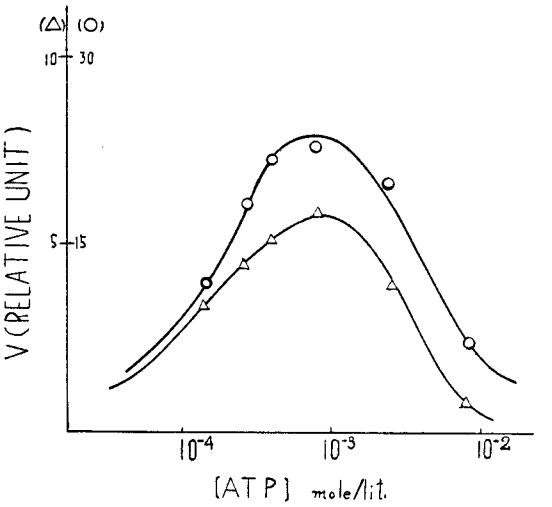
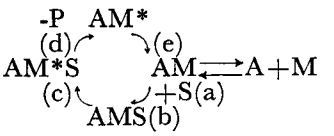


FIG. 7. Relationship between ATPase activity and ATP concentration.
pH 6.5 (veronal-acetate), 25°.,
$[K^+ + Na^+] = 0.15$ *mole/lit.* (△).
$[K^+ + Na^+] = 0.15$ *mole/lit.* $+ [CaCl_2] = 5 \times 10^{-2}$ *mole/lit.* (○).

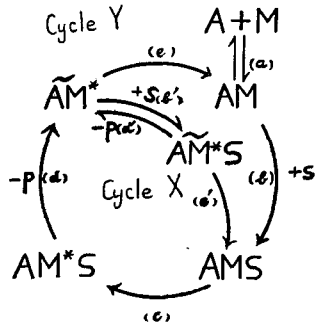
DISCUSSION

In the previous paper (*1*), the interaction between actomyosin and ATP (in the presence of 0.5 *M* KCl) has been represented schematically as follows:



However, it is well known (*2*) that ATP causes the so-called " superprecipitation " of actomyosin in the presence of about 0.1 *M* KCl, a phenomenon considered to represent a model of muscular contraction and, on the other hand, inorganic pyrophosphate deforms AM to AM* but does not cause the superprecipitation of actomyosin. Considering these facts, it may be natural to presume that upon the splitting of ATP, actomyosin becomes energy-rich and actomyosin in such a state has a certain life; further it seems that the superprecipitation is produced by

the polymerization of energy-rich actomyosin. If this be the case, the interaction of actomyosin to ATP may be shown schematically as follows:



(Affix~represents the energy-rich state.)

In this schema, the outside cycle Y (b, c, d, e) corresponds to the sequence of a single muscular twitch (see (*1*)) and the inside cycle X (c, d, b′, d′, e′) corresponds to that of the contraction in general. Therefore, if the polymerization of energy-rich actomyosin $\widetilde{AM}$* were sufficiently speedy in comparison with the ATPase action (d,d′) and the concentration of the contracted actomyosin [AMcont] is equal to [$\widetilde{AM}$*] plus [$\widetilde{AM}$*S], the following relationships should hold for the reaction cycle in the presence of the sufficient amount of ATP and Mg^{2+},

$$k_c \gg k_d;\ k_b'\,[S] \gg k_d,\ k_d'.\#$$

Accordingly, in the steady state of reaction,

$$[AMS] \ll [AM^*S],\ [\widetilde{AM}^*] \ll [\widetilde{AM}^*S].$$

That is,

$$[AM_{cont}] \fallingdotseq [\widetilde{AM}^*S],\quad [AM_{rel}]\#\# \fallingdotseq [AM^*S].$$

Hence,

$$\frac{d[AM_{cont}]}{dt} = k_d[AM^*S] - k_e,\ [\widetilde{AM}^*S]$$

$$= k_d[AM_{rel}] - k_e,\ [AM_{cont}] = 0,\#\#\#$$

$$\frac{[AM_{cont}]}{[AM_{rel}]} \fallingdotseq \frac{k_d}{k_e'}$$

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k_c, k_d, k_b', k_d, are the velocity constants of the reaction steps (c), (d), (b′) and (d′) in the schema proposed above. *Cf.* (*1*), pages 48, 54.

[AM rel] represents the concentration of the relaxed actomyosin.

(### See next page)

As is evident from the equation just derived, any alteration in the condition which would cause any increase in the value of k_d should raise the ratio $[AM_{cont}]$ / $[AM_{rel}]$ and hence lead to a circumstance more favourable for the contraction of actomyosin and *vice versa*.

In the previous paper (*1*), it has been shown that Ca^{2+} and Mg^{2+} exert scarecely influence upon the life of $\widetilde{AM}^*$, *i.e.*, upon the velocity of the reaction $AM^* \rightarrow AM$. Accordingly, it may be deduced that the life of $\widetilde{AM}^*S$, *i.e.*, the velocity constant k_e' of the reaction $\widetilde{AM}^*S \rightarrow AM^*S$ is scarcely affected by these cations. If so, under the ionic conditions in which k_d is large (*i.e.*, the ATPase activity is high), actomyosin will contract, and under the ionic conditions in which ATPase activity is low, actomyosin will relax. In this connection, Korey (*6*) showed that the fibers preserved in glycerol retain their ability to contract when their ATPase activity is above a difinite value and below this level, contraction is absent. Perry (*7*) observed that shortening of myofibrils is most marked at approximately that concentration of $MgCl_2$ which gives optimum ATPase activity.

Recently, Bozler (*8*) has found in the presence of 0.16 *M* KCl and 2 per cent ATP that Mg^{2+} (10^{-2} *M*) was required for the relaxation of the glycerol-treated muscle fiber and that Ca^{2+} in very low concentration (0.5×10^{-3} *M*) produced a rapid contraction. The present authors showed in the former section that the ATPase action of some actomyosin preparations is inhibited by 10^{-2} *M* Mg^{2+} and the addition of 10^{-3} *M* Ca^{2+} thereto results in a reactivation to a level several times (3~7 times) that of the ATPase activity in the presence of Mg^{2+} alone. Bozler's result may be explained by supposing the same behavior of

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Foot noted (continued)

For convenience's sake, the polymeryzation process $n\widetilde{AM}^*S \rightleftarrows (\widetilde{AM}_{cont})$, the mechanisms of which has not yet been ascertained, is neglected in the present consideration. However, if the muscle contraction is brought about by the polymerization of $n\widetilde{AM}^*S$ and the equilibrium of the polymerization is rapidly established, and if the inactivation process of polymers occurs much slower than that of monomer, so that it may be neglected, the following relations may exist in the steady state:

$$\frac{d\,[AM^*S]}{dt} = k_e'\,[\widetilde{AM}^*S] - k_d\,[AM^*S] = 0, \qquad \frac{[AM^*S]_n}{[AM_{cont}]} = K.$$

Hence

$$\frac{\sqrt[n]{[AM_{cont}]}}{[AM_{rel}]} = \frac{k_d}{k_e'\sqrt[n]{K}}$$

This relation shows that when n is large even a minute change of k_d (or k_e') causes a vast change in [AMcont]/[AM rel].

Mg^{2+} in the reaction of actomyosin-ATPase of their glycerol-treated muscle fibers.

Bozler (*9*) has found also that in the presence of Mg^{2+} ,a high concentration (2 per cent) of ATP was required for the relaxation of the glycerol-treated muscle fiber. This is also in good accordance to our result that the higher concentrations of ATP inhibit the ATPase action.

Contraction of intact muscle seems also not to differ, in this respect, from that in glycerol-treated muscle: Ca^{2+}, at isosmotic concentrations, causes shortening of the intact muscle fiber while Mg^{2+} prevents it (*10*).

It is expected that the simple correspondence of the ATPase activity to the intensity of contraction ($[AM_{cont}]/[AM_{rel}] \fallingdotseq k_d/k_e'$) can be observed only in such a case that the polymerization of energy-rich actomyosin $\widetilde{AM}$*S proceeds very rapidly as compared with the ATPase action, while such a correspondence can not be observed when the speed of the polymerization process is slower than that of ATPase action. In this connection, Bowen (*11*, *12*) observed that phosphorylysis does not occur directly concomitantly with the shortening of myosin B threads. Bowen's result is probably an example of the latter case.

SUMMARY

1. Effects of Mg^{2+} and Ca^{2+} on muscle ATPase were investigated in detail. It was noticed that in some actomyosin prparations a small amount of Ca^{2+} (10^{-3} *M*) enhances the ATPase action of actomyosin in the presence of physiological concentrations of K^+ (*ca.* 0.1 *M*) and Mg^{2+} (10^{-2} *M*).

2. At lower concentrations (less than 5×10^{-4} *M*) of ATP, the relation of ATPase activity to the ATP concentration obeys Michelis-Menten's formula. Based on the change of *Km* caused by the addition of Ca^{2+}, the velocity constants k_1* and k_1*', of the binding reaction between the deformed actomyosin (AM*) and ATP (S), *viz.*,

$$AM^* + S \underset{k_1^{*\prime}}{\overset{k_1^*}{\rightleftarrows}} AM^*S$$

were calculated. These calculated values were roughly in accord with those of the binding reaction between ATP and undeformed actomyosin which were estimated in the previous paper (*1*) by measuring the light-scattering change of actomyosin solution.

3. At the concentration of ATP higher than 10^{-3} *M*, the ATPase activity decreases with the increase of the ATP concentration.

4. Comparing these results with the effects of ATP, Ca^{2+} and Mg^{2+} on the contraction and relaxation of the glycerol-treated muscle fiber, it was deduced that the muscular contraction is equivalent to the polymerization of energy-rich actomyosin which is produced by the splitting of ATP.

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ADDENDUM

After this paper was written, we read the excellent review by Weber and Portzehl (*Advances in Protein Chem.*, **8**, 161 (1952)) entitled "Muscle Contraction and Fibrous Muscle Protein". They have also regarded the breakdown of ATP as the cause of contraction. Their view is chiefly based on the experiments concerning the effects of inorganic polyphosphates and Salyrgan which are non-physiological substances or poisons, while our view is based on the experiments concerning the effects of calcium and magnesium ions in physiological concentrations.

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