JB Reflections and Perspectives

J. Biochem. 2009;146(1)7–11
doi:10.1093/jb/mvp081

Yuji Tonomura: A Pioneer in the Field of Energy Transduction in Muscle Contraction

Hirofumi Onishi[*,†]

ERATO 'Maéda Actin-Filament Dynamics' Project, Japan Science and Technology Agency, c/o RIKEN Harima Institute at Spring-8 Center, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan

Late Professor Yuji Tonomura has made a great contribution in the study of energy transduction in muscle contraction. He was the investigator who first proposed that a myosin–phosphate intermediate is produced subsequently to the Michaelis–Menten complex in the pre-steady state of the myosin ATPase reaction and that it is a key intermediate for muscle contraction. Here, his proposed intermediate will be viewed from the prospective of today's understanding of actomyosin ATPase kinetics and in the context of myosin motor domain crystal structures.

Key words: actomyosin, ATP hydrolysis, initial burst of P_i liberation, muscle contraction, myosin.

Abbreviations: NTP, *p*-nitrothiophenol; TCA, trichloroacetic acid.

DAYBREAK FOR MUSCLE RESEARCH

Muscle contraction results from a thermodynamic coupling between the hydrolysis of ATP and the conformational transitions of actomyosin (a complex of actin and myosin molecules). In 1939, Engelhardt and Ljubimowa first isolated an enzyme of ATP hydrolysis 'myosin' from muscle tissues (*1*). In the 1940s, Albert Szent-Györgyi advanced the biochemistry of 'myosin'. His colleague Straub isolated actin from 'myosin', showing 'myosin' was a complex between actin and true myosin. Szent-Györgyi demonstrated that ATP allows actomyosin threads to contract *in vitro*. He published a book titled with 'Chemistry of Muscle Contraction' Academic Press Inc. in 1947. Professor Tonomura (Fig. 1) was strongly impressed by this book and started kinetic studies of myosin ATPase. Throughout his life, he was challenged by a fundamental question in muscle contraction, 'How myosin converts the chemical energy of ATP into force generation to move actin filaments in muscle'.

Tonomura's greatest contribution in science is his proposal that a myosin–phosphate complex is rapidly produced in the pre-steady state of myosin ATPase reaction. Weber and Hasselbach had reported that the activity of myofibrils (minced fragments of muscle fibre) or actomyosin immediately after the addition of ATP was several times higher than the steady state activity (*2*). Impressed by this earlier work, he started extensive studies of this hydrolytic reaction with various samples, that is, myofibrils, actomyosin and purified myosin, with a variety of nucleotides and in a wide range of salt conditions and temperatures. His findings can be summarized as follows:

1. Purified myosin exhibited an initial burst of inorganic phosphate (P_i) liberation when its ATPase reaction was stopped with trichloroacetic acid (TCA) (*3*, *4*). The amount of P_i liberated was a constant value of 1 mol/mol of myosin over wide ranges of ionic strength, pH and temperatures, when the Mg^{2+} concentration was higher than 1 mM (*3*, *4*). Therefore, he hypothesized that P_i is derived from the terminal phosphate of ATP.
2. When a small amount of ATP was added to the reaction mixture in advance, the initial burst of P_i liberation did not occur upon the repeat addition of ATP (*5*). After the ATP was exhausted, the initial burst reappeared when ATP was subsequently added (*5*). Therefore, vanishing of the initial burst was not due to an accumulation of hydrolysis products.
3. No muscle model underwent contraction on addition of ATP under conditions in which an initial burst of P_i liberation was not observed, or on addition of ATP analogues which caused no initial burst from the myosin–ATP analogue system (*6*).
4. Decomposition of the myosin–phosphate complex could be accelerated by more than 100-fold when actin bound to myosin (*7*).

Novel concepts were produced by his findings:

(a) After ATP binds to the active site of myosin, the Michaelis–Menten complex rapidly converts into a stable myosin–phosphate complex prior to the liberation of P_i in the normal conditions.
(b) The myosin–phosphate complex is labile to acidic environments so that the rapid P_i liberation results from terminating the ATPase reaction with TCA.
(c) The ATPase pathway via the myosin–phosphate intermediate is closely connected to muscle contraction.

*To whom correspondence should be addressed.
Tel: +81-791-58-1350; Fax: +81-791-58-1360;
E-mail: honishi@spring8.or.jp;
onishi.hirofumi@h.mbox.nagoya-u.ac.jp
†Present address: The Structural Biology Research Center and Division of Biological Science, Nagoya University, Graduate School of Sciences, Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8601 Japan.

Fig. 1. **Late Prof. Yuji Tonomura (1923–1982); 1958–1963, Professor, Hokkaido University; 1963–1982, Professor, Osaka University.**

Tonomura's pioneering works provided a fruitful decade starting from 1965 for kinetic studies of myosin and actomyosin ATPase. Enormous amounts of works were accumulated and filed in two monographs written by himself and his colleagues (*8*, *9*). Here, I will not get into the details of his works, but rather his works will be viewed back from the point of view of today's knowledge.

KINETIC STUDIES OF MYOSIN AND ACTOMYOSIN ATPase

Tonomura perhaps imagined that in the myosin–phosphate intermediate, myosin and phosphate formed a covalent bond interaction, which was known in those days as a high-energy chemical bond, for example, phosphoryl compounds in the metabolic pathway of glucose, or carboxyl phosphate intermediates of membrane Na^+, K^+-ATPase. When myosin was incubated with *p*-nitrothiophenol (NTP) in the presence of MgATP, NTP-myosin was formed (*10*). This finding led him to conclude that a nucleophilic attack by NTP converts a labile myosin–phosphate intermediate into a stable NTP-myosin. Moreover, nitrophenylation of 1 mol of

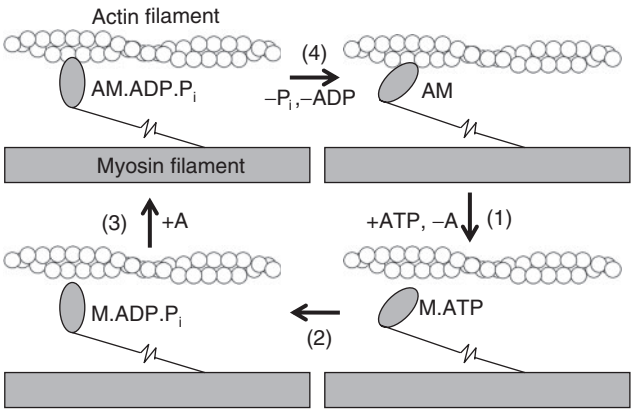

Fig. 2. **The Lymn–Taylor model for cross-bridge cycling (*11*).** (1) The binding of ATP dissociates a cross-bridge (myosin head) from actin. (2) ATP hydrolyses into ADP and P_i, and it changes an angle of the cross-bridge into 90°. (3) The cross-bridge rebinds to actin. (4) The products are released from the cross-bridge and it leads to a return of the cross-bridge to the 45° position. In this transition, actin is moved past myosin. [Adapted from Geeves and Holmes (*30*).]

phosphorylation site per mole of myosin completely prevented the initial burst of P_i liberation, whereas this modification did not affect the steady-state ATPase activity of myosin (*10*). Therefore, he proposed that ATP hydrolyses in two different routes: (a) simple hydrolysis of the Michaelis–Menten complex and (b) hydrolysis in which the Michaelis–Menten complex is converted to a phosphoryl myosin prior to the P_i liberation. Later, Lymn and Taylor (*11*) proposed a one-route hypothesis of actomyosin ATPase via a myosin-product complex. Tonomura's group tried to explain two different routes of ATP hydrolysis by assuming two non-identical heads of the molecule (*12*). The two groups were engaged in a long controversy during the 1970s and 1980s. One-route mechanism is now popular.

In order to interpret the movement of a myosin head and the sliding movement of an actin filament, which was established by physiological studies (*13*–*15*), Lymn and Taylor (*11*) proposed a mechanism for actomyosin ATPase in which ATP hydrolysis is coupled to cross-bridge cycling (shown in Fig. 2). The binding of ATP to the active site of the myosin head is a necessary step for the dissociation of the actomyosin complex (step 1). On the other hand, actin promotes product release by binding to the myosin-products complex (steps 3 and 4). Coupling to this process, the myosin head undergoes a rowing-like stroke so as to move the actin filament by approximately 10 nm. This unitary process is referred to as the 'power stroke'. Bagshaw and Trentham (*16*, *17*) showed that the ATPase reaction includes two-step processes for ATP binding and ADP release. The ATP-binding process is composed of the formation of the Michaelis–Menten intermediate and the subsequent isomerization. The isomerization can be measured by the ATP-induced fluorescence enhancement of myosin tryptophan as will be discussed later. The ADP release occurs in a similar manner but in the backward direction of the ATP binding process. Nowadays, it is believed that

$$M+ATP \overset{(1)}{\rightleftharpoons} M.ATP \overset{(2)}{\rightleftharpoons} M^{*}.ATP \overset{(3)}{\rightleftharpoons} M^{**}.ADP.P_i$$
$$\overset{(4)}{\rightleftharpoons} M^{*}.ADP+P_i \overset{(5)}{\rightleftharpoons} M.ADP \overset{(6)}{\rightleftharpoons} M+ADP$$

Scheme 1. **ATP hydrolyses in a single pathway of six elementary steps. Conformations distinguishable by tryptophan absorbance or fluorescence are indicated by * and ****.

ATP hydrolyses in a single pathway of at least six elementary steps (Scheme 1).

STRUCTURAL STUDIES OF MYOSIN HEADS

Webb and Trentham (*18*) investigated the exchange of oxygen-18 [^{18}O] between H_2O and P_i during the myosin–ATPase reaction. The pattern of exchange indicated that $M^{*}{\cdot}ATP + H_2O$ and $M^{**}{\cdot}ADP{\cdot}P_i$ are interconverted directly, without any different chemical species, between these intermediates. Therefore, a phosphoryl intermediate in which P_i binds to myosin with a covalent bond cannot be produced in the hydrolytic process by myosin. How is Tonomura's myosin–phosphate intermediate understood in the context of today's knowledge? The answer was provided by X-ray crystal analyses of the *Dictyostelium* myosin motor domain complexed with MgADP and aluminium fluoride ($MgADP{\cdot}AlF_4^-$) and with MgADP and vanadate ($MgADP{\cdot}VO_4^-$) by Rayment and his colleagues (*19*, *20*). AlF_4^- and VO_4^- have the similar structure to P_i and stabilize the binding of MgADP to the active site of myosin. It is thus expected that myosin motor domain-MgADP complexes with these P_i analogues are analogous to the myosin–substrate complex or the myosin-products complex. In these complexes, the converter[1] was rotated by 70° compared to the myosin head in the near-rigor (left in Fig. 3) or rigor (right in Fig. 3) complex. Therefore, these complexes are referred to as the 'pre-power stroke' state (centre in Fig. 3). Since the bond distance (2.0 Å) between the aluminium or vanadium atom and the terminal oxygen on the β-phosphate of MgADP was longer than the corresponding bond distance (1.7 Å) in the structure of MgATP free from myosin, Rayment (*19*, *20*) concluded that motor domain complexes with $MgADP{\cdot}AlF_4^-$ and $MgADP{\cdot}VO_4^-$ are models for the transition state of ATP hydrolysis (left in Fig. 4), but not for the pre-hydrolytic state (M.ATP shown in Scheme 1 and Fig. 3). These crystal structures suggest the following:

1. In the transition state, MgADP and γ-phosphate have no covalent bonds with amino-acid residues, but they are trapped with a network of many hydrogen bonds within the active site of myosin (left in Fig. 4). When myosin is denatured by TCA, destruction of hydrogen bonds results in the initial burst of P_i liberation in the past discovered by Tonomura.

[1] The C-terminal segment of the motor domain (colored blue in Fig. 3) is called the converter, because it converts linear displacement of the relay helix (colored orange in Fig. 3) into its rotation (arrow in Fig. 3).

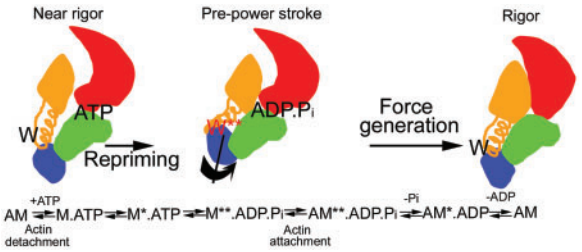

Fig. 3. **Chemo-mechanical coupling in the actomyosin ATPase cycle.** Positions of subdomains in the three myosin states are shown at the top. Binding of ATP to the rigor state opens the actin-binding cleft, causing myosin to dissociate from actin in the near rigor state. The near rigor state isomerizes into the pre-power stroke conformation, repriming the converter (and thus the lever arm), and allowing hydrolysis of ATP. Relative to the converter, the rotation necessary from the near rigor state to the pre-power stroke state is indicated by the arrow. Rebinding of actin to the pre-power stroke state causes some uncharacterized structural isomerizations into the rigor state. Lever arm movements and force generation on actin are produced during these isomerizations. The location of Trp-501 and its conformational change are indicated by *W* and *W***, respectively. [Models for near rigor, pre-power stroke, and rigor states are adapted from Coureux *et al*. (*31*).]

2. When the products are released from the complex between actin and a myosin head in the post-hydrolytic state ($AM^{**}{\cdot}ADP\ {\cdot}P_i$ shown in Fig. 3), the rotation of the converter generates force to move actin filaments ('power stroke'). No lever arm was contained in the motor domain construct which Rayment employed in the crystallization (*19*, *20*), but later Dominguez and Cohen (*21*) confirmed the rotation of the converter by using the smooth muscle myosin motor domain construct with a part of the lever arm.

The crystal structure of the post-hydrolytic state $M^{**}{\cdot}ADP\ {\cdot}P_i$ is still unavailable, but the process from the transition state (left in Fig. 4) to the post-hydrolytic state (right in Fig. 4) can be inferred by molecular modelling (*22*). Tonomura's idea that the energy necessary for force generation is preserved within a myosin-products intermediate until P_i is released from the intermediate is exactly correct. However, the present understanding is that the energy is not stored in a high-energy phosphate bond, but preserved as reverse swing of the lever arm in a metastable complex $M^{**}{\cdot}ADP\ {\cdot}P_i$.

Finally, I focus on which transition in the actomyosin ATPase cycle is responsible for repriming of the lever arm. Morita (*23*) and Sekiya and Tonomura (*24*) found that the UV spectrum of the myosin tryptophan is altered when myosin is mixed with ATP. This was the first report suggesting that a conformational change is induced by the binding of ATP to the active site of myosin. Although the location of the ATP-sensitive tryptophan in myosin was not identified until much later, the ATP-induced fluorescence change of the tryptophan was frequently used in kinetic studies of myosin and actomyosin ATPase (*8*, *9*, *11*, *16*, *17*, *25*). The ATP-sensitive tryptophan (Trp-501) was first demonstrated in the crystal structure of the *Dictyostelium* myosin motor domain complex with $MgADP{\cdot}VO_4^-$ (*20*). This residue sits on a long α-helix connecting the active site and the converter,

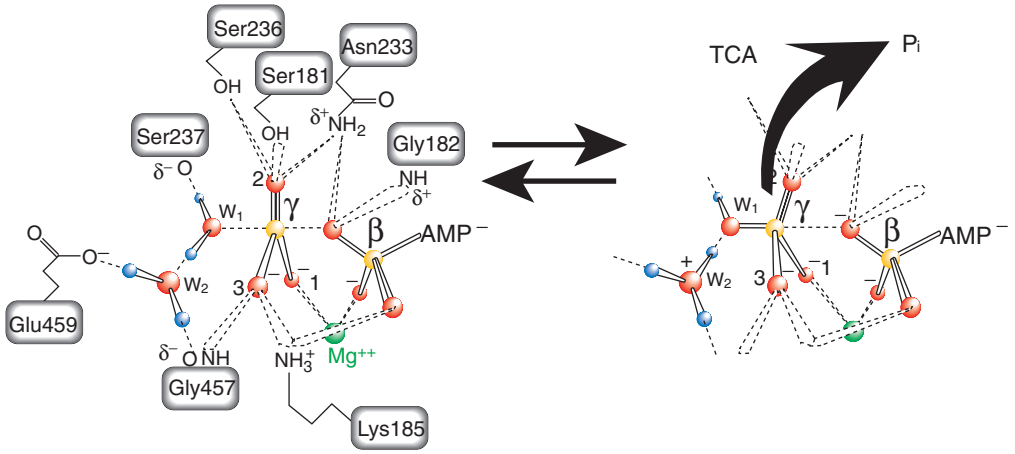

Fig. 4. **Structural models of the transition (left) and the post-hydrolysis (right) states (*32*).** The transition state model was produced from the crystal structure of the *Dictyostelium* myosin motor domain compound with MgADP.VO_4^- (*20*). δ^+ and δ^- indicate positive and negative charges, respectively, which are induced by the resonance structures of the peptide bonds. Covalent bonds are shown as filled lines, and hydrogen bonds and ionic interactions in dashed lines. The transition state shows two interesting features: (1) the attacking water (w_1) is now oriented to carry out its attack on the γ-phosphate, and (2) the γ-phosphate is readied for the attack from w_1 by changing its configuration into a trigonal bipyramidal. Amino-acid residues are not shown in the right panel. A stoichiometric amount of P_i (initial burst) is released from the right complex by terminating the ATPase reaction with TCA.

thus named as the 'relay helix' (shown as orange helix in Fig. 3). Because of this result, it is widely accepted that the ATP-induced fluorescence change reflects a local conformational change of the relay helix. Using the *Dictyostelium* myosin motor domain fused with two different fluorescent probes at different positions, Suzuki *et al*. (*26*) demonstrated that the reverse rotation of the converter (and thus the lever arm) occurs at the isomerization of the myosin-substrate complex (the transition from M·ATP to M^*·ATP in Scheme 1). Based on these studies, it is believed that repriming of the lever arm occurs prior to the hydrolytic step of ATP. If this is true, the binding energy of ATP to myosin could be a driving force of the conformational change, which results in the movement of the lever arm. Therefore, the most reasonable explanation could be that when actin promotes product release from M^{**}·ADP·P_i, the potential energy stored for the next power stroke discharges to move actin. Mysteriously, however, so far, all crystal structures, which have the bound nucleotide in the pre-hydrolytic state, had a rotation angle of the converter the same as that for the near-rigor (left in Fig. 3) or rigor (right in Fig. 3) complex, but not like that for the pre-power stroke state (centre in Fig. 3) (*19*, *27*, *28*).

As described earlier, crystal structures of the myosin head or its fragments in the post-hydrolysis state are unavailable. A large rotation of the converter upon the liberation of P_i may prevent the crystal from growing. A high-resolution structure of the post-hydrolysis state will be necessary in order to complete understanding of the lever arm movements coupled to the ATPase cycle of myosin. No one has succeeded to grow a crystal of the actomyosin complex so far. Using the fit of the nucleotide-free structure of the myosin head (S1) into the cryo-electron microscope 3D reconstruction of S1-decorated actin, Holmes *et al*. (*29*) made the actin–myosin complex model at the last stage of the power stroke. The refinement of this model will be indispensable for the atomic level understanding of the cross-bridge cycle in muscle. The site-directed mutagenesis will be a useful tool in identifying key residues in ATP hydrolysis at the active site, force transmission in the relay helix-converter system and actin activation of myosin ATPase. Computer modelling experiments are applicable in tracing of an energy minimized pathway between two snapshots inferred from X-ray crystallography and electron microscopy. Knowledge of energy transduction in muscle contraction has increased explosively since Tonomura's generation as described here, but more studies are needed to reach the goal.

ACKNOWLEDGEMENT

The author thanks emeritus Profs. Kazuya Taniguchi and Fumi Morita of Hokkaido University, Sapporo and Dr Thomas P. Burghardt of Mayo Clinic, Rochester, MN, USA, for their helpful suggestions on this manuscript.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Engelhardt, W.A. and Ljubimova, M.N. (1939) Myosin and adenosine triphosphatase. *Nature* **144**, 668–669
2. Weber, A. and Hasselbach, W. (1954) Increased rate of ATP-splitting by myosin and actomyosin gels at the onset of the splitting. *Biochim. Biophys. Acta* **15**, 237–245
3. Kanazawa, T. and Tonomura, Y. (1965) The pre-steady state of the myosin-adenosine triphosphate system. I. Initial rapid liberation of inorganic phosphate. *J. Biochem.* **57**, 604–615
4. Tonomura, Y., Kitagawa, S., and Yoshimura, J. (1962) The initial phase of myosin A-adenosinetriphosphatase and the possible phosphorylation of myosin A. *J. Biol. Chem.* **237**, 3660–3666

5. Tonomura, Y. and Kitagawa, S. (1960) The initial phase of actomyosin-adenosinetriphosphatase. II. Factors influencing activity. *Biochim. Biophys. Acta* **40**, 135–140
6. Ikehara, M., Ohtsuka, F., Kitagawa, S., Yagi, K., and Tonomura, Y. (1961) Interaction between synthetic ATP analog and actomyosin system. *J. Am. Chem. Soc.* **83**, 2679–2686
7. Imamura, K., Kanazawa, T., Tada, M., and Tonomura, Y. (1965) The pre-steady state of the myosin-adenosine triphosphate system. III. Properties of the intermediate. *J. Biochem.* **57**, 627–636
8. Tonomura, Y. (1972) *Muscle Proteins, Muscle Contraction and Cation Transport* University of Tokyo Press, Tokyo
9. Tonomura, Y. (1986) *Energy-Transducing ATPases—Structure and Kinetics.* Cambridge University Press, Cambridge
10. Tonomura, Y. and Kanazawa, T. (1965) Formation of a reactive myosin-phosphate complex as a key reaction in muscle contraction. *J. Biol. Chem.* **240**, 4110–4112
11. Lymn, R.W. and Taylor, E.W. (1971) Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* **10**, 4617–4624
12. Inoue, A., Takenaka, H., Arata, T., and Tonomura, Y. (1979) Functional implications of the two-headed structure of myosin. *Adv. Biophys.* **13**, 1–194
13. Huxley, A.F. and Niedergerke, R. (1954) Structural changes in muscle during contraction: interference microscopy of living muscle fibres. *Nature* **173**, 971–973
14. Huxley, H. and Hanson, J. (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976
15. Huxley, H.E. (1969) The mechanism of muscular contraction. *Science* **164**, 1356–1365
16. Bagshaw, C.R., Eccleston, J.F., Eckstein, F., Goody, R.S., Gutfreund, H., and Trentham, D.R. (1974) The magnesium ion-dependent adenosine triphosphatase of myosin. Two-step processes of adenosine triphosphate association and adenosine diphosphate dissociation. *Biochem. J.* **141**, 351–364
17. Bagshaw, C.R. and Trentham, D.R. (1974) The characterization of myosin-product complexes and of product-release steps during the magnesium ion-dependent adenosine triphosphatase reaction. *Biochem. J.* **141**, 331–349
18. Webb, M.R. and Trentham, D.R. (1981) The mechanism of ATP hydrolysis catalyzed by myosin and actomyosin, using rapid reaction techniques to study oxygen exchange. *J. Biol. Chem.* **256**, 10910–10916
19. Fisher, A.J., Smith, C.A., Thoden, J.B., Smith, R., Sutoh, K., Holden, H.M., and Rayment, I. (1995) X-ray structures of the myosin motor domain of *Dictyostelium discoideum* complexed with MgADP.BeF_x and MgADP.AlF_4^-. *Biochemistry* **34**, 8960–8972
20. Smith, C.A. and Rayment, I. (1996) X-ray structure of the magnesium(II).ADP.vanadate complex of the *Dictyostelium discoideum* myosin motor domain to 1.9 Å resolution. *Biochemistry* **35**, 5404–5417
21. Dominguez, R., Freyzon, Y., Trybus, K.M., and Cohen, C. (1998) Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state. *Cell* **94**, 559–571
22. Grigorenko, B.L., Rogov, A.V., Topol, I.A., Burt, S.K., Martinez, H.M., and Nemukhin, A.V. (2007) Mechanism of the myosin catalyzed hydrolysis of ATP as rationalized by molecular modeling. *Proc. Natl Acad. Sci. USA* **104**, 7057–7061
23. Morita, F. (1967) Interaction of heavy meromyosin with substrate. I. Difference in ultraviolet absorption spectrum between heavy meromyosin and its Michaelis-Menten complex. *J. Biol. Chem.* **242**, 4501–4506
24. Sekiya, K. and Tonomura, Y. (1967) Change in ultraviolet absorption spectrum of H-meromyosin induced by its binding with substrate and competitive inhibitor. *J. Biochem.* **61**, 787–795
25. Werber, M.M., Szent-Györgyi, A.G., and Fasman, G.D. (1972) Fluorescence studies on heavy meromyosin-substrate interaction. *Biochemistry* **11**, 2872–2883
26. Suzuki, Y., Yasunaga, T., Ohkura, R., Wakabayashi, T., and Sutoh, K. (1998) Swing of the lever arm of a myosin motor at the isomerization and phosphate-release steps. *Nature* **396**, 380–383
27. Bauer, C.B., Holden, H.M., Thoden, J.B., Smith, R., and Rayment, I. (2000) X-ray structures of the apo and MgATP-bound states of *Dictyostelium discoideum* myosin motor domain. *J. Biol. Chem.* **275**, 38494–38499
28. Gulick, A.M., Bauer, C.B., Thoden, J.B., and Rayment, I. (1997) X-ray structures of the MgADP, MgATPγS, and MgAMPPNP complexes of the *Dictyostelium discoideum* myosin motor domain. *Biochemistry* **36**, 11619–11628
29. Holmes, K.C., Angert, I., Kull, F.J., Jahn, W., and Schröder, R.R. (2003) Electron cryo-microscopy shows how strong binding of myosin to actin releases nucleotide. *Nature* **425**, 423–427
30. Geeves, M.A. and Holmes, K.C. (2005) The molecular mechanism of muscle contraction. *Adv. Protein Chem.* **71**, 161–193
31. Coureux, P.D., Sweeney, H.L., and Houdusse, A. (2004) Three myosin V structures delineate essential features of chemo-mechanical transduction. *EMBO J.* **23**, 4527–4537
32. Onishi, H., Mochizuki, N., and Morales, M.F. (2004) On the myosin catalysis of ATP hydrolysis. *Biochemistry* **43**, 3757–3763