



## Commentary and Perspective (Invited)

# On the functioning mechanism of an ATP-driven molecular motor

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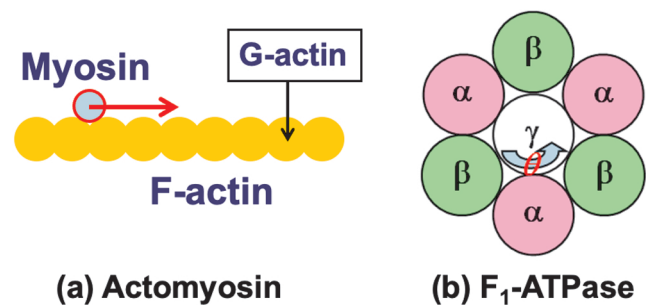
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## Introduction

A molecular motor, an ATP-driven protein or protein complex, fulfills its function by utilizing the ATP hydrolysis cycle comprising the following elementary processes: (1) the binding of ATP to the motor, (2) ATP hydrolysis ( $\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i$ ), and (3) dissociation of ADP and  $\text{P}_i$  from the motor. Paradigmatic examples which have received extensive investigations are myosin achieving unidirectional movement along filamentous actin (F-actin) and  $\text{F}_1\text{-ATPase}$  whose  $\gamma$  subunit accomplishes unidirectional rotation within the  $\alpha_3\beta_3$  complex (see Fig. 1). Though they are simpler than the sarcomere and the  $\text{F}_0\text{F}_1\text{-ATP}$  synthase, the investigations provide physical insights into and much useful information on the functioning mechanism of the motor. In the prevailing view, the motor converts the free energy of ATP hydrolysis to mechanical work that must be performed against the viscous resistance force by water during the unidirectional movement or rotation. The motor is highlighted as the system of interest and the aqueous solution surrounding it is regarded as the external system. In this article, the author suggests a new view by pointing out that the prevailing view is problematic and inconsistent with some of experimental observations. Detailed discussions are given in recent books written by the author [1,2] and references therein, and only the physical essence is recapitulated here.

Let us take protein folding as an example. The system of interest is composed of not only the protein but also water



**Figure 1** Functional expression of a molecular motor utilizing ATP hydrolysis cycle. (a) Unidirectional movement of myosin, an ATP-driven protein, along F-actin. The ATP hydrolysis cycle occurs for myosin. In the real system, F-actin possesses a double helical structure. (b) Unidirectional rotation of  $\gamma$  subunit within  $\alpha_3\beta_3$  complex of  $\text{F}_1\text{-ATPase}$ , an ATP-driven protein complex. The ATP hydrolysis cycle occurs for the  $\alpha_3\beta_3$  complex. The  $\gamma$  subunit rotates in the counterclockwise direction when  $\text{F}_1\text{-ATPase}$  is viewed from the  $\text{F}_o$  side ( $\theta$  denotes the rotation angle arbitrarily defined for the  $\gamma$  subunit).

in which it is immersed. An ensemble of folded structures is stabilized as the equilibrium state where the free energy of the protein-water system is minimized. The folding is an irreversible process accompanied by a decrease in system free energy, i.e., a spontaneously occurring process. No input of free energy (or energy) is required. On the other hand, the structures of a molecular motor before and after its functional expression are the same. Hence, one is inclined to think that a structure stabilized in the equilibrium state must be destructed for the functional expression and input of free energy is required for the destruction. This thought might have led to a proposition of the prevailing view. It should be noted, however, that the aqueous solutions before and after the functional expression are not the same: One ATP molecule is

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hydrolyzed during the functional expression.

The ATP hydrolysis can hardly occur without a catalyst. The molecular motor acts as the catalyst and becomes involved in the ATP hydrolysis cycle described above. The system of interest consists of not only the motor but also the aqueous solution of ATP, ADP, and Pi in which it is immersed. The change in system volume upon each elementary process in the ATP hydrolysis,  $\Delta V$ , is small and the system pressure  $P$  is only 1 atm with the result of  $|P\Delta V| \ll k_B T$  where  $k_B$  is the Boltzmann constant and  $T$  is the absolute temperature [2]. Hence, even in the case of  $\Delta V > 0$ , the system performs essentially no mechanical work (for  $\Delta V < 0$ , the external system performs mechanical work on the system of interest). During the ATP hydrolysis cycle, the motor exhibits sequential changes in its conformation for minimizing the system free energy to which the translational, configurational entropy of water makes a dominant contribution. The force or torque which moves the protein or rotates a protein in the complex is generated by water. The functional expression of the motor, upon which the system free energy decreases by the free energy of ATP hydrolysis, is also a spontaneously occurring process.

### Inconsistency of prevailing view with some of experimental observations

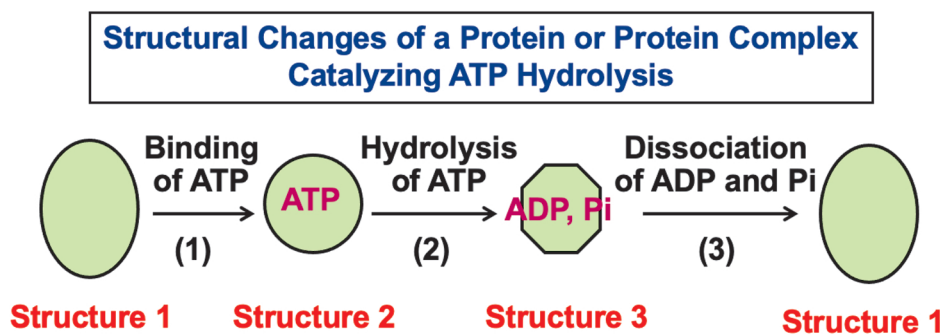
Iwaki *et al.* [1] experimentally studied the unidirectional movement of myosin along F-actin by adding sucrose to the aqueous solution. At a sucrose concentration of 1 mol/L, the viscous resistance force by water becomes about six times stronger because the viscosity of aqueous solution is made about six times higher. According to the prevailing view, the movement of myosin would be stopped or remarkably affected. However, it was observed that the sucrose addition has virtually no effects on the movement. By experimental studies using high-speed atomic force microscopy, Kodera *et al.* [3] showed that no input of free energy is required for the unidirectional movement of myosin along F-actin. Strikingly, even in the absence of

ATP, when the trailing head of myosin V is artificially detached from F-actin, myosin V makes the unidirectional movement. The observations in these experimental studies coincide with not the prevailing view but the new view explained below (refer to [2] for more details).

### Involvement of a molecular motor in ATP hydrolysis cycle

Myosin and the  $\alpha_3\beta_3$  complex in  $F_1$ -ATPase catalyzes the ATP hydrolysis. Let us suppose that the solution is under the condition that the ATP concentration ( $C_{ATP}$ ) is sufficiently high and the ADP and Pi concentrations ( $C_{ADP}$  and  $C_{Pi}$ , respectively) are sufficiently low. Under this solution condition, the frequency of the ATP hydrolysis reaction is much higher than that of the ATP synthesis reaction, and the overall reaction is ATP hydrolysis which can be regarded as an irreversible process accompanied by a decrease in system free energy [2,4,5]. The binding free energy of ATP or ADP and a molecular motor M is negative. However, the free-energy change upon the ATP-M binding is negative whereas that upon the ADP-M binding is positive [2]. For example, the frequency of ATP binding to M is much higher than that of ATP dissociation from M.

Taken together, each of elementary processes (1), (2), and (3) in the ATP hydrolysis cycle is accompanied by a decrease in system free energy and it spontaneously occurs (see Fig. 2). Without ATP, ADP, and Pi, structure 1 of myosin or the  $\alpha_3\beta_3$  complex would remain stabilized and unchanged. However, it undergoes a structural change upon each elementary process. The cycle depicted in Figure 2 is repeated. The net decrease in system free energy upon a single cycle in which one ATP molecule is hydrolyzed,  $\Delta G$ , is  $\sim -20k_B T$  ( $T=298$  K) [2,6].  $\Delta G_{(1)} + \Delta G_{(2)} + \Delta G_{(3)} = \Delta G$  where  $\Delta G_{(j)}$  denotes the free-energy decrease upon elementary process ( $J$ ) ( $J=1, 2, 3$ ). The absolute value of free energy of ATP hydrolysis in myosin or the  $\alpha_3\beta_3$  complex,  $|\Delta G_{(2)}|$ , is considerably smaller than  $|\Delta G|$ . More



**Figure 2** Structural changes of myosin or  $\alpha_3\beta_3$  complex in  $F_1$ -ATPase catalyzing ATP hydrolysis. The hydrolysis can be accelerated by not an isolated  $\beta$  subunit but a  $\beta$  subunit in the complex [2]. Myosin or the  $\alpha_3\beta_3$  complex exhibits a structural change upon each of the elementary processes (1), (2), and (3).

detailed descriptions are given in subsections 2.1.1 and 2.1.2 in [2] entitled “Thermodynamics of ATP hydrolysis reaction” and “ATP hydrolysis cycle where a molecular motor acts as a catalyst for hydrolysis reaction”, respectively.

### Critical role of water entropy in functional expression of a molecular motor

For a molecular motor to fulfill its function, myosin and the  $\alpha_3\beta_3$  complex need to coexist with F-actin and the  $\gamma$  subunit, respectively, as illustrated in Figure 3 (we consider two systems).

System (I). The position of F-actin is fixed whereas that of myosin is variable. The system free energy  $F$  is strongly dependent on the structures of myosin and F-actin and the position of myosin. Suppose that ATP, ADP, and Pi are not present in the aqueous solution. Once  $F$  is minimized, the structure and position of myosin and the structure of F-actin remain unchanged though they exhibit fluctuations: Myosin is strongly bound to the so-called rigor binding site of F-actin [1,2]. In aqueous solution of ATP, ADP, and Pi, however, elementary processes (1), (2), and (3) in the ATP hydrolysis cycle sequentially occur. Upon each occurrence, myosin undergoes a structural change with the result that  $F$  no longer takes the lowest value (a structural change of F-actin also occurs, but that of myosin is emphasized here). Therefore, for each new system configuration, myosin is driven to move for updating its position so that  $F$  can be minimized again. (More strictly, upon each elementary process in the ATP hydrolysis cycle, the structure and position of myosin are simultaneously changed for minimizing  $F$ .)

System (II). The positions of the  $\alpha_3\beta_3$  complex and the  $\gamma$  subunit are fixed but the orientation of the  $\gamma$  subunit is variable. The system free energy  $F$  is strongly dependent on the structures of the  $\alpha_3\beta_3$  complex and the  $\gamma$  subunit and the orientation of the  $\gamma$  subunit. Upon each elementary process in the ATP hydrolysis cycle, the  $\alpha_3\beta_3$  complex undergoes a structural change (a structural change of the  $\gamma$  subunit also

occurs, but that of the  $\alpha_3\beta_3$  complex is emphasized here).  $F$  does not take the lowest value for each new system configuration, and the  $\gamma$  subunit is driven to rotate for updating its orientation so that  $F$  can be minimized again.

$F$  is expressed as

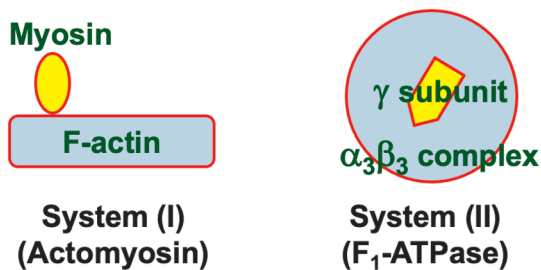
$$\begin{aligned} F &= E_C - TS_C + \mu_H, \quad \mu_H = E_H - TS_H, \\ F &= E_C + E_H - TS_C - TS_H \end{aligned} \quad (1)$$

where  $E_C$  is the conformational (intramolecular) energy,  $S_C$  is the conformational entropy, and  $\mu_H$ ,  $E_H$ , and  $S_H$  are the hydration free energy, energy, and entropy, respectively.  $E_C$ ,  $S_C$ ,  $\mu_H$ ,  $E_H$ , and  $S_H$  are defined for the combination of F-actin and myosin in system (I) or that of the  $\alpha_3\beta_3$  complex and the  $\gamma$  subunit in system (II).  $|S_H|$  of a solute denotes the magnitude of water-entropy loss caused by the solute insertion. In general, a change in conformational energy  $\Delta E_C$  and that in hydration energy  $\Delta E_H$  are compensating. ( $\Delta X$  signifies the change in  $X$  caused by changes in the structure and position of myosin or by those in the structure of the  $\alpha_3\beta_3$  complex and the orientation of the  $\gamma$  subunit.) That is,  $\Delta E_H > 0$  for  $\Delta E_C < 0$  and  $\Delta E_H < 0$  for  $\Delta E_C > 0$ , and  $\Delta E_C + \Delta E_H$  is usually positive but much smaller than  $|-T\Delta S_H|$  [2]. In a structural change of a protein, when two oppositely charged groups of the protein are buried and contacted, for example,  $E_C$  decreases due to a gain of electrostatic attractive interaction between the two groups. However, the gain accompanies a loss of group-water electrostatic attractive interactions, causing an increase in  $E_H$ . This is why  $\Delta E_C$  and  $\Delta E_H$  are compensating. On the other hand,  $|\Delta S_C|$  is significantly smaller than  $|\Delta S_H|$  [2]. Therefore, when we discuss  $\Delta F$ , the water entropy  $S_{\text{Water}}$  can be regarded as a principal component of  $F$ :

$$F \sim -TS_{\text{Water}}. \quad (2)$$

The structure and position of myosin in system (I) or the structure of the  $\alpha_3\beta_3$  complex and the orientation of the  $\gamma$  subunit in system (II) are determined so that the water entropy can be maximized.

According to our experience in studies on microscopic, biological self-assembly processes,  $\Delta S_H$  is the most important quantity in arguing their mechanisms [2]. For the binding of an oncoprotein (MDM2) and the extreme N-terminal peptide region of a tumor suppressor protein p53 (p53NTD) [7], for example,  $\Delta E_C \sim -622k_B T$  ( $T=298$  K),  $\Delta E_H \sim 632k_B T$ ,  $\Delta E_C + \Delta E_H \sim 10k_B T$ ,  $\Delta S_H \sim 132k_B$ , and  $\Delta S_C \sim -59k_B$  ( $\Delta X$  signifies the change in  $X$  upon the binding). These quantities were calculated using our state-of-the-art theoretical method [7,8] where molecular models are adopted for water, the structures of biomolecules (the MDM2-p53NTD complex and isolated MDM2 and p53NTD) are treated at the atomic level, and the structural



**Figure 3** Two systems considered. System (I) (actomyosin): myosin and F-actin. System (II) (F<sub>1</sub>-ATPase):  $\gamma$  subunit incorporated within  $\alpha_3\beta_3$  complex. Actomyosin and F<sub>1</sub>-ATPase are immersed in aqueous solution of ATP, ADP, and Pi ( $C_{\text{ATP}}$  is sufficiently high, and  $C_{\text{ADP}}$  and  $C_{\text{Pi}}$  are sufficiently low).

fluctuation of the biomolecules in water are taken into account with the aid of molecular dynamics (MD) simulations with all-atom potentials. The conformational-entropy loss for the biomolecules,  $\Delta S_C$ , was calculated by means of the Boltzmann-quasi-harmonic method [9] combined with the MD simulations. Therefore, the calculated values given above are quantitatively reliable. Taken together, the fundamental, qualitative aspects of a biological self-assembly process can be argued with the emphasis on  $\Delta S_H$ . The author notes, however, that  $\Delta E_C + \Delta E_H$  and  $\Delta S_C$  should be taken into account when quantitatively accurate evaluation of the free-energy change is undertaken.

### Force or torque generation by water for moving or rotating a protein

We assume that myosin and F-actin or the  $\alpha_3\beta_3$  complex and the  $\gamma$  subunit (see Fig. 3) take prescribed structures. In system (I),  $F$  is a function of the Cartesian coordinates of the center of gravity of myosin,  $(x, y, z)$ . The origin of the coordinate system  $(0, 0, 0)$  is chosen to be, for example, the left edge of F-actin. The  $x$ -axis is taken to be in the direction of forward movement of myosin along F-actin.  $F(x, y, z) - F(+\infty, +\infty, +\infty)$  represents the spatial distribution of the water-mediated potential, the potential of mean force (PMF), between F-actin and myosin. The mean force (MF) acting on myosin,  $\mathbf{f}$ , is expressed as

$$\begin{aligned} \mathbf{f} &= f_x \mathbf{i} + f_y \mathbf{j} + f_z \mathbf{k}, \\ f_x &= -\partial F / \partial x, \quad f_y = -\partial F / \partial y, \quad f_z = -\partial F / \partial z \end{aligned} \quad (3)$$

where  $\mathbf{i}$ ,  $\mathbf{j}$ , and  $\mathbf{k}$  denote the direction unit vectors.  $\mathbf{f}(x_0, y_0, z_0)$  represents the force induced between F-actin and myosin averaged over all the possible configurations of water molecules in the whole system with  $(x, y, z)$  being fixed at  $(x_0, y_0, z_0)$ . Namely, myosin feels a potential or force field due to the presence of F-actin near it.  $S_{\text{Water}}$  is largely dependent on  $(x, y, z)$ , and the entropic force expressed by Eqs. (2) and (3) and the PMF (i.e., the entropic interaction) are significantly strong.

Upon a structural change of myosin illustrated in Figure 2,  $F(x, y, z) - F(+\infty, +\infty, +\infty)$  and  $\mathbf{f}(x, y, z)$  also change. That is, the potential or force field felt by myosin exhibits sequential changes during the ATP hydrolysis cycle. If myosin was isolated, its motion would be random. When it is near F-actin, however, its motion is influenced by the potential field. It can overcome only a free-energy barrier whose order of magnitude is  $k_B T$ . We illustrated that the sequential changes mentioned above lead to the unidirectional movement of myosin subfragment 1 (S1) along F-actin, by which S1 sometimes moves by more than a single step (the step size equals the size of a G-actin molecule) and even backward in accord with the

experimental result for S1 forcibly attached to F-actin using a novel technique [1,10]. In a strict sense, the structure of myosin exhibits not a step change but a gradual one. When the effect of this gradual change is taken into account, the potential field felt by myosin gradually shifts in the negative direction as myosin moves and its structural change proceeds, since the structure of myosin changes so that  $F$  can become as low as possible. Myosin then exhibits a biased Brownian motion. The picture of the unidirectional movement argued in [1] is to be revisited by accounting for the aforementioned potential shift in a future study.

Here, let us consider a large hard sphere and a planar hard wall immersed in small hard spheres forming the solvent. The position of the wall is fixed but that of the large sphere is variable. It is assumed that the interaction potentials include no soft, repulsive or attractive terms. In this model system, all the possible system configurations share the same energy and the system behavior is purely entropic in origin. Nevertheless, an entropic force acts on the large sphere (the MF does not possess an energetic component) [2]. It is a function of the surface separation between the wall and the large sphere,  $h$ . It is oscillatory with a periodicity of the diameter of small spheres ( $d_s$ ), strongly attractive for small  $h$ , and rapidly damped with an increase in  $h$ . The entropic force becomes stronger with increasing  $\eta_s$  ( $\eta_s$  is the packing fraction of small spheres) and decreasing  $d_s$ . By virtue of the hydrogen-bonding network, water can exist as a dense liquid at ambient temperature and pressure despite its exceptionally small molecular diameter. The entropic force becomes strongest when the solvent is water.

Aqueous solution under the physiological condition contains NaCl at a concentration of  $\sim 0.15$  mol/L). The electrostatic interaction is screened by not only water molecules but also cations and anions. As a consequence, it becomes over two orders of magnitude weaker and much shorter-ranged than in vacuum [11]. The entropic interaction mentioned above is significantly stronger than the screened electrostatic interaction. Further, upon the addition of salts whose cations or anions are strongly hydrated, the entropic interaction is significantly enhanced primarily due to the increased packing fraction of aqueous solution [12]. The emphasis placed on the entropic interaction for actomyosin can be rationalized by, for example, the experimental data manifesting that the binding of myosin to F-actin is entropically driven [13].

In system (II),  $F$  is a function of the rotation angle  $\theta$  defined for the  $\gamma$  subunit (see Fig. 1(b)). The mean torque acting on the  $\gamma$  subunit,  $\tau(\theta)$ , is expressed as

$$\tau = -\partial F / \partial \theta. \quad (4)$$

$\tau(\theta_0)$  represents the torque acting on the  $\gamma$  subunit averaged over all the possible configurations of water molecules in

the whole system with  $\theta$  being fixed at  $\theta_0$ . In this case,  $F$  takes a sharp minimum at  $\theta=\theta_{\min}$  and  $\tau(\theta_{\min})=0$  [2]. Since  $S_{\text{Water}}$  in Eq. (2) is largely dependent on  $\theta$ , the entropic torque expressed by Eqs. (2) and (4) is significantly strong. Upon a structural change of the  $\alpha_3\beta_3$  complex illustrated in Figure 2,  $\tau(\theta)$  and  $\theta_{\min}$  also change. In a single ATP hydrolysis cycle, the torque acts on the  $\gamma$  subunit in the counterclockwise direction and  $\theta_{\min}$  increases by  $120^\circ$  [2].

### Physical origin of hydrophobic effect and its essential role in biological self-assembly

It is worthwhile to revisit the importance of the water entropy in arguing self-assembly processes in biological systems. The hydration of a solute molecule (e.g., a protein) with a fixed structure can be decomposed into the following two processes [8].

Process 1. The cavity, which matches the solute structure at the atomic level, is created in water. The cavity can be modeled as a set of fused, neutral hard spheres corresponding to the atoms constituting the solute molecule. This process is the hydrophobic hydration.

Process 2. This process consists of subprocesses 2-vdW and 2-ES. In subprocess 2-vdW, the solute-water van der Waals (vdW) potential is incorporated: The hard-sphere potential between an atom in the solute molecule and a water molecule (i.e., the atom-water hard-sphere potential) is replaced by the Lennard-Jones potential. In subprocess 2-ES, the solute-water electrostatic (ES) potential is incorporated: A prescribed partial charge is given to each atom in the solute molecule for taking account of the atom-water ES potential.

The thermodynamic quantities of hydration in processes 1 and 2 can be calculated with sufficient accuracy and very high speed by our hybrid of the angle-dependent integral equation theory combined with the morphometric approach and the three-dimensional reference interaction site model theory [8]. Molecular models are employed for water. We showed the following: (i) The hydration properties relevant to process 1 depend on  $T$  and  $P$  much more strongly than those relevant to process 2; and (ii) in such processes as the protein-peptide binding [7], protein folding [2], and pressure [14] and cold [15] denaturation of a protein, the hydration entropy in process 1,  $S_{\text{H1}}$ , plays a pivotal role.

The presence of a solute generates a space which is inaccessible to the centers of water molecules in the whole system. The volume of this space is referred to as the "excluded volume (EV)". When the EV reduces, the total volume available for the translational displacement of water molecules increases. The presence of a water molecule also generates an EV for the other water molecules. Thus, all the water molecules are entropically correlated. We refer to this entropic correlation as the "water crowding". The hydrophobic effect is governed by

the EV-dependent term of  $S_{\text{H1}}$  (i.e., the term which is dependent on the EV of the cavity) and the translational, configurational entropy is a dominant component of  $S_{\text{Water}}$ . A biological self-assembly process occurs so that the water crowding can be mitigated as much as possible [2]. Conventionally, the hydrophobic effect is discussed only in terms of the hydration layer near a solute. However, this conventional method is not capable of elucidating the effects of lowered temperatures and elevated pressures on the structure formed by a biological self-assembly process [14,15]. Let us take system (I) where the structure and position of F-actin are fixed, for example. For a prescribed structure of myosin, the degree of water crowding is strongly dependent on the position of myosin. The entropic potential is positive when the degree is higher than that in the reference case whereas it is negative when the degree is lower than that in the reference case (in the reference case, myosin is infinitely separated from F-actin). This is the physical origin of the formation of spatial distribution of the entropic potential.

### Comments on more complex systems

Properties of the aqueous solution in a cell where a molecular motor works can be considerably different from those in an in-vitro experiment. There are significantly many biomolecules in cytoplasm. It should be noted that the surfaces of most of these biomolecules are hydrophilic. In general, when hydrophilic solutes are present in aqueous solution at significantly high concentrations, the total packing fraction of the solution becomes considerably higher. Therefore, the entropic effect discussed in this article should become even larger in a cell. As an evidence, when a highly hydrophilic cosolvent is added to aqueous solution, the entropic gain upon protein folding is magnified, leading to higher thermostability of a protein [16].

In general, when a solute (solute 1) is immersed in water, it exhibits only a random motion. When solute 1 and another solute (solute 2) are immersed in water, however, a water-mediated force (the mean force (MF)) is induced between the two solutes. The MF becomes stronger as the sizes of the two solutes increase. When the position of solute 2 is fixed, solute 1 exhibits an ordered, constrained motion due to the presence of the force field. Since myosin and F-actin are both quite large, the MF is significantly strong for actomyosin.

The muscle contraction is driven by the sliding of myosin and actin filaments in a myofibril (by a decrease in the sarcomere length). These filaments are even larger than myosin and F-actin referred to above, with the result that the force of muscle contraction is very strong. Here, let us consider the following thought experiment. Suppose that a weight is suspended by thread connecting it with muscle.

When the muscle contraction occurs, the weight is raised against the gravity. However, this large mechanical work is performed by neither the free energy of ATP hydrolysis nor muscle. The author emphasizes that the strong force for the muscle contraction is generated by water though the force generation is triggered by the ATP hydrolysis. The large mechanical work is performed by nothing but water. The mechanism of muscle contraction can be unveiled only by looking at the system comprising muscle and the aqueous solution. The system performs only small mechanical work even if the system volume increases, because the pressure is only 1 atm. On the contrary, it is possible that the system volume decreases ( $\Delta V < 0$ ) upon the muscle contraction.

### Concluding remarks and perspective

In the previous studies on actomyosin and  $F_1$ -ATPase, the attention was focused on the conversion of free energy of the ATP hydrolysis to mechanical work and the critical role of water was not suitably taken into account (besides the papers by the author and coworkers, there is only one paper [17] showing the crucial importance of hydration of myosin and F-actin using an approach based on statistical mechanics; see [2] for a detailed discussion). Not only the proteins or protein complexes but also the aqueous solution of ATP, ADP, and Pi in which they are immersed forms the system. Importantly, the system performs essentially no mechanical work. The ATP hydrolysis involving the proteins or protein complexes spontaneously occurs, which is accompanied by a decrease in system free energy. The most serious drawback of the prevailing view is that the concept of formation of the force or torque field by water is completely missing in it.

A force or torque field is formed for the key protein (myosin in actomyosin or the  $\gamma$  subunit in  $F_1$ -ATPase). The characteristics of the field are dependent primarily on the structure of myosin or that of the  $\alpha_3\beta_3$  complex. Upon each of the elementary processes in the ATP hydrolysis cycle, myosin or the  $\alpha_3\beta_3$  complex undergoes a structural change. As a consequence, the force or torque field exhibits sequential changes during the cycle, leading to the unidirectional movement or rotation of the key protein. Thus, the key protein is moved or rotated for increasing the translational, configurational entropy of water governing the system free energy. If an external force or torque is imposed on the key protein, the average movement distance or rotation angle for a number of ATP hydrolysis cycles should decrease.

For  $F_1$ -ATPase, the author argued that the following four cases can be elucidated within the same theoretical framework [2]: (A) The  $\gamma$  subunit rotates in the normal direction when the overall reaction in the aqueous solution is ATP hydrolysis (the inverse rotation rarely occurs); (B) it rotates in the inverse direction when the overall reaction is

ATP synthesis; (C) it rotates in the normal and inverse directions with the same frequency (i.e., its rotation is directionally random) under the solution condition that the ATP hydrolysis and synthesis reactions are in equilibrium; and (D) even when the overall reaction should be ATP hydrolysis, ATP is synthesized from ADP and Pi through forcible rotation of the  $\gamma$  subunit in the inverse direction by means of external torque imposed on it, which is stronger than the entropic torque by water. (Cases (B) and (C) have not been corroborated by experiments.) The mechanism in case (A) can be summarized as follows [2].  $S_{\text{water}}$  is maximized in the stabilized structure of the  $\alpha_3\beta_3\gamma$  complex. In this catalytic dwell state, ATP just before the hydrolysis reaction, ATP, and Pi are bound to the three  $\beta$  subunits, respectively. The atoms in these  $\beta$  subunits are closely, moderately, and loosely packed, respectively. This nonuniformity in the packing structure and the structural asymmetry of the  $\gamma$  subunit play imperative roles. Due to the occurrence of the elementary processes in the ATP hydrolysis cycle, the chemical compounds bound to the three  $\beta$  subunits sequentially change, in concert with which the  $\alpha_3\beta_3$  complex structure also undergoes sequential changes. The  $\gamma$  subunit is driven to take a particular orientation in accordance with the structure of the  $\alpha_3\beta_3$  complex so that the maximized value of  $S_{\text{water}}$  can be retained. In a single cycle, the  $\gamma$  subunit exhibits a  $120^\circ$  rotation.

In conclusion, the author requests that the prevailing view be reconsidered and intends to further improve the new view. In the near future, the effects of  $E_C + E_H$  and  $-TS_C$  (see Eq. (1)) on the functional expression of a molecular motor are to be investigated for the improvement.

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