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The unit event of sliding of the chemo-mechanical enzyme composed of myosin and actin with regulatory proteins

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Abstract

Various myosin–actin systems do not always show the same sliding behaviors. To make the situation clear, discussions are concentrated on the unit event of sliding of the chemo-mechanical enzyme composed of a single myosin head and a single actin filament with regulatory proteins. The popular idea of the one-to-one correspondence between the chemical state and the physical state or between the chemical reaction step and the physical conformational change is reexamined. It is likely that the sites and the modes of interaction between myosin head and actin filament during the ATP hydrolysis are more multiple and variable, and the input–output coupling in the chemo-mechanical enzyme is loose.
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Preface

I remember conversations with Dr. S. Ebashi I had after the Muscle Biochemistry Meeting in 1962. At this meeting he presented, based on his own careful experiments, a new idea that calcium ion is the key signal for muscle contraction. But, most people did not agree. One of the reasons was that the in vitro contraction and the ATPase activity of purified myosin and actin did not depend on the presence or absence of calcium ions. After this meeting, at the Boston airport he said to me "I will immediately start the experiment to look for a third protein which makes myosin and actin sensitive to calcium ion. It must be in the side of actin filaments." Since 1954, my main research subject had been the mechanism of the G-F transformation of actin and the dynamics of F-actin. I had never thought about the possibility of the presence of the third protein. What I could say was only to encourage him. After a couple of years, Dr. Ebashi reached the finding of native tropomyosin and then troponin, as the third protein he expected [1]. I heard his presentation of hot data on native tropomyosin in the Biochemistry Meeting at New York in 1964. I wish to emphasize that his finding was not an accidental one, but was based on his way of logical thinking and hard efforts for careful experiments. He established his calcium ion-troponin-actin filament story on regulation of contraction of the striated muscle [2]. He beautifully predicted the structure of the actin filament with tropomyosin and troponin, although later, troponin was found to be composed of three subunits.

He had a fine sense of physics. He liked to show the experimental data, for example, on the effect of calcium ion not only at the physiological condition, but also in the wide range of the environmental condition, the protein concentration, the salt concentration, the ATP concentration, etc. Such a manner of experiments is important for understanding the molecular mechanism.

On the other hand, he was always critical about easy generalization of observed phenomena and the mechanism. In fact, in all myosin–actin systems the initial signal is calcium ion and the final response is the regulation of myosin–actin sliding, but the route connecting them is different in different kinds of cells.

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Already more than 40 years passed since his discovery. Many data have been accumulated, but the molecular mechanism of regulation of the sliding between myosin and actin filaments by the regulatory proteins, troponin, and tropomyosin, depending on calcium ions has not yet been made fully understandable.

Here, I like to discuss about unit events of single chemo-mechanical enzymes composed of myosin molecules and actin filaments with regulatory proteins and raise some questions concerning the input–output coupling in those enzymes, the answer of which may give a clue for understanding the molecular mechanism of the chemo-mechanical free energy conversion and its regulation.

Introduction

In l939, Engelhardt demonstrated that the main protein component in muscle fibers called ''myosin'' had an enzymatic activity of the hydrolysis of ATP, which had been identified as the energy source of muscle contraction [3]. Myosin was regarded as the chemo-mechanical enzyme. It was extracted from muscle fibers into a solution of high salt concentration. If this solution of myosin was injected into a solution of low salt concentration, myosin molecules were insoluble and formed a thin gel. When ATP was added, the gel showed contraction.

The classical myosin was found to be composed of two kinds of proteins; in addition to myosin, the new protein ''actin''. Szent-Gyorgyi showed that the mixture of myosin and actin at low salt concentrations gave a turbid solution and formed dense compact aggregates upon addition of ATP [4]. He called this phenomenon ''superprecipitation''.

Both the gel contraction and the superprecipitation of the complex of myosin and actin were irreversible phenomena; after finishing the hydrolysis of ATP, the initial state was not recovered. On the other hand, in the case of simple enzymes, after catalyzing the chemical reaction, the state of enzyme molecules returns to the initial state.

In 1954, A. Huxley, and H. Huxley and J. Hanson found that the contraction of muscle cells occurred as a result of sliding between thick filaments and thin filaments aligned in parallel [5,6]. Interaction between myosin molecules regularly arranged in thick filaments and actin molecules in thin filaments coupled with the hydrolysis of ATP on myosin molecules produced the sliding. The states of myosin molecules and actin molecules return to those before sliding and only the shift of the relative position of two filaments remains as a result of sliding. In this sense, the sliding system maintained the character as an enzyme.

Construction of chemo-mechanical enzyme

About 40 years after the finding of sliding, the in vitro reconstruction of the ''chemo-mechanical enzyme'' composed of a single myosin molecule and a single actin filament was successfully performed in Yanagida's laboratory [7]. In a salt solution, an actin filament was fixed on a plate. A single head of myosin molecule, called S-1, was attached to the end of a thin needle placed perpendicular to the actin filament, where the other end of the needle was fixed.

Upon addition of ATP to the solution, an ATP molecule is bound to the myosin head and hydrolyzed into ADP and inorganic phosphate (P), both of which are kept bound on the myosin. In this state, the end of the needle is artificially moved onto the actin filament. The myosin head on which ATP is reversibly hydrolyzed to ADP and P begins to interact with actin molecules and the sliding force is generated. Then, the myosin head moves on the actin filament and the thin needle is bent. When P, and then ADP, are dissociated from the myosin head after sliding of a certain distance, the binding of the head with actin molecule is made tight and the sliding stops. Or, if a needle having a large elastic modulus against bending is used, the sliding stops when the sliding force of the myosin head and the elastic force of the bent needle are balanced. Then, ADP and P are both dissociated from the head and the myosin head is kept bound tightly. Anyway, some mechanical energy is stored in the bent needle. When a new ATP molecule comes to be bound with the myosin head, the head having ATP is readily dissociated from the actin molecule. The needle becomes free and returns to the initial position; both the myosin head and the actin filament also return to the original states. In this process the elastic energy in the needle can be used to do some mechanical work in an arbitrary way. Thus, the chemical free energy released during the hydrolysis of one ATP molecule is partly converted into the sliding movement and the mechanical energy.

The unit event in the chemo-mechanical enzyme

The term ''chemo-mechanical enzyme'' can be reasonably given to the above system. It must be remarked that the chemical free energy converted into the sliding or the mechanical work during the unit event is not much larger than, or sometime of the same order as, the average energy of thermal fluctuation, kT. In the ordinary experimental condition, the efficiency of the conversion of the chemical free energy released in the hydrolysis of one ATP molecule to the mechanical work was estimated to be about 15–20%.

Using fluorescently labeled myosin head and ATP or ADP, the movement of the head on the actin filament and the binding-dissociation of ATP or ADP on the head were directly observed during sliding with spatial and temporal resolutions of nm and ms. It was reported in 1999 that the sliding happens stepwise, sometimes including the backward step; each step is about 5 nm, which corresponds to the size of actin monomer in the actin filament [8]. The sliding produced by the hydrolysis of one ATP molecule consists of a few or several steps, in such a way that the total distance is variable and often attains 20 nm or longer.

Most people, however, had a different idea about how the sliding occurs. In the current opinion, the sliding has

been supposed to be caused by a specific conformational change in the myosin head kept bound on the actin filament directly coupled with a specific step of the ATP hydrolysis reaction. In this line, the lever arm (or head rotation) mechanism or the walking (or hand-over-hand) mechanism was proposed and became popular [9,10]. Such a mechanism produces a single step sliding of a definite magnitude. Actually, experimental data appeared to support the lever arm mechanism or the walking mechanism. Even so, when a needle having a large elastic modulus of bending is used, the sliding distance must become short. Therefore, the myosin head must have flexibility somewhere in the structure in addition to the principal conformational change.

There is a big family of myosin distributed in various kinds of cells. Not all members of the family show the same behaviors of sliding on actin filaments. How are the input, the chemical reaction, and the output, the sliding or the mechanical work, coupled in the unit events of the chemo-mechanical enzymes composed of various myosin molecules and actin filaments? In the first case where the sliding consists of multiple small steps, partially oscillatory, there is no one-to-one correspondence between the chemical state and the physical state or between the chemical step and the physical conformational change. The input–output coupling is loose. On the other hand, in the second case such correspondence may be satisfied, and the input-output coupling is tight. This problem will be discussed later again.

The chemo-mechanical enzyme with regulatory proteins

In the striated muscle, regulatory proteins, troponin and tropomyosin are bound on each actin filament. Troponin molecules are composed of three subunits, TnC, TnI and TnT, and tropomyosin molecules make a two stranded coiled-coil structure [11]. Each actin filament is a two stranded helical polymer of actin monomers. On every half pitch of the helix, the length of which is about 37 nm, one pair of troponin molecules and tropomyosin strands are bound. Therefore, a set of a half pitch of actin filament with a pair of troponin and tropomyosin and a single myosin head must be the smallest unit of the chemo-mechanical enzyme in which the sliding behavior depends on the calcium ion concentration.

The interaction of the myosin head with the actin filament for sliding is inhibited by troponin–tropomyosin in the absence of calcium ions. Based on the structural analyses, the steric blocking by positioning of tropomyosin on the actin filament was proposed as the mechanism of inhibition [12]. When troponin binds calcium ions, the position of tropomyosin is shifted to release the steric blocking. However, some other experiments, for example, the fluorescent energy transfer measurements gave no indication of the change of the position of tropomyosin on the actin filament [13]. It was found also that the actin filament with troponin and tropomyosin is more rigid than the pure actin filament and is made more flexible by binding of calcium ion with troponin [14].

Usually, the actin filament with troponin and tropomyosin is supposed to make a transitional change between two states following binding and dissociation of calcium ions on troponin. The experiments using the smallest unit of the chemo-mechanical enzyme with regulation defined above must be useful to make the situation clearer. How many actin monomers along the filament are influenced by binding and dissociation of calcium ions on one pair of troponin? At intermediate concentrations of calcium ions, each troponin molecule quickly repeats binding and dissociation of calcium ion. In this condition, does the actin filament repeat transitions between two discrete states, on and off, or does the actin filament assume some intermediate state? Which does happen the sliding of the on–off type or of a slow speed and a short distance? It has been suggested that the actin filament with troponin and tropomyosin assumes three kinds of states, blocked, closed and opened, corresponding to in the absence of calcium ion, in its presence, and in the presence of both calcium ion and tightly bound myosin head [15]. Significance of these states must be examined also in the above smallest system. If the normal sliding of the single myosin head occurs without coexistence of other heads, the third state is not required for sliding or it is realized before the head enters into the tight binding with the actin filament. Then, the one-to-one correspondence between the physical conformation of the filament and the binding state of calcium ion is not necessarily satisfied. It may be probable that the sites of the myosin head and the actin molecule to interact each other are not unique but multiple or variable [16]. There is an evidence for that different members of myosin family may interact with the actin filament at different sites. For example, the interaction of plant myosin with actin filament is completely inhibited by tropomyosin [17].

The free energy conversion in the unit event

As described in previous sections, various manners of sliding of myosin heads on actin filaments and various modes of inhibition of the sliding by troponin–tropomyosin have been reported. Is there any basic principle in the sliding mechanism and the regulatory mechanism? To extract the basic principle, if it exists, what kind of question must be raised?

What does happen in the unit event of the chemo-mechanical enzyme if the chemical potential difference in the ATP hydrolysis is decreased? In practice, it is very difficult, almost impossible to make the chemical potential difference close to zero by decreasing the ATP concentration and increasing the ADP and P concentrations without any disturbance on the state of the enzyme. Therefore, at present what we can do is to attempt some imaginative experiments (Gedanken experiments).

It is likely that the sliding speed and the force for sliding decrease with decreasing chemical potential difference. At zero chemical potential difference, unidirectional sliding between the myosin head and the actin filament must not occur. In the case of the multiple stepwise sliding, it is most probable that the number of steps and the total distance of sliding decreases with decreasing chemical potential difference and finally the directional sliding changes to the bi-directional Brownian movement and the average of the mechanical energy of bending of the needle tends to $(1/2)kT$. What kind of mechanism does such performance make possible?

On the other hand, if the sliding is totally due to a specific conformational change in the myosin head directly coupled with a specific intermediate step in the ATP hydrolysis on the myosin head, the sliding distance and the force generated, and the mechanical work also may be independent of concentrations of ATP, ADP and P, or the chemical potential difference in the ATP hydrolysis. With decreasing the chemical potential difference, the efficiency of the chemical free energy conversion into the mechanical work increases towards 100% or more. Under such a condition, the reverse reaction to the synthesis of ATP associated with the sliding in the opposite direction may occur. Does the definite one-step sliding occur in both directions equally when the chemical potential difference approaches to zero? It seems difficult to suppose such behaviors without contradiction with thermodynamic principles.

It is more likely that there are multiple and variable states in the myosin head and the actin filament interacting during sliding and the hydrolysis of ATP. Anyway, it is a difficult problem to be solved; how does the chemical potential difference in the ATP hydrolysis influence the sliding behaviors?

The input–output coupling, tight or loose

I have been interested in the input–output coupling in molecular machines in living cells. More than 20 years ago, I took up this problem and proposed the idea of loose coupling in relation to the mechanism of the three kinds of molecular machines, the bacterial flagellar motor, the F1Fo ATP synthetase or the Fl ATPase, and the myosin–actin sliding system [18].

The experiment to investigate the input–output relation in the myosin–actin sliding was undertaken for the first time about thirty years after the finding of sliding. Using the myosin and actin filaments aligned in parallel, Yanagida et al. demonstrated that the sliding distance per ATP molecule hydrolyzed is remarkably longer than that expected based on the lever-arm mechanism or the walking mechanism [19]. The loose coupling was suggested, and a thermal ratchet mechanism was proposed [20]. Later, various kinds of the ratchet mechanism have been presented [21]. As described already, the direct observation of multiple-step sliding in the unit event gave a more reliable evidence for the loose coupling.

Rotation is a kind of sliding. In the flagellar motor the direct measurement of the input, the number of protons flowing in the rotating motor, is too difficult to get the answer to the question about the input–output coupling. Up to the present, various mechanisms have been proposed on the motor rotation; most of them assumed the tight coupling in the unit event, that is, a definite angle of rotation by the flow of a definite number of protons. However, recent structural studies suggest that the proton channel where the electrochemical potential difference exists and the site where the torque for rotation is generated have no direct interaction in the motor structure [22]. It seems difficult to assume a direct one-to-one correspondence between the flow of individual protons and the stepwise rotation of the motor.

In the Fl ATPase, the rotation of the inner subunit was directly observed during the hydrolysis of ATP [23]. People have no doubt about the tight coupling and believe that three ATP molecules are hydrolyzed during one rotation. The observed three-step rotation strongly supports this idea. The unit event is 120° rotation [24]. Further, the experiments showed that with decreasing concentration of ATP, the resting time of rotation becomes longer, but once the unit event of rotation occurs, the angle of rotation and the generated torque are not different from those under the ordinary condition of the chemical potential difference. Then, the same problem about the efficiency arises as in the myosin–actin sliding, if the unit event of rotation is always tightly coupled with the hydrolysis of one ATP molecule.

Kinesin molecules or dynein molecules slide on microtubules with the hydrolysis of ATP.

In these cases also, sliding behaviors are not always similar.

I wish to emphasize that the question whether the input–output coupling is tight or loose is valuable to be asked in various kinds of molecular machines in living cells. This question is related to the reexamination of the idea of the one-to-one correspondence between the chemical state and the physical state in those machines. Most people assume such correspondence. However, the correspondence may be multiple and variable.

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