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# A MOLECULAR MECHANISM OF MUSCLE CONTRACTION

By

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## Introduction

Modern development in biochemistry on muscle contraction is founded on the well known works of ENGELHARDT and SZENT-GYÖRGYI. ENGELHARDT and LJUBIMOVA[1] have found that structural protein of muscle, myosin, catalyzes hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic orthophosphate ($P_i$). SZENT-GYÖRGYI and his associates[2] have shown that the muscle protein known in early literature as myosin consists of pure myosin and F-actin, and, subsequently, the more appropriate term actomyosin was adopted to denote the complex. Their extensive studies have also established the following important facts: (i) Actin exists in two states, globular (G) and fibrous (F) from, (ii) in 0.6 M KCl actomyosin shows decrease in viscosity on adding ATP while in 0.1M KCl the suspension of actomyosin becomes clear ("clearing response") and superprecipitates on addition of a large and a small amount of ATP, respectively, and (iii) "glycerinated muscle" which is obtained by treating psoas muscle with 50% glycerine aqueous solution is mostly composed of actomyosin and contracts in the presence of ATP. Further investigations to compare glycerinated muscle fibers with living muscle in their mechanical and chemical properties were substantially made by WEBER's group[3)4)] and enable us to assert that essentially the same reaction participates in the contraction of both living muscle and the glycerinated muscle fibers. On the basis of these ENGELHARDT-SZENT-GYÖRGYI-WEBER experiments, it has been generally accepted that the interaction between actomyosin and ATP is the primary reaction in muscle contraction. However, the reaction mechanisms of the hydrolysis of ATP by myosin (myosin ATPase reaction) and of the change in the structure of actomyosin by the addition of ATP are not fully elucidated, and the physiological roles of these two reactions in muscle contraction are still obscure. Because of its intricacy, it may be understandable that various have been proposed

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as biochemical mechanism of muscle contraction.

We have been engaged on the physicochemical study on the myosin-actin-ATP system since 1949, and have acute feeling of the necessity to reconsider the implications of experimental results. Thus, several principal proposals from other groups as mechanisms of muscle contraction are scrutinized on the basis of our results, and also in the present review is presented a new molecular mechanism that seems to be pertinent from our observations.

This review consists of the following four chapters. The first is devoted to the mechanism of the changes in the size and shape of actomyosin on addition of pyrophosphate ($PP_i$) or ATP and of the dependence of the ATPase activity at the steady state on the colloidal state of actomyosin. The mechanisms of muscle contraction proposed by MORALES and BOTTS and by WEBER are discussed from those results. The second deals with the phosphorylation of myosin by ATP and the importance of the phosphorylation in muscle contraction. From analysis of the effects of modifiers on the ATPase activity and of the effects of ATP analogs on the actomyosin systems, the phosphorylation of myosin is shown as the primary reaction of muscle contraction. The third is concerned with the structure and function of myosin molecule. Particularly, the dynamic changes in the molecular structure of myosin are shown under various conditions. In the fourth, a new molecular mechanism of muscle contraction is proposed mainly on the experimental results obtained in this laboratory and the "sliding model" of HANSON and HUXLEY.

## I. Interaction of Actomyosin and ATP

Actomyosin is obtainable not only by direct extraction from muscle but it can also be reconstituted from separately purified F-actin and myosin. The former is usually called as myosin B and the latter as reconstituted actomyosin. The determinations of dependence on angle of intensity of light scattering[5] and of rotatory diffusion constant[6] of myosin B have shown that the length of the class of main components of myosin B is comparably constant and its configuration can be represented as a worm-like chain of the length of 7,500 Å, though the components are heterogeneous with respect to the molecular weight ($6.6\text{–}26 \times 10^7$).

MORALES and BOTTS[7][8] have assumed entropy and the electrostatic repulsion as the main factors in determining the molecular shape of myosin B, based on; (i) constancy of molecular weight of myosin B in 0.6 M KCl in spite of its elongation on the absorption of ATP[9][10], and (ii) negative electronic charge of 3–4 of ATP at neutral pH. With the additional fact that the binding of ATP to myosin is stronger than that of ADP, they proposed a molecular model

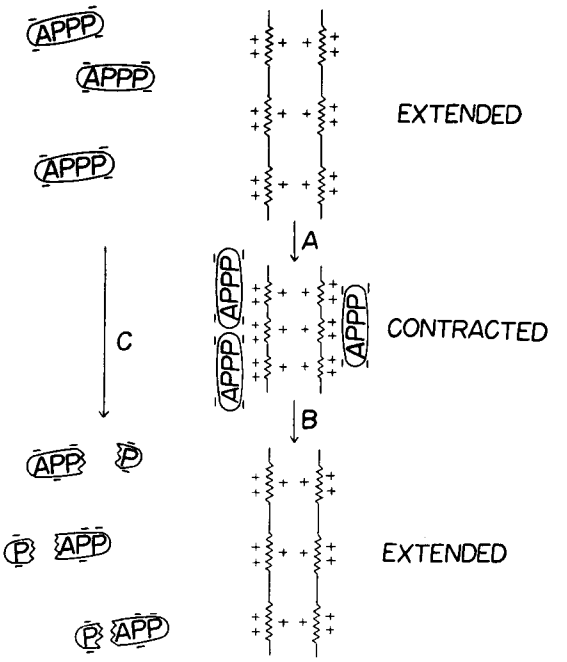


**Fig. 1.** Molecular mechanism of contraction of muscle model by ATP proposed by MORALES and BOTTS.

of muscle contraction, as shown in Fig. 1. Myosin B is negatively charged in 0.6 M KCl and at neutral pH. When negatively charged ATP is absorbed on myosin B, the protein is elongated by the increase of intramolecular electrostatic repulsive force. However, in the physiological medium where a concentration of Mg ion is high, myosin B is positively charged due to the absorption of Mg ion. When negatively charged ATP is absorbed on the positively charged myosin B, the electrostatic repulsion decreases and the protein is contracted by entropy effect. When ATP is hydrolyzed to ADP and $P_i$, the products are desorbed from myosin B, and the protein regains the positive charge and is extended by the electrostatic repulsion. This mechanism is based on clear concepts of physical chemistry and has been accepted as one of the best organized one.

As is well known, $PP_i$ produces the same changes in the size and shape of myosin B as ATP does. Since $PP_i$ is not hydrolyzed by myosin B-ATPase, whereas ATP is hydrolyzed, $PP_i$ is a favorable reagent for the elucidation of the molecular mechanism of the change of myosin B. According to our results[5)11)12)] on the effect of $PP_i$ on the intensity of light scattered by myosin

B solution, the conclusion of MORALES' group is correct at least in the changes in the size and shape of the class of main components of myosin B on the addition of $PP_i$. The salting-out analysis of myosin B[13] has also shown that myosin B does not dissociate into myosin and F-actin in the presence of $PP_i$.

However, there are several facts against the mechanism of MORALES and BOTTS: (i) The results of equilibrium dialysis and of light scattering studies[14] showed that the binding of 1 mole of $PP_i$ or ATP produces changes in shape of myosin B of $5.6 \times 10^5$ g, that is, 1 mole of $PP_i$ or ATP binds to 1 mole of myosin in myosin B particle, since myosin B is mostly composed by myosin and the molecular weight of myosin is $4\text{–}6 \times 10^5$. MORALES and his coworker calculated the amount of ATP bound to the contractile protein to contract muscle based on their mechanism, but the value estimated was several ten times as high as the one necessary to change the shape of myosin B determined as above. Moreover, they did not take into account the presence of a high concentration of Mg ion in the physiological medium. At neutral pH and in the presence of Mg ion, ATP is actually in the form of $MgATP^{2-}$ or $MgATP^-$, whereas they assumed ATP as $ATP^{4-}$ or $ATP^{3-}$. Therefore, the decrease in electrostatic repulsive force by the absorption of $MgATP^{2-}$ or $MgATP^-$ must be much less than that calculated by MORALES and BOTTS. (ii) The method of light scattering was firstly applied by us[14a)15)16)] to the kinetic analysis of the reaction of myosin B with ATP. A more detailed investigation recently performed on the myosin B–$PP_i$ system[16a)] has given more strong support to our reaction mechanism on the myosin B–ATP system. The result showed that the reaction between myosin B and $PP_i$ consists of two steps; the binding step of the two molecules and the succeeding deformation step of the myosin B–$PP_i$ complex: $MB + PP_i \rightleftharpoons MB \cdot PP_i \rightleftharpoons MB^* \cdot PP_i$. One of the bonds between actin and myosin, probably an salt linkage containing $-NH_3^+$, is broken at the first step and consequently the structure of the myosin B–$PP_i$ complex becomes loose. The factor determining the molecular shape of the myosin B–$PP_i$ complex at the deformation step was shown to be large decreases in entropy and in electrostatic repulsive force as advocated by MORALES. The electrostatic repulsive force is not, however, caused directly by the binding of $PP_i$ or ATP but may be dominantly attributable to that existing between carboxyl groups of the protein, since Salyrgan, which contains no net charge, produced the same change in the shape of myosin B as $PP_i$ did, and since the equilibrium constant of the deformation step was independent of pH from 6.0 to 9.6.

CHURNEY[17] observed contraction of glycerinated muscle fibers by $SO_4^{2-}$, $Fe(CN)_6^{3-}$ or $Fe(CN)_6^{4-}$, and LAKI and BOWEN[18] by $I^-$ or $SCN^-$. In a series of ATP analogs, the reciprocal MICHAELIS constants ($\bar{K}_m$) of the hydrolysis and

the abilities of contracting the muscle model decreased in the same order of ATP, UTP, ITP and $TP_i$. MORALES and BOTTS[8] have regarded these results as the strong supports for their idea that the decrease in electrostatic repulsive force by absorption of anion is the primary event in muscle contraction. However, it is already well known that KI and KSCN can depolymerize F-actin, and it has also recently been observed by us[19] that the helical content of myosin is conspicuously decreased by the addition of several salts. Consequently the contraction produced by inorganic anion may not be in the close relation to the physiological contraction, even though observed phenomena are superficially similar to each other. Furthermore, according to our experiments using synthetic ATP analogs, there are several remarkable differences between the order of the values of $\overline{K}_m$ and of extents of contraction by these chemicals (see below). Therefore, MORALES' opinion that muscle contracts by the absorption of ATP due to the decrease in the electrostatic repulsive force is hardly acceptable, though his idea that the shape of myosin B is essentially determined by entropy and the electrostatic repulsion could be correct.

The class of main components of myosin B is elongated by the addition of ATP of $PP_i$, without changing the molecular weight, as described above. However, according to the ultra-centrifugal analysis[5], $PP_i$ dissociates 20% of myosin B (the class of dissociable components whose molecular weights are smaller than those of the main components) into F-actin and myosin. In the presence of $PP_i$ the viscosity of myosin B solution decreased immediately after subjection to shearing stress[20] and the rotatory diffusion constant of myosin B at a high velocity gradient was extremely larger in the presence than in the absence of ATP[6]. These results indicate that even the structure of the main components of myosin B becomes rather loose on the addition of ATP or $PP_i$. Gergely[21][22] has demonstrated that a larger portion of reconstituted actomyosin dissociates into F-actin and myosin on the addition of ATP or $PP_i$. As will be described later, the plasticizing effect of ATP on muscle model, an important step involved in muscle contraction, may be attributed to the breaking by ATP of a bond between F-actin and myosin.

Another famous proposal on the mechanism of muscle contraction, which is in opposition to the MORALES and BOTTS theory, is the one by H. H. WEBER. His proposal is based on the extensive experimental results obtained in his laboratory on the contraction of muscle models by ATP. PORTZEHL[23] contracted the muscle model by the addition of ATP and relaxed it by a further addition of a SH reagent. The relaxation was also obtained when an inorganic polyphosphate was added, which is not hydrolyzed by myosin. She determined the ATPase activity of the contracted and of the relaxed glycerinated fibers,

and observed that the activity of the former was much higher than that of the latter. A similar relation[25] between ATPase activity and contraction has later been observed with the MARSH factor[24], a physiological relaxing factor. ULBRECHT et al.[26][27] showed that the power of contraction of muscle fibers depends on temperature in the same way as the rate of splitting. HASSELBACH[28] observed that the velocities of hydrolysis and the tensions caused by various nucleotide triphosphates (NTP) are in the same decreasing order of ATP≈acetyl ATP≥CTP≫ITP>GTP. From these and several other results, WEBER[3] has drown the following three conclusions; (i) polyphosphate is binary functional on muscle model; the plasticizing effect and the contracting one, (ii) the plasticizing effect is produced by the breakdown of one bond in actomyosin caused by the absorption of polyphosphate, and (iii) the contraction is coupled with the hydrolysis of ATP or other NTP. The distinction of the function of NTP into the two effects is his remarkable contribution to muscle biochemistry, and his interpretation of the plasticizing effect is reasonable. However, other several investigations are disadvantageous to his last conclusion.

It is generally accepted that the "clearing response" of the suspension of actomyosin on the addition of a high concentration of ATP corresponds to the relaxation of muscle model[29]. For example, according to our own results[30], the "clearing response" was accelerated conspicuously by ethylenediamine tetraacetate (EDTA) and moderately by 1, 2-diaminocyclohexane-N, N'-tetraacetate or by $PP_i$, and was observed clearly in the presence of Mg ion, fairly in Mn ion and not in Ca ion. The "clearing response" produced by MgATP in the presence of EDTA was inhibited by a further addition of a minute amount of Ca ion. The results are in accord with those obtained on the relaxation of muscle model. At various temperatures and in the presence or the absence of a chelator, the activity of myosin B-ATPase was much higher in the superprecipitated state, which corresponds to contraction, than in the "clearing response", which corresponds to relaxation. But, BOWEN[31] showed that, under some conditions, ATP splitting and the contraction of muscle model can be dissociated, thereby disproving any simple coupling between these two processes. There is also a very doubtful point in the WEBER'S explanation; the contraction or the superprecipitation is caused by the high rate of ATP splitting and the relaxation or the "clearing response" by the inhibition[32] of ATPase with a high concentration of ATP.

It has been observed by us[30] that in the presence of an appropriate concentration of ATP the superprecipitation commences several minutes after ATP addition and the rate of ATP hydrolysis increases abruptly accomanying the appearance of superprecipitation, and that the activity of ATPase of super-

precipitated myosin B is exceedingly higher than that of "clearing response", even under the same experimental condition of both the systems except for the colloidal state of myosin B. From these findings it seems reasonable to conclude that, contrary to the WEBER theory, the colloidal state of myosin B is determined and as the result the activity of ATPase is changed by the concentration of ATP. Furthermore, the experiments using ATP analogs, which will be described later, distinctly dissociated the NTP splitting from the tension development.

WEBER has assumed that actomyosin dissociates into F-actin and myosin during the "clearing" phase, since in the presence of Mg ion the ATPase activity of myosin is much lower than that of actomyosin[32]. An analysis of effect of divalent cation and ATP analog on the "clearing response" of myosin B has also led us to conclude[30] that the binding of myosin to actin is weakened in the state of "clearing response", and that the so called substrate inhibition of ATPase is due to the transition in ATPase from the myosin type to the actomyosin type. Recently MARUYAMA and GERGELY[32a] and we[32b] have demonstrated by viscometric and ultra-centrifugal analyses that in "clearing response" reconstituted actomyosin is dissociated into myosin and F-actin. The dependence of ATPase activity (v) of myosin on the ionic radii (r) of divalent metal ions has been represented by us[33] as a bell-shaped curve with its maximum at 0.95 Å. In the presence of a divalent cation, of which radius is apart 0.3 Å from the maximum, myosin-ATPase is immensely at a low rate. The v-r curve of actomyosin-ATPase is much flatter than that of myosin-ATPase, though its maximum is also at 0.95 Å. Then, the activity of actomyosin-ATPase is remarkably higher than myosin-ATPase in the presence of a cation of radius of 0.65 Å, *i.e.* Mg ion. (The molecular mechanism of the transition of ATPase from the myosin type to the actomyosin one will be discussed later).

We[34][35] have recently demonstrated that Ca tightly bound to myosin is an essential factor for the "clearing response" of actomyosin. 2 moles of Ca and a trace amount of Mg were found in $4.2 \times 10^{5}$ g of myosin. When myosin was treated by p-chloromercuribenzoate (PCMB) and then by cysteine, the SH content of the myosin remained unchanged, but divalent cations were no longer detectable in the modified myosin. The ATPase activity of this modified myosin was the same as that of the original myosin at various experimental conditions and in the presence of a modifier, such as EDTA or PCMB. The removal of "intrinsic" metals from myosin produced no change in the viscosity, the sedimentation constant and the extent of binding to F-actin. Furthermore, it has recently been demonstrated[19] that the optical rotatory dispersion of myosin does

not change by the treatment with PCMB and $\beta$-mercaptoethanol. In spite of these similarities, actomyosin composed of the Ca free myosin and F-actin superprecipitated without showing the "clearing response" immediately after the addition of a high concentration of ATP in the presence of Mg ion and 0.075 M KCl. In addition, the ATPase activity of actomyosin reconstituted from the Ca free myosin was about 20 to 30 times as high as the control actomyosin. The ATPase activity and the colloidal state of actomyosin reconstituted from the Ca free myosin did not change even on the addition of EDTA in the presence of Mg ion and ATP. These results suggest that both the breakdown of one bond in actomyosin by the binding of ATP and the combination of a relaxing factor (high ATP, EDTA, or the MARSH factor*) to "intrinsic" Ca are necessary for the "clearing response" of actomyosin or the relaxation of muscle model.

BOZLER[40] has proposed the reaction scheme of a relaxing factor (Fig. 2), on the basis of his findings that the glycerinated fibers relax on the addition of EDTA in the presence of MgATP and contracts on the further addition of a trace amount of Ca ion and that EDTA binds to the fibers fairly tightly. BOZLER's mechanism accords essentially with our results. PARKER and GERGELY[41]

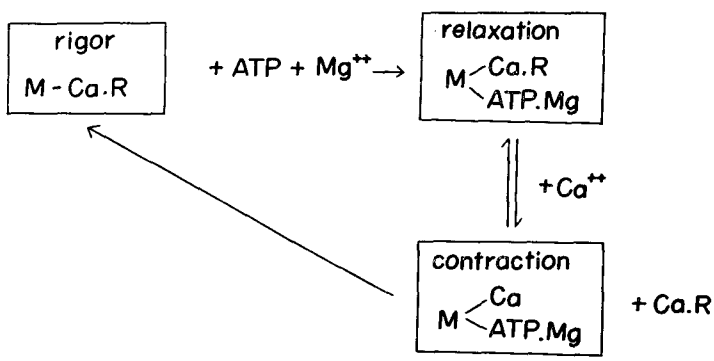


**Fig. 2.** Reaction mechanism of relaxing factor proposed by BOZLER. M and R represent muscle contractile protein and relaxing factor, respectively.

have recently observed the relaxation of muscle model by the relaxing factors after the removal of free Ca from the model and suggested that the Ca tightly bound to the protein is necessary to the relaxation. On the other hand, A. WEBER[42] and EBASHI[43] have separately observed the complete inhibition of the superprecipitation of actomyosin on the addition of MgATP by the exhaustive removal of free Ca ion by a chelator. In their experiments, the ionic strength of

*) See refs. 24, 36–39 for physiological relaxing factors.

the reaction medium was kept rather high, and the addition of Ca ion on the washed actomyosin showed only 3 times activation in its ATPase, whereas our modified actomyosin showed 20 to 30 times activation. So far it is not obvious whether the relaxing factor removes free Ca or it binds to the Ca tightly bound to myosin. This must be the most important point in the molecular explanation of muscle relaxation, but we will provisionally take the latter view, since this point is not essential to the mechanism of muscle contraction developed in this paper.

## II. Phosphorylation of Myosin as Primary Reaction of Contraction

As described in the preceding chapter, the primary event in muscle contraction cannot be attributed to the shape change of actomyosin by ATP or to ATP-splitting in the steady state. Therefore, it is necessary to find out a new reaction which is coupled with muscle contraction in a simple way. On this point, ULBRECHT *et al.*[44)45)] firstly made interesting observation that the exchange reaction between ATP and $ADP^{32}$ is catalyzed by myosin B and glycerinated muscle fibers, and ascribed this exchange reaction to the phosphorylation of actin (A) by ATP: $A+ATP \rightleftharpoons A\text{-}P+ADP$. On the other hand, HANSON and HUXLEY[46)47)] have demonstrated from their excellent electron-microscopic studies on muscle fibers that muscle contraction is due to a sliding of the actin filaments past the myosin filaments. On the basis of these two experimental results, WEBER[48)] has recently proposed his new reaction mechanism of muscle contraction (Fig. 3): An acid group of actin is phosphorylated by ATP, and this phosphate residue is exchanged with a sulfhydryl group of myosin and then the linkage between actin and myosin is displaced to a definite direction and consequently the actin filament slides past the myosin filament. The exchange reaction observed by ULBRECHT *et al.*, however, cannot be attributed to actin, because neither F-actin[44)] nor purified myosin B[49)] catalyzed the exchange reaction, but might be due to sarcosome contaminated in their preparations.[49a)]

The second important reaction is the initial rapid liberation of $P_i$ from the myosin-ATP system which was first observed by WEBER and HASSELBACH[50)]. This phenomenon has extensively been investigated by us[51-53)]. The rate of liberation of $P_i$ immediately after the addition of ATP was several times as high as that of the constant value ultimately attained at the steady state. The quantity of the initial burst, *i. e.*, the intercept of the ordinate obtained by extrapolating the linear $P_i$-liberation to zero time, was 1–2 moles per $10^5$ g of myosin. The initial burst of $P_i$-liberation was abolished by the addition of a SH reagent, such as PCMB or Oxarsan, and of a chelator. The initial burst

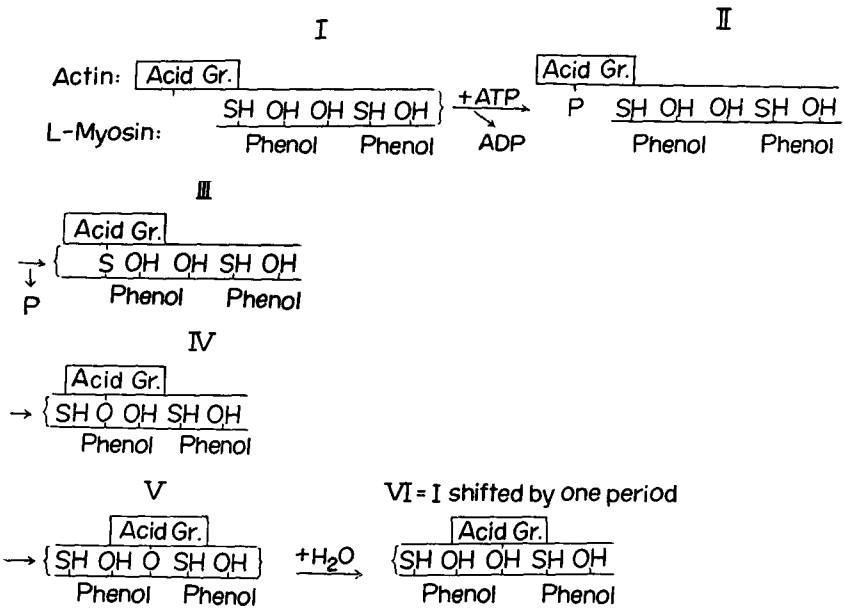


**Fig. 3.** Sliding mechanism of contraction recently proposed by WEBER.

disappeared after exhaustive dialysis of myosin solution, and was recovered by the addition of a minute amount of Mg ion or large amount of Mn ion, but not by Ca ion. These findings indicate that the initial burst is closely coupled with muscle contraction, because the inhibitors of the initial burst of $P_i$-liberation, such as EDTA, $PP_i$, Oxarsan and PCMB, also inhibit the contraction of the muscle models, and because the effect of divalent cations upon the recovery of the diminished initial burst of the dialyzed myosin is quite compatible with their effects on the contraction of the dialyzed muscle models; isolated myofibrils and glycerinated fibers, which are depleted of Mg ion by dialysis, are unable to contract on the addition of ATP without further supplement of Mg ion which cannot be replaced by Ca ion.

The initial rapid liberation of $P_i$ could not be explained by the hypothesis that the transition of dephosphorylation rate is accompanied by the change in a enzyme itself from a highly active state to a less active one[33]. Furthermore, from the analysis of the effects of several modifiers and the dialysis on the initial and the steady state of ATPase, it was concluded that the mechanisms of these two ATPases differ substantially from each other. As the initial burst requires sulfhydryl group of the protein and Mg ion, we[53] have assumed that a phosphoryl myosin is produced by ATP and the initial extra-splitting is the

result of the breakdown of the phosphoryl myosin by the addition of trichloroacetic acid which is added to the mixture to stop the reaction. The result that the same initial rapid splitting of ATP was repeatedly obtained on the further addition of fresh ATP after complete hydrolysis of the old ATP indicates instability of the assumed phosphoryl myosin. Therefore, an attempt has recently been made by us[32b)54)] to give more direct evidence as to the phosphorylation of myosin by ATP.

In the presence of Mg ion and at pH 8, linear liberation of total and free $H^+$ was observed and there was no initial extra-liberation of $H^+$, whereas the initial extra-liberation was of course observed in $P_i$-liberation. This result can easily be explained, if the initial ATPase is nothing but a transferring reaction of the terminal phosphate of ATP to an anionic group of myosin, which dissociates completely at pH 8, probably a carboxyl group. In addition to the above result, an interesting observation was made; a nucleophilic reagent, p-nitrothiophenol (PNT), specifically bound to myosin in the presence of Mg ion and ATP. The amount of bound PNT was almost equal to that of the extra-liberation of $P_i$. The combination of PNT to myosin occurred not as a simple absorption but as a covalent linkage, since PNT-myosin was extremely stable at pH 6.5 and 0°, though it was unstable in alkaline solution, and since its ATPase activity was 3.6 and 100 times as high as those of the control in the absence and the presence of Mg ion, respectively and equal to that of the control in the presence of EDTA, though the addition of free PNT promoted ATPase in the presence of Mg ion only slightly. This specific binding of PNT was completely inhibited by EDTA, thus indicating that PNT-myosin was produced by a nucleophilic attack of PNT on the phosphoryl myosin. These two results support quite strongly our concept that the mechanism of extra-splitting of ATP at the initial state is different from that of splitting at the steady state and that the initial extra-splitting of ATP is due to the phoshorylation of myosin*). Our finding that the pH dependency of the initial burst was quite similar to that of the steady ATPase[51)] indicates that both the processes share the common ATP-binding site of myosin molecule, *i. e.*, myosin is a double headed enzyme and the reaction of myosin with ATP can be schematized as in Fig. 4. It is interesting to note that according to this mechanism PCMB and EDTA react on the myosin-ATP system as

*) The above results can also be explained by a binding of ADP to an undissociated group of myosin at the initial phase. However, BOYER and DEMPSEY[55)] have observed that for each mole of $P_i$ split one mole of $O^{18}$ is incorporated into $P_i$ even under the condition where the quantity of extra-liberation of $P_i$ is of the same order of magnitude as that of $P_i$-liberation by the steady ATPase. This indicates that the phosphoryl myosin, but not the ADP-myosin, is produced by ATP at the initial phase.

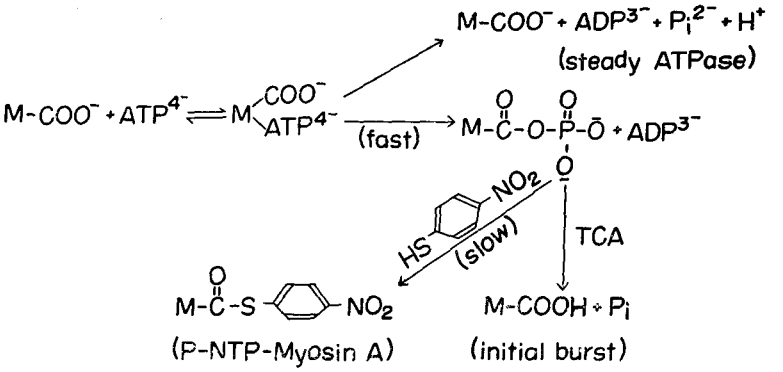


**Fig. 4.** Reaction of myosin with ATP.

"uncouplers".

Concerning the phosphorylation process of myosin the following two discussions may be interesting. The first is the absence of the ATP–$ADP^{32}$ exchange reaction in the myosin-ATP system[44)56)]. It can be explained by assuming that the phosphate bond formed on myosin is not a "high energy" one, such as a carboxyl phosphate bond (Fig. 4), but a "low energy" one or that rate constant of the breakdown of $M<^{ATP^{4-}}_{COO^-}$ into $M\text{-}COO^- + ADP^{3-} + P_i^{2-}$ is much higher than that of its dissociation into $M-COO^- + ATP^{4-}$ [57)]*).

The second problem is to interprete at the molecular level the transition of ATPase from the myosin type to the actomyosin type. In the case of superprecipitated actomyosin, the initial burst of $P_i$-liberation was not observed, the hydrolysis of ATP proceeded at very high rate, and the initial burst of $P_i$-liberation of myosin seemed to proceed continuously[32b)]. The rate of binding of PNT to actomyosin in the presence of ATP and Mg ion was much higher in the state of "clearing response" than in the state of superprecipitation[32b)]. The rate of initial burst was not affected by the addition of Mg or Ca ion, when a minute amount of Mg ion was present in the preparation[52)]. On the other hand, the remarkable contrast of the Ca and Mg ion effect on myosin-ATPase is not observed on actomyosin-ATPase; both Ca and Mg ions activate actomyosin-ATPase. These results suggest that, when myosin binds to F-actin, the phosphoryl myosin is rapidly hydrolyzed and consequently the dominant route of ATP splitting by actomyosin is the cycle via the intermediate phosphoryl myosin (see Fig. 4). Dinitrophenol (DNP)-activation of myosin-ATPase and

*) MOMMAERTS and NANNINGA[57)] deduced this conclusion from their direct measurement of the rate constant of the binding of ATP to myosin together with the conventional MICHAELIS kinetics of ATPase. Their deduction may, however, be inconclusive, since the existence of the initial phase of ATPase was neglected by them.

inhibition of actomyosin-ATPase[58] are also explained by a catalyzed cleavage of the phosphoryl myosin by DNP. Furthermore, the relaxing factor may hinder the formation of phosphoryl myosin by its binding with the Ca tightly bound to the protein.

The following alternatives may serve as the explanations why the binding of actin promotes the cleavage of the phosphoryl myosin and, thus, the cycle of ATP-splitting via the phosphoryl myosin (Fig. 5). One possibility is the change in secondary structure of myosin caused by its binding to actin (see below). As illustrated in (a) of Fig. 5, the carboxyl phosphate on the myosin

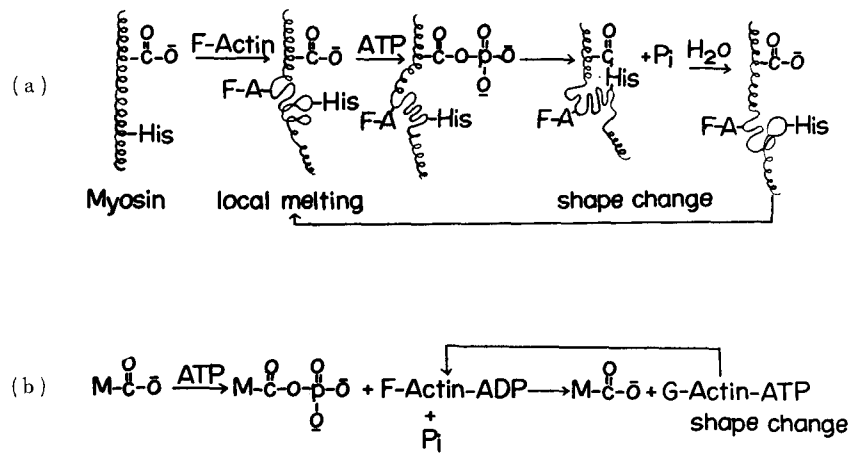


**Fig. 5.** Molecular mechanisms of effect of actin on phosphoryl myosin.

molecule could be rapidly broken by the action of histidyl residue to form acyl-imidazole bond. The histidyl residue might become accecible to the phosphoryl group by the local melting of secondary structure of myosin due to the binding to actin. This mechanism is suggested from the fact that acyl AMP is known to react easily with imidazole group to from acyl imidazole[59] which is hydrolyzed rapidly[60], and from the possibility that the phosphorylation of myosin is coupled with the configurational change of the myosin molecule. The second mechanism is the coupling of G⇌F transformation of actin with the phosphorylation of myosin ((b) in Fig. 5). The phosphate group on myosin is firstly transferred to ADP in F-actin molecule and $P_i$ is released after G-actin thus produced is repolymerized to F-actin (G·ATP→F·ADP+$P_i$)[61]. The merits and shortcomings of these two hypotheses will be discussed in detail in the last chapter of this review, because these hypotheses are of fundamental importance in the discussion of the mechanism of muscular contraction at the

TABLE I. Interaction between ATP Analogs and Actomyosin Systems

| Comp. | Structure | | | Decrease in light-scattering | Hydrolysis | Contraction | Relaxation |
|---|---|---|---|---|---|---|---|
| | Base | Sugar | Phosphate | | | | |
| ATP | 6-$NH_2$-purine | D-ribose | -P-P-P | + | + | + | + |
| VIIIa | 6-$NHCH_3$-purine | D-ribose | -P-P-P | + | + | + | − |
| VIIIb | 6-$N(CH_3)_2$-purine | D-ribose | -P-P-P | + | + | + | − |
| ITP | 6-OH-purine | D-ribose | -P-P-P | | + | + | − |
| UTP | 2,6-$(OH)_2$-pyrimidine | D-ribose | -P-P-P | | + | + | − |
| X | 2-OH-pyrimidine | D-ribose | -P-P-P | ± | − | − | |
| RTP | — | D-ribose | -P-P-P | | ± | | |
| $TP_i$ | — | — | -P-P-P | − | ± | − | |
| Diacetyl ATP | 6-$NH_2$-purine | 2′, 3′-diacetyl-D-ribose | -P-P-P | | + | + | + |
| Deoxy ATP | 6-$NH_2$-purine | Deoxy-D-ribose | -P-P-P | + | + | + | + |
| VIb | 6-$NH_2$-purine | -$(CH_2)_4$-O- | -P-P-P | + | + | − | − |
| VIa | 6-$NH_2$-purine | -$(CH_2)_3$-O- | -P-P-P | + | + | − | − |
| VIc | 6-$NH_2$-purine | -$(CH_2)_2$-O- | -P-P-P | + | + | − | − |
| IX | 6-$NH_2$-purine | D-ribose | -P-P-S | | | − | |

molecular level.

Our conclusion that the phosphorylation of myosin by ATP is the primary event in muscle contraction has further been supported from the study of interaction between the actomyosin systems and ATP analogs, including several new synthetic ones. On the columns 1 and 2 of Table I are shown the abbreviations and the structures of the analogs whose properties were investigated by us[62)63)]. The intensity of light scattered by myosin B was decreased by the addition of the compounds listed in Table I, except for $TP_i$, X and IX, to the same extent as by ATP. The steady velocities of hydrolysis of the compounds by myosin B were of the same order of magnitude as that of ATP under various conditions, except for $TP_i$, RTP, X and IX. The strength of bindings of these compounds to myosin B as judged by the change in light-scattering was in the decreasing order of deoxy ATP$\geqslant$ATP$\geqslant$VIIIb$>$VIc$>$UTP$\geqslant$VIb$\geqslant$VIa$\geqslant$ITP$>$$TP_i$, and the reciprocal of the MICHAELIS constant diminished from ATP to ITP in the following sequence ATP, VIIIb, RTP, VIb, $TP_i$, ITP. In spite of these facts, VIa, VIb and VIc, where the ribosyl group of ATP is displaced by the chain of $-(CH_2)_n-O-$, did not contract myofibrils, while ATP, VIIIa, VIIIb, UTP, ITP, diacetyl ATP[64)] and deoxy ATP contracted myofibrils. The tension development by ITP was about 50% of that by ATP and the quantity of the initial burst observed with ITP was about 30% of the quantity for ATP, whereas VIc showed neither the contracting ability nor the initial burst. It should be noted that there exist several compounds which can not contract myofibrils, even though they change the size and shape of actomyosin to the same extent as ATP and are hydrolyzed at comparable rate to ATP, and that the quantity of initial burst of splitting of the substrate shows good correspondence with the degree of muscle contraction. Our studies on ATP analogs, therefore, raise an objection to both the MORALES and BOTTS and the WEBER theories and provide strong arguments in favor of our view that the initial burst is one of the necessary reactions to muscle contraction. The substrate inhibition of the hydrolysis, which corresponds to muscle relaxation, was not observed for all the analogs investigated, except for ATP, deoxy ATP, and diacetyl ATP. It can be said that requirement of the chemical structure of substrate for muscle relaxation is more specific than the requirement for muscle contraction; the presence of adenine base is necessary to the appearance of this phenomena, in addition to the presence of ribosyl group.

Several objections have been raised by MORALES and BOTTS[8)65)] against the hypothesis that the phosphorylation of myosin by ATP is the primary event of muscular contraction. First BOWEN[31)] observed that under several conditions the hydrolysis of ATP can be dissociated from the tension development. How-

ever, BOWEN measured only the rate of ATPase at the steady state, and the steady ATPase cannot be coupled in any simple way with muscle contraction, as discussed above. Second the elegant experiments of KOSHLAND[56)66)] showed that myosin cannot catalyze the exchange reaction between ATP and ADP. From this result MORALES and BOTTS deduced the conclusion that the steady state concentration of the phosphoryl myosin is negligibly small. However, the phosphoryl myosin does exist and the KOSHLAND experiments do not necessarily mean that the concentration of the phosphoryl myosin is small, as described above. Third, a recent investigation has shown that, when ATP is hydrolyzed through a route involving the myosin-ATP complex (but not the phosphoryl myosin), the free energy drop of the binding is a large fraction of the free energy drop in going from initial to hydrolysis equilibrium; $-\Delta F^{\circ}$ of the ATP hydrolysis is 6～8 Kcal/mole and $-\Delta F^{\circ}$ of the binding of ATP to myosin is at least 6～7 Kcal/mole. However, in living muscle or muscle model myosin exists always as the complex with actin or with ATP. Therefore, $-\Delta F^{\circ}$ of the binding of ATP to actomyosin in muscle may be much smaller than that of binding of ATP to myosin at the steady state, although the $-\Delta F^{\circ}$ value of the binding cannot be determined by the kinetic methods because of the complicated mechanism of actomyosin ATPase (see p. 266 and p. 273) (Free energy changes of the steps of ATP splitting by actomyosin via phosphoryl myosin will be shown later).

## III. Molecular Structure and Function of Myosin

In the preceding chapter the coupling of phosphorylation of myosin with muscle contraction was demonstrated on the basis of kinetic or phenomenological evidences obtained from the study on the actomyosin-ATP system and the muscle model. However, to develop a molecular model of muscle contraction, detailed investigations on the molecular structure and function of F-actin and of myosin, which constitute the contractile protein actomyosin, are urgently needed. The molecular structure and function of myosin will be principally described in this chapter, since the role of myosin molecule in muscle contraction seems to be more important than that of actin.

Myosin prepared in our laboratory under the precisely controlled conditions showed following physicochemical properties. The fresh preparation was ultracentrifugally monodisperse, and a chromatogram[67)] on DEAE cellulose column revealed one major peak (the $\alpha$ one of BRAHMS), which contained about 95% of the total area, and a minor peak. The size and shape of myosin fluctuated to some extent from one preparation to another. The mean values of the weight average molecular weight, radius of gyration, sedimentation constant and

intrinsic viscosity were $5.9\times10^5$*), 1,058 Å, 6.11 S, and 2.30 (100 ml/g), respectively[69]. It could be deduced from the relationship between these physical constants that the myosin molecule has a shape of intermediate between a drained random coil and a rigid rod, *i. e.*, it is considerably flexible. If we adopt the rigid rod as the model of myosin molecule, the length of myosin is calculated to be 3,665 Å.

One of the most important features of the molecular structure of myosin is in the point that the shape of myosin is very susceptible to changes in solvent system[69]. For instance, soon after the addition of 10 volume % of dioxane to 0.6 M KCl solution of myosin enhancement of ATPase activity, increase in radius of gyration by several % and decrease in viscosity were observed concomitantly. These changes were followed by a gradual decrease in the radius of gyration to 70–80% of the original value, an increase in viscosity and inhibition of ATPase activity**). The excess right-handed helical content, which was estimated by the DOTY method from the optical rotatory dispersion curve, was also changed by the addition of 10 volume % of dioxane[35a]. The helical content of myosin was about 60%. Immediately after the addition of dioxane, it increased by 4–5% and then gradually decreased. After the lapse of two to three hours, the helical content was only slightly smaller (1–2%) than the original one[35a], but the ATPase activity was abolished almost completely. The changes of essentially similar nature were also observed after the addition of other organic solvents[35a]. From these findings it may be deduced that the secondary structure around the ATPase active site is especially susceptible to the change of environment and that the structure around the active site plays an important role with respect to the maintenance of the molecular shape of

*) The problem of the molecular weight of myosin is yet unsettled. KIELLEY and HARRINGTON[68] have determined the molecular weight of subunit in guanidine-hydrochloride solution to be $2\times10^5$. We have measured the weight of myosin per binding of 1 mole of $PP_i$[14], 1 mole of tightly bound Ca[34], and 1 mole of lysyl residue which is specific to trinitrobenzenesulfonate binding (seen below), and obtained the same value, $2.1\times10^5$. Therefore, the myosin molecule in the solution seems to be dimer or trimer of the subunit.

**) Recently M. F. MORALES and K. HOTTA (personal communication) have demonstrated that dioxane contains a small amount of peroxide and the dioxane effect observed by us is mimicked by $H_2O_2$. The effects of dioxane on ATPase activity and optical rotatory dispersion of myosin were, however, essentially unchanged by its purification by treatments with $Ag_2O$, KOH, Na metal and Fe powder, though the content of peroxide was reduced from 1.5 to smaller than 0.25 μmoles/ml. and, therefore, the concentration of peroxide in the reaction mixture became to be lower than $2.5\times10^{-5}$ M. The effect of purified dioxane on ATPase was not influenced by the addition of 10 mM KCN, which completely inhibits the $H_2O_2$ effect. Furthermore, the dioxane effect disappears to some extent after dilution. Therefore, it must be concluded that the dioxane effect is substantially attributed to the solvent effect of dioxane itself and not to peroxide contained in dioxane.

myosin molecule as a whole.

The investigations on the heat, acid and alkali denaturations of myosin-ATPase by measurement of optical rotatory dispersion[67], by cellulose column chromatography[67] and by kinetic method[71], have also revealed that the secondary structure around the active site of ATPase is particularly sensitive to these treatments. A similar conclusion was also deduced from analysis of effects of PCMB on ATPase activity and helical content of myosin[35a]. On the addition of high concentration of LiBr, LiCl, KSCN or KI, the helical content of myosin reduced to almost zero, the viscosity decreased to 1/4 of the original value[19] and $S_{w,20}$ increased. Even with these salts, the ATPase activity disappeared in the range of low concentration of salt where the physical properties of myosin remained almost unaffected. Furthermore, we[71a] have recently observed that the helical content of myosin decreases and increases by several % on the addition of $PP_i$ and ATP, respectively, and that the content of so-called "abnormal" tyrosyl residue increases remarkably on the addition of $PP_i$ or ATP, though until recently it is generally considered that $PP_i$ or ATP does not change the structure of myosin. These marked dynamic behaviors of myosin molecule might offer a new aspect for the elucidation of the role of myosin molecule in muscle contraction.

Myosin-ATPase has several characteristic features which are not observed on other enzymes. The pH activity curve has a particular shape with a maximum at around pH 6.8 and a minimum at around pH 7.8 and increases monotonously in higher pH region[33]. Furthermore, PELLETIER and OUELLET[72] have recently shown that the pH dependence of $K_m$ of ATPase is given by a bell shaped curve with a maximum at around pH 8. The maximum velocity and the $K_m$ of ATPase are increased by the addition of various reagents, such as PCMB[73] and EDTA[74-76], and in the presence of these modifiers the pH activity curve lacks the minimum at neutral pH. In the preceding review[77] we have interpreted these characteristics of ATPase on the basis of the following reaction mechanism:

$$\begin{array}{c} E+S \rightleftharpoons ES_1 \longrightarrow E+P\ ^{*)}\ . \\ \quad\quad\ \ \Updownarrow \\ \quad\quad\ ES_2 \end{array}$$

ATP (S) binds to myosin at the base and at the TP group. The binding of TP occurs at two sites of myosin, site 1 and site 2. When the binding occurs at site 1, the substrate is hydrolyzed, and when it occurs at site 2 a stable enzyme-substrate complex ($ES_2$) is formed. The assumption that the binding at

*) As mentioned in the preceding chapter, myosin is a double-headed enzyme. This mechanism is the one for the simple hydrolysis of ATP.

the TP part is mediated by divalent cation is consistent with the result of the kinetic analysis of the effect of Ca ion on ATPase[33] and with the similarity of the effects of various divalent cations upon hydrolysis of ATP, $TP_i$ and VIb[63].

One important point in the ATPase problem, that has not been treated in our preceding review[77], is the binding of NTP at the base moiety with myosin. BLUM[78][79] has developed a theory that the interaction with myosin of a substrate at the 6 position of the base plays an important role in myosin-NTPase. According to our recent studies[63], the rates of ITPase and of ATPase in the presence of a modifier which binds to site 2 were the highest and that of dimethyl ATP was smaller than them, while those of RTP and $TP_i$ the lowest. This observation clearly indicates that the rate of breakdown of the complex $ES_1$ depends on the binding of substrate at the 6 position of the base on myosin. The rate of hydrolysis of VIb was lower than ITP and higher than RTP. This may suggest that the strength of binding to myosin of VIb at the base is weaker than ATP due to loss of configurational entropy of the chain, $-(CH_2)_4-O-$. Analysis of the pH dependence of hydrolysis has also supported this conclusion. The findings, that VIb and VIc do not contract muscle model and that VIc shows no initial rapid splitting, suggest that the binding of the base moiety is prerequisite to the phosphorylation of myosin and muscle contraction.

The fact that the binding of adenine with myosin is rather weak is clearly shown in our experimental results[63] that ATPase was not inhibited by the addition of adenine, even when molar concentration of adenine was 160 times as high as that of ATP. Therefore, we have assumed that myosin binds with NTP at the 6 position of the base by a hydrogen bonding. Recently we[34][80] have found that trinitrobenzenesulfonate (TBS) binds specifically and stoichiometrically to the amino group of one lysyl residue in one myosin subunit, which is located at the neighbor of the binding site of adenine base. The determination of the amino acid sequence around the trinitrophenyl-lysine (TNP-lysine) is currently being in progress in our laboratory. Several TNP-peptides have been isolated and their amino acid compositions and the N-terminal amino acids have been determined. The typical TNP-peptide isolated is Ser. (Asp. Glu. Ser. Gly. TNP-Lys)[81]. The serine residue, which may be situated at one turn apart in $\alpha$-helix from the TNP-Lys residue, might be responsible for the hydrogen bond formation with the 6 position of the base of NTP.

One of the most important features of myosin is its ability to interact with actin. Recently, a detailed physicochemical investigation on the binding of myosin to F-actin has been made by us[70]. By the light-scattering method, the binding ratio of myosin to F-actin was determined to be 3.7 : 1 by weight[9][70]. Since the molecular weight of actin monomer is $6\text{–}7\times10^4$ and

TABLE II. Thermodynamic and Kinetic Constants of Binding of Myosin to F-Actin

| $K_{9^\circ}$ | $\Delta H$ | $\Delta S$ | $k_{9^\circ}$ | $\Delta H^{\ddagger}_{\ddagger}$ | $\Delta S^{\ddagger}$ |
|---|---|---|---|---|---|
| (mole/l) | (Kcal/mole) | (cal/mole/deg) | (l/mole/sec) | (Kcal/mole) | (cal/mole/deg) |
| $4.6\times10^{-7}$ | +57.5 | +234 | $1.9\times10^{5}$ | +11.5 | +6.2 |

that of myosin $5\text{–}6\times10^5$, the above value means that one molecule of myosin binds to an actin dimer. The rate and the equilibrium constants of the binding reaction were determined under various conditions by the use of the light-scattering method. The velocity of the binding was proportional to both the concentration of myosin and of actin, and the rate constant increased with temperature. The equilibrium constant also increased remarkably with elevation of temperature. The kinetic and thermodynamic constants are listed in Table II. The large increase in entropy (+234 *e. u.*) and enthalpy is the most distinct feature of this binding reaction.

In actomyosin formation, electrostatic effects appear to play a dominant role, because the binding strength decreased with increasing ionic strength and increased with decreasing dielectric constant of the medium. When sulfhydryl groups of myosin were attacked by SH reagent or when one amino group of a lysine residue in one actin monomer was modified by TBS[82], actomyosin formation was inhibited. Accordingly it was concluded that the salt linkage between a sulfhydryl group of myosin and an amino group of actin is one of the bonds essential to actomyosin formation. This conclusion was consistent with the pH dependence of the rate and the equilibrium constant.

When myosin and F-actin were mixed, the helical content was reduced by several percent. The same reduction in the helical content of myosin was observed on the addition of $PP_i$[71a] which is known to bind to myosin competitively with F-actin. Therefore, the increase in entropy with actomyosin formation might be due to the local melting of the secondary structure of myosin (*cf.* 83). On considering together with the fact that the slight change in secondary structure around the ATPase active site produces a profound change in the molecular shape of myosin, this slight but distinct alternation of secondary structure of myosin by its binding to F-actin seems to be of fundamental importance for elucidation of molecular model of muscle contraction.

## IV. A Molecular Mechanism of Muscle Contraction

A molecular model of muscle contraction is presented in this chapter based

on our experimental results. The primary reaction of muscle contraction and the molecular structure of myosin have already been discussed in the previous chapters. The following three facts seem to be important concerning muscle contraction.

(i) The structural proteins, actomyosin and myosin, are flexible molecules. The shape of the myosin B–ATP or $-PP_i$ complex is principally dominated by the intramolecular electrostatic force and entropy. The secondary structure of myosin around the active site is quite flexible, and the molecular shape of myosin depends strongly on the conformation around the active site. The conformation of myosin is changed by its combination to F-actin or with ATP or $PP_i$. As pointed out by MORALES[8], it seems to be impossible to explain shortening of muscle only by formation of covalent linkage in the contractile protein, without changing shape of the protein (for example WEBER's new scheme (Fig. 3)). (ii) Myosin is a double headed enzyme carrying a phosphorylating "head" and a hydrolyzing "head". The phosphorylation by ATP of the protein at the former is the primary event in muscle contraction. The binding of actin promotes the cleavage of the phosphoryl myosin and, subsequently, the cycle of ATP splitting via the phosphoryl myosin. (iii) There are two kinds of sites, a and b, in myosin molecule to bind to F-actin. The bond at site a is stable even at a high concentration of KCl, and is cloven by ATP binding to this site. The "intrinsic" Ca tightly bound to myosin is involved in site b. The strength of the bond at site b depends on electrostatic effect and hence the bond can exist only at a low concentration of KCl. This is broken by combination of the relaxing factor with the "intrinsic" Ca. When both of these bonds at site a and b are split, the rate of ATP hydrolysis of the myosin-actin-ATP system decreases and this state corresponds to the relaxation (Fig. 2).

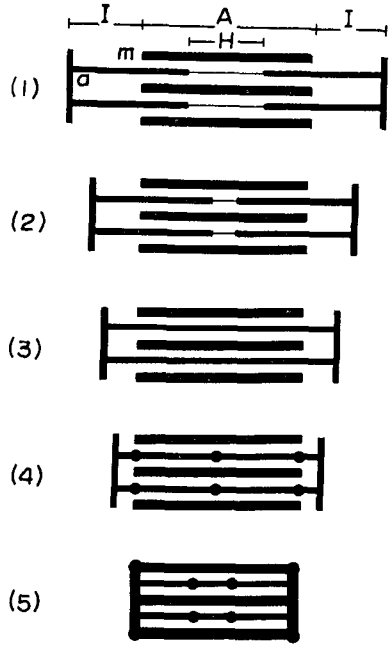


**Fig. 6.** Sliding model of muscle contraction by HANSON and HUXLEY. a and m denote actin and myosin filament, respectively.

The relative values of the resistance to stretch of glycerinated muscle fibers are about 2, 1 and 0 in rigor, contraction and

relaxation, respectively[48]. Thus, the binding force between actin and myosin decreases in this order: Both of the bindings at the two sites may exist at rigor, either of them at contracted, and none at relaxed state.

The localization of myosin and actin in muscle fibers and the movement of these proteins on shortening and lengthening of muscle have been clearly demonstrated by the excellent electron-microscopic study of HANSON and HUXLEY[46)47)]. The resulting picture of a fibril is shown schematically in Fig. 6. The essentials of the scheme are: (i) A fibril is constructed from the thick filament of myosin and the thin filament of actin and (ii) when the muscle lengthens or shortens, the filaments of these two types slide past one another.

Two possible mechanisms can be considered for sliding of the filaments. HUXLEY[84)] explained the shortening by the decrease in free energy due to increase in number of the bonds between myosin and actin. It is well known that myosin is composed of L- and H-meromyosins, and that only H-meromyosin shows ATPase activity and binding ability with actin[85)]. From experiments on myofibrils treated with antibodies of meromyosins, MARSHALL[86)] and A. G. SZENT-GYÖRGYI[87)] have recently suggested that myosin filament in a sarcomere is composed of meromyosins in the order of L–H–L. The length of the myosin filament in one sarcomere is about 14,000 Å, and the length of myosin molecule, when assumed as the rigid rod, was found to be 3,665 Å. However, since the myosin molecule is flexible to some extent, it may not be unreasonable to conclude that two myosin molecules are arranged in one sarcomere as L–H–H–L. As described above, $PP_i$ binds to myosin competitively with actin and one mole of $PP_i$ binds to one mole of myosin in myosin B particle, and the binding between myosin and actin is inhibited by the modification with TBS of one lysine residue in one actin monomer. Thus it must be concluded that the binding between myosin and actin takes place at the specific sites of these proteins. If we accept these two conclusions, the maximum number of the pair of binding sites of actin to two myosin molecules in one sarcomere becomes to be 2 regardless of the extent of the overlapping of the two filaments. Even though nonspecific weak bonds between actin and myosin could exist, it seems impossible to proceed the overlapping of the actin filament and the myosin filament by the binding energy of these proteins beyond the point where the binding number of the pair of sites per two myosin molecules in one sarcomere becomes 2.

Another way in which muscle contracts by the sliding mechanism is the operation of a cycle, in which a slight change of molecular length of either actin or myosin takes place, simultaneously with the dissociation and the recombi-

nation of the proteins. In the HANSON and HUXLEY[46] and the AUBERT[88] theory, actin is the principal participant in this type of mechanism, and the change in the length of actin is produced by the G–F transformation. This mechanism corresponds to our explanation (b) of ATPase of the actomyosin type shown in Fig. 5, where the phosphorylation of myosin is coupled with G⇌F transformation of actin. The mechanism is very attractive, since it is well known that G–F transformation of actin is usually coupled with the dephosphorylation of ATP to ADP[61]. Furthermore, the G–F transformation of actin is accelerated by myosin[89] and the rotatory diffusion constant of unit in F-actin is greatly increased on binding of F-actin with myosin[90]. Consequently, if the phosphate on myosin molecule is transferred directly to ADP of F-actin bound with myosin, a marked change in shape of F-actin can be produced and muscle can shorten. The absence of the change in ATP–ADP content at the contraction phase can also be easily explained by this mechanism (*cf.* 91).

MARTONOSI *et al.*[92] have demonstrated the predominance of F-actin in living muscle from the absence of the ADP*–ADP exchange reaction. We have measured the content of G-actin in the superprecipitated actomyosin by stopping the G–F transformation of actin with EDTA[93] and found that the content of G-actin is negligible[94] (smaller than 5%). Therefore, in the superprecipitated actomyosin or living muscle the rate of polymerization of actin must be much higher than that of depolymerization. The rate of ATP splitting by actomyosin is of the order of 1.5 moles/second/mole of myosin, and actin monomer binds with 1 subunit of myosin. On adopting the scheme (b) in Fig. 5, the rate of depolymerization of actin should, therefore, be at least of the same order to that of ATP splitting (1.5 $sec^{-1}$), and also the rate constant of actin polymerization should be much higher than this value. The observed rate constant is, however, extensively smaller than 1.5 $sec^{-1}$, though the transformation can be accelerated by myosin[89] and F-actin[95]. Furthermore, MARTONOSI and GOUVEA[95a] have recently reported that the transformation of ATPase from the myosin type to the actomyosin one occurs on the addition of actin whose polymerizability is inhibited by photo-oxidation. These two results seem to be very serious shortcomings of the mechanism in which G⇌F transformation of actin is coupled with contraction. Proprieties of the mechanism of this type may be further examined by investigating properties of actomyosin composed of myosin and actin of which polymerization is not coupled with ATP-splitting[96].

The other explanation of contraction from the structural change of the protein is based on the hypothesis ((a) in Fig. 5) that the conformation of phosphoryl myosin is largely changed on its binding to F-actin. This mechanism

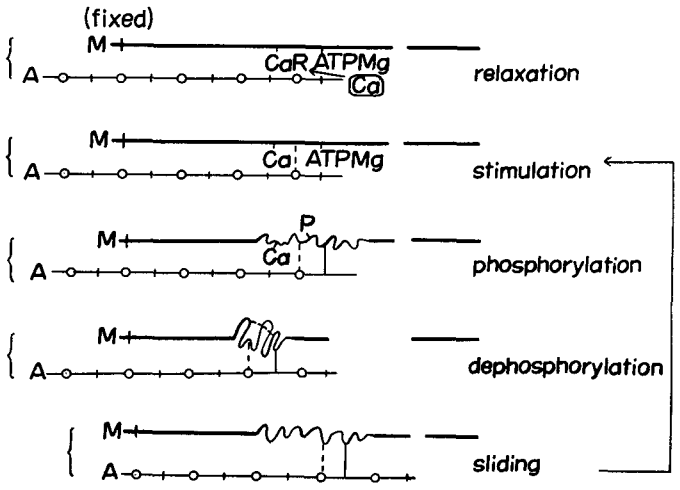


**Fig. 7.** A molecular mechanism of muscle contraction proposed by us. Details see text.

is suggested from the facts; the flexibility of the secondary structure of myosin around the active site, the remarkable dependence of molecular shape of myosin on the conformation of the active site, and the changing in the secondary structure of myosin on its binding to actin. Moreover, it can give a reasonable explanation of the fact that the molecular shape of myosin does not change largely but the colloidal state of actomyosin changes conspicuously on the addition of ATP.

A molecular model of muscle contraction deduced from the above general idea is illustrated in Fig. 7. The system works as follows: (i) Initially, the binding sites, a and b, of myosin to actin are occupied by ATP and the relaxing factor, respectively, and the phospholylation of myosin is inhibited. (ii) When Ca ion is set free or free Ca ion penetrates from sarcoplasma into sarcomere by the excitation, as suggested by HEILBRUNN[97], SANDOW[98], NIEDERGERKE[99][100] and BIANCHI[101], the relaxing factor is released from the binding site b by its combination with free Ca ion. (iii) A linkage between myosin and actin is formed at site b and myosin is phosphorylated by ATP. The local concentration of ATP around site a is lowered for the moment, and the possibility of the binding of ATP to site a is reduced. The linkage between myosin and actin is formed at site a, and consequently the secondary structure around the active site of myosin-ATPase becomes loose. (iv) The phosphoryl myosin tends to shorten by the formation of acyl-histidyl bond or by entropy effects. To change the shape of myosin, the binding site of myosin must move on to an

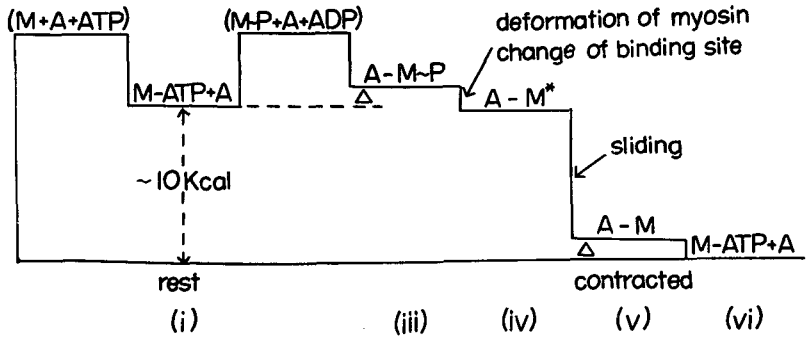


**Fig. 8.** Loss of free energy and production of work, as system goes through cycle shown in Fig. 7.

actin site which is located near the Z membrane. The equilibrium of actomyosin is in favour of the combined state, because of the low local concentration of ATP. But actomyosin is in a dynamic state of dissociation and recombination, and the movement of the binding site of myosin on the actin filament may waste a small amount of free energy, since the binding sites are situated on the actin filament regularly and closely to each other. (v) By the hydrolysis of acyl-histidine linkage or the dephosphorylation, the myosin filament regains the original form and the actin filament slides past the myosin filament. When the local concentration of ATP is recovered, the cycle is repeated through the steps (ii) to (v). (vi) When Ca ion, which is released by the excitation, returns to the original bound state, the relaxing factor binds at site b and ATP hydrolysis is inhibited. Then ATP binds to site a, and actin is detached from myosin and muscle lengthens passively. Fig. 8 shows schematically the loss of free energy and production of work, as the system goes through the cycle. It must be added that this reaction cycle is consistent with the one previously proposed by us[16)101a)101b)] and shows molecular changes of the steps, such as the "deformation" and the "activation" one, of the previous mechanism.

Recently, NASSOW[102)], ALEXANDROW[102)] and SEGAL[103)] have explained various physiological phenomena, such as excitation and transmission of nerve and contraction of muscle, by reversible denaturation of protein from their extensive but indirect evidences. Our mechanism of contraction is consistent with their assumption in point of the change in secondary structure of protein. Furthermore, RACKER[104)] and LEHNINGER[105)] have pointed out the similarity of the properties of mitochondrial ATPase and of myosin-ATPase, and LEHNINGER[105)] suggested that the shape change of the mitochondrial protein is produced by

the formation of a phosphoryl protein as an intermediate in oxidative phosphorylation. The analogy of the molecular mechanism of muscle contraction and of oxydative phosphorylation is very interesting from a point of view of general biochemistry.

Finally, the thermodynamic property of the chemical reaction coupled with the contraction in living muscle will be discussed. In the reaction mechanism described above, the overall chemical reaction coupled with muscle contraction is the hydrolysis of ATP. According to the analysis done by HILL[106] and WILKIE[107] on living muscle, the ratio of $\Delta F$ and $\Delta H$ of the chemical reaction coupled with the contraction is in range from 0.4 to 1.65. According to MORALES *et al.*[8)108], on the other hand, $-\Delta F$ and $-\Delta H$ of the hydrolysis of ATP under the physiological condition are 9.4 and 3.7 Kcal/mole, respectively, and subsequently the $\Delta F/\Delta H$ is 2.5. Therefore, the hydrolysis of ATP cannot directly be coupled with contraction *in vivo*. We[109] have recently found that creatine-kinase combines tightly with myosin and that, when ATP is supplied through creatine-kinase system, the MICHAELIS constant of myosin ATPase is about 1/50 of the value obtained from ATP alone as the substrate. These findings suggest that, in living muscle, ATP is supplied to myosin through the creatine-kinase system and that the overall chemical reaction coupled with contraction is dephosphorylation of creatine phosphate (CrP) derived from the corporated reaction of $CrP + ADP \rightleftharpoons Cr + ATP$ and $ATP + H_2O \rightarrow ADP + P_i$. The values $-\Delta F$ and $-\Delta H$ of the hydrolysis of CrP under the physiological condition are about 10 and 9.2 Kcal/mole[110], respectively, and $\Delta F/\Delta H$ is 1.1. This value is in the range of values obtained by HILL and WILKIE. CARLSON and SIGER[111] have analyzed the changes in content of phosphate compounds during contraction of living muscle, and showed a linear relation between the amount of CrP breakdown and the number of muscle twitch. This result can reasonably be understood by the coupling of hydrolysis of CrP with muscle contraction, as described above, though they suggested $G \rightleftharpoons F$ transformation of actin as the primary reaction of muscle contraction.

## Conclusion

A molecular mechanism of muscle contraction is presented on the basis of our experimental results on the myosin-actin-ATP system and of the HANSON-HUXLEY sliding model. The most important points of this mechanism are the phosphorylation of myosin by ATP and the shape change of phosphoryl myosin which takes place after the minute change in the secondary structure of myosin by its combination to actin. Our mechanism seems to involve little conflict with the experimental facts, though at present the molecular mechanisms of

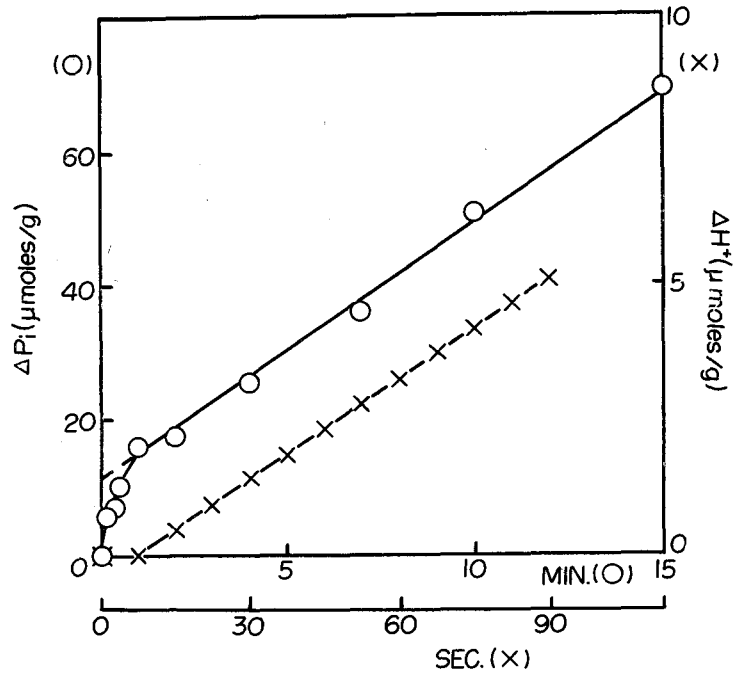


**Fig. 9.** Liberation of $P_i$ (○) and $H^+$ (×) from myosin-ATP system. 0.5 M KCl, 2 mM $Mg^{++}$, 1 mM ATP. pH 8.2, 20°. Concentration of myosin A: 3 mg/ml for $P_i$-liberation and 0.8 mg/ml for $H^+$-liberation.

some steps are hypothetical.

## Addendum

Recently, BENDALL (Biochem. J., **81**, 520 (1961)) has concluded that the initial burst of ATPase observed by us is due to the product inhibition. Here is added a discussion on this point, since the mechanism of the initial phase of ATPase is one of key-points of the molecular mechanism of muscle contraction presented in this review.

Firstly, BENDALL's experiments were performed at higher concentration of protein and in much lower ionic strength than our measurements were made. The appended figure shows the time-course of $P_i$-liberation from myosin-ATP system with that of $H^+$-liberation, as a typical example of our results. As clearly seen in this figure, $P_i$-liberation shows a remarkable initial burst but no burst is seen in $H^+$-liberation. The molar concentrations of products in this experiment were lower than 20 and 1.4% of the initial concentration of ATP

for $P_i$- and $H^+$-liberations, respectively. Furthermore, the steady velocity of $P_i$-liberation agrees exactly with that of $H^+$-liberation. Therefore, the initial burst of $P_i$-liberation cannot be attributed to the product inhibition.

Secondly, as already mentioned, actomyosin ATPase shows no burst of $P_i$-liberation in superprecipitated or contracted state and it shows a remarkable burst only in state of "clearing response" or relaxed state. BENDALL could not observe the initial burst of our type, since his measurements were apparently made on contracted myofibrils.

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