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Energy transformation in biological molecular motors

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Abstract

Biological molecular motors transform the metabolic free energy into the directed movement. The physical principles governing this transformation are very different from the principles underlying the manmade macroscopic motors. Theoretical analysis shows that the internal thermal diffusion in motor proteins is a key element of the process, and the chemical energy performs no mechanical work directly but instead it is used for rectifying the diffusion. A few specific motor systems are considered to illustrate the general principle. The principle of rectified thermal diffusion has recently received a great support from the single-molecule studies.

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Keywords: Rectified diffusion; Allosteric transition; Motor protein; Brownian ratchet

Contents

1. Introduction

In the biological molecular motors, the chemical energy is transformed into the directed movement. There is no need to argue that molecular motors represent a key element of the phenomenon of life. Studying the molecular motors

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has been one of the major topics of biophysics over the past decades, and enormous progress has been achieved. This progress is related, first of all, to understanding the structural organization and mechanics of the motor action, and is due to the great variety of experimental techniques used in these studies (see [1–3] and references therein). Experimental studies have not been capable, however, to reveal fundamental principles of chemical energy transformation into directed movement. Therefore, our current knowledge of the issue is based, mainly, on the theoretical analysis. This theoretical analysis is very fruitful, however, and has made it possible to formulate the basic physical principles of the molecular motors.

A very general consideration of the problem allows one to conclude that biological molecular motors cannot use principles of manmade macroscopic motors. A chemical reaction, which delivers the energy for each cycle of a molecular motor, causes displacement of the participating atoms in the scale of one tenth of nanometer, while one step of displacement in a motor protein usually extends to a few nanometers. Also, the reaction cannot push the two parts of a protein to the distance of a few nanometers, since motion in this scale has no inertia: a directed movement of a protein subunit lasts as long as a force is acting on it. All forces which can be associated with a chemical act are short-range forces which nearly disappear at distances smaller than one nanometer. This is true even for electrostatic forces, since the electric charges are effectively screened under ionic conditions in the living cell. In the absence of inertia and long-range forces, directly created by a chemical act, the manmade macroscopic motors would never work. Thus, different principles have to be used in molecular motors.

The fundamental idea explaining the action of biological molecular motors is that chemical energy stored in ATP molecules (and other "energy-charged" molecules) is not used for the mechanical work directly, but instead is responsible for rectifying thermal motion, or diffusion, of the motor elements. The idea of a key role of rectified thermal diffusion was pioneered by Huxley [4]. Another model, based on thermal motion, originates to the Feynman's thermal ratchet (also called the Brownian ratchet) [5–7]. Although the principle of the thermal ratchet has been used in manmade devices, some of its features make its use in the biological motors very problematic. The term "Brownian ratchet" has been used in much broader sense in recent years, however, referring to any use of thermal motion in molecular motors. Definitely, the idea of rectified thermal diffusion is more general than the Brownian ratchet principle. In different forms it has been formulated and analyzed by many researches [8–13]. The principle of rectified thermal motion was used to design artificial molecular motors where chemical energy is transformed into directed movement [14–18]. Before recently, however, there have been no direct experimental data showing that biological molecular motors are based on the same principle. This is probably a reason that the idea has not become a commonly accepted principle of biological molecular motors. In many reviews on biological molecular motors and textbooks the act of transforming chemical energy into mechanical work is simply called by a mysterious term "Power stroke". The situation has been changing recently when the single-molecule technique has started bringing quantitative data which support the principle of rectified thermal diffusion. The idea of using chemical energy to rectify thermal diffusion and its application to biological molecular motors is a topic of this review.

2. Movement without inertia

The fact that movement in the molecular scale has practically no inertia strongly restricts possible mechanisms of large-scale conformational changes in proteins and in molecular motors in particular. This fact is due to extremely high viscosity of water in the molecular scale.

Let us consider movement of a bead in a viscous medium. The movement is described by Newton's second law:

$$m\frac{\mathrm{d}v}{\mathrm{d}t} = F, \tag{1}$$

where m and v are bead's mass and velocity, t is time and F is the force acting on the bead. Let us assume that at moment $t = 0$ the velocity of the bead is v_0 and the frictional force, $F = -fv$, where f is the frictional coefficient, is the only force acting on the bead. Under these conditions the solution of Eq. (1) is

$$v = v_0 \exp(-t/\tau), \tag{2}$$

where $\tau = m/f$. Thus, the velocity exponentially diminishes with time. The value of f for a bead is specified by Stokes' equation:

$$f = 6\pi\eta r, \tag{3}$$

where r is the bead radius and η is the viscosity of the medium. If we also express m through the bead radius and density, ρ, we obtain that

$$\tau = \frac{2}{9} r^2 \rho / \eta. \tag{4}$$

Eq. (4) shows that τ is proportional to r^2, so it diminishes dramatically when we go from the scale of our macroscopic world to the molecular scale. For $r = 1$ nm (the radius of a small protein), $\eta = 0.01$ poise (the viscosity of water) and $\rho = 2$ g/cm, τ equals 4.4×10^{-13} s $\cong 0.5$ ps. The bead participates, however, in a fast Brownian motion caused by the interaction with water molecules. We can estimate the average velocity of the bead movement. It is known that at thermodynamic equilibrium its average kinetic energy equals $3/2 k_B T$. Although the velocity associated with this energy is large, for the above time interval τ the bead with this velocity is displaced by ≈ 0.02 nm only. This is a short distance even for the molecular scale, and by passing such infinitesimal distance the bead essentially "forgets" its initial velocity. We can say that, within a good approximation, the bead immediately "forgets" the direction of its movement in water, as if it does not have any inertia. We conclude from this analysis that a directed movement of a protein or its large part over a distance larger than 0.1 nm is only possible if a directed force is permanently applied during the movement. Since a chemical reaction in general, and ATP hydrolysis in particular, cannot produce such a force, the molecular motors cannot be based on directed movement. On the other hand we know that large, determined displacements of protein parts, the allosteric conformational transitions, is a usual, well-understood phenomenon (see review [19] and references therein). Let us consider these transitions in details.

3. Allosteric transitions in proteins

The tertiary and quaternary structures of proteins are determined by rather weak interactions including hydrogen bonds, van der Waals bonds, hydrophilic and hydrophobic interactions. These structures are subject to thermal perturbations since the energy associated with these individual interactions is of the order of only several $k_B T$. Thus, proteins are dynamic molecules constantly exhibiting thermal fluctuation of their conformations. Although the fluctuations occur around a family of conformations with the lowest free energy, for some proteins these fluctuations result in large global changes of their conformations. We can accept, for the purpose of this analysis, that the distribution of protein conformations is the equilibrium Boltzmann distribution (although sometimes the barriers between different conformations are so high that they prevent the transitions between these conformations during the protein lifetime and actual conformational distribution is different from the equilibrium one). The equilibrium distribution is specified by the free energy potential. This potential can be changed substantially by interaction with another molecule, which can be a small ligand or a part of a large molecule. Fig. 1 suggests one way of affecting the global free energy landscape by binding a small ligand with a specific protein site. As a result of the binding, the equilibrium conformational distribution is also changed, so the protein undergoes an allosteric transition. The term means, usually, that binding at one site of a protein induces a conformational change at a different site. Of course, an allosteric transition is a result of chaotic thermal motion on a new free energy landscape created by the ligand binding (or releasing). Although the motion is chaotic at each moment, its result, specified by the free energy landscape, can be a well determined change of protein conformation.

A real example of a well-studied allosteric transition is shown in Fig. 2. A large conformational change in the ribose-binding protein (RBP), from an open to a closed conformation, occurs upon binding of ribose to the protein binding site [20]. Both open and closed conformations of the protein are known from the X-ray analysis [21,22]. Recently Levy and co-workers managed to compute the free energy landscapes for both ribose-free and ribose-bound RBP [23]. Within the first approximation, the conformational changes can be described as changes of the angle between two subunits of the protein, θ. The definition of the angle is diagrammed in Fig. 2(b). The free energy landscapes, $\Delta G(\theta)$, are shown in Fig. 3. The figure shows that the ligand binding results in a large change of the free energy profile, $\Delta G(\theta)$. As a result of this change of $\Delta G(\theta)$ the most probable conformation of the protein changes from the closed, with $\theta = 109°$–$112°$, to open, centered at $\theta = 122°$. This change entails the thermal diffusion of the protein subunits.

In the above example, the ligand binds with a protein conformation which corresponds to the free energy minimum. Of course, it is not always the case. In another scenario, which is probably even more important for the molecular motors, a ligand binds only with a relatively rare conformation of a protein, stabilizing this conformation. Although

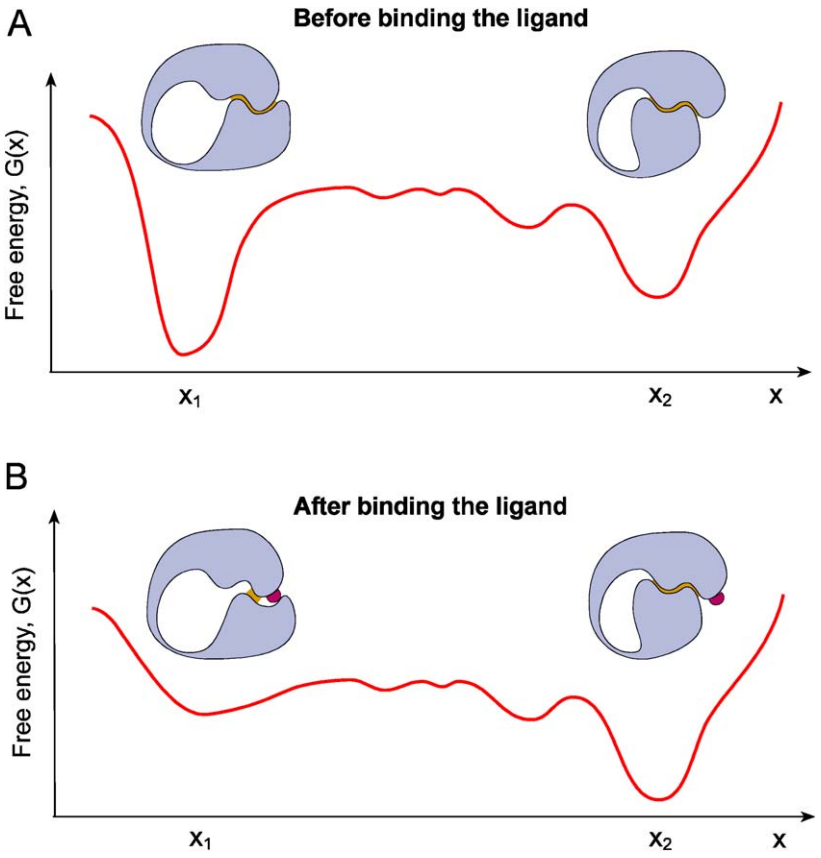

Fig. 1. Diagram of an allosteric transition in a protein. In this idealized picture, conformational changes in the protein are specified by coordinate x. (A) Two free energy minima, at $x = x_1$ and $x = x_2$, correspond to two global states of the protein, shown above the potential. The interaction between the protein surfaces, marked by dark yellow, stabilizes both conformations of the protein. In the absence of the bound ligand $G(x_2) > G(x_1)$, and therefore conformation 1 has higher probability. (B) Binding of a small ligand at the specific site of the protein prevents the proper interaction of the protein surfaces in state 1 but does not affect the interaction in state 2. By this way the ligand binding changes $G(x)$, so that $G(x_2) < G(x_1)$ for the ligand-bound protein and state 2 becomes the most probable. The transition from state 1 to state 2 occurs by thermal diffusion in the protein conformational space. (For interpretation of the references to colour in this figure legend, the reader in referred to the web version of this article.)

the free energy of this rare conformation is larger than the minimum free energy of the ligand-free protein, thermal motion makes the conformation accessible. In this case the conformational transition occurs by the diffusion-capture mechanism. A higher binding constant is required to stabilize a ligand-bound state of the protein in the latter case.

Cycles of biological molecular motors represent sequences of allosteric transitions, driven by thermal diffusion on the changing free energy landscape and changing boundary conditions. Following Fox [12], we refer to this mechanism as rectified thermal diffusion. Let us consider the sequence of such transitions in some details for the myosin-actin filaments of the skeletal muscle.

4. Skeletal muscle

The skeletal muscle is one of the well-studied systems that transform chemical energy into directed mechanical movement. We will consider it here as an example of a motor protein transforming chemical energy into directed motion.

The major structural elements of huge multinucleated muscle cells are myofibrils. Myofibrils have a cylindrical structure 1–2 μm in diameter. Hundreds of parallel myofibrils are packed in each muscle cell, and their length can be as long as the length of the cells. A myofibril is a long chain of repeated contractile units, sarcomeres, each about 2.2 μm long. To analyze muscle contraction we need to consider one sarcomere, since all of them are identical and perform the same work. A diagram of the sarcomere is shown in Fig. 4.

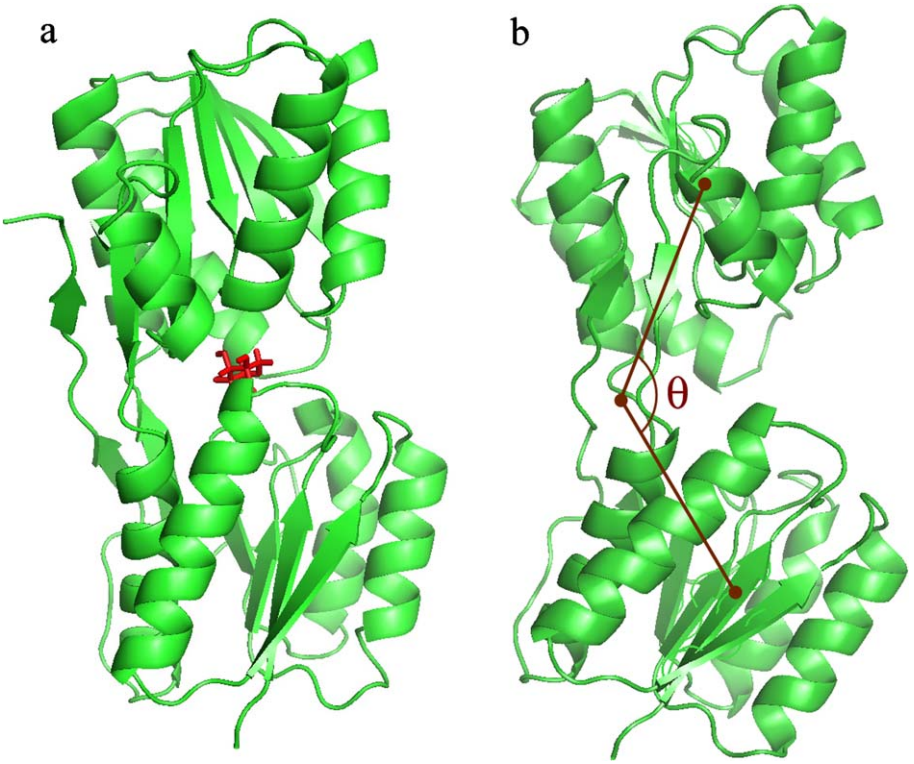

Fig. 2. Two conformations of ribose binding protein (RBP). (a) A diagram of the closed crystal structure of RBP is shown together with bound ribose (red). (b) The open crystal structure of RBP. Also shown is the definition of angle θ which specifies the conformational change from the open to closed structure. The brown lines connect the centers of the bottom and top domains of RBP to the center of mass of the hinge region. (For interpretation of the references to colour in this figure legend, the reader in referred to the web version of this article.)

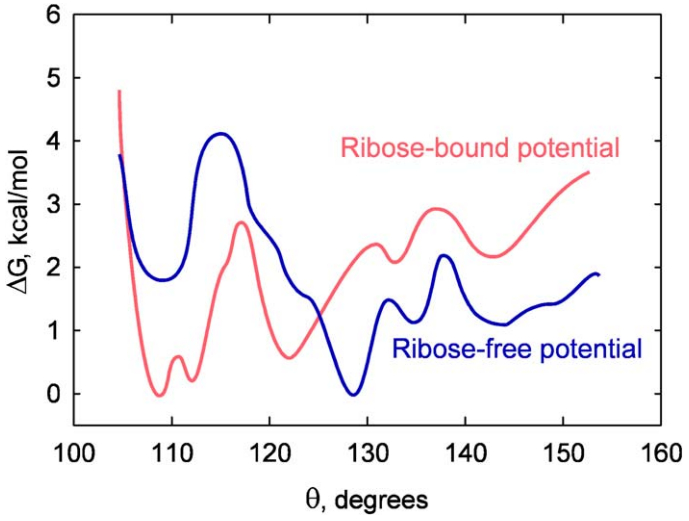

Fig. 3. Computed free energy potential for the ribose-free and ribose-bound RBP [23]. The change of the potential upon ribose binding correlates well with crystal structures of the closed and open conformations shown in Fig. 2.

Each sarcomere is formed by the large, precisely ordered array of parallel and partly overlapping actin and myosin filaments. Actin filaments in the left and right halves of sarcomere are oppositely oriented and attached at their plus ends to a Z discs at each end of the sarcomere. The capped minus ends of the actin filaments extend in

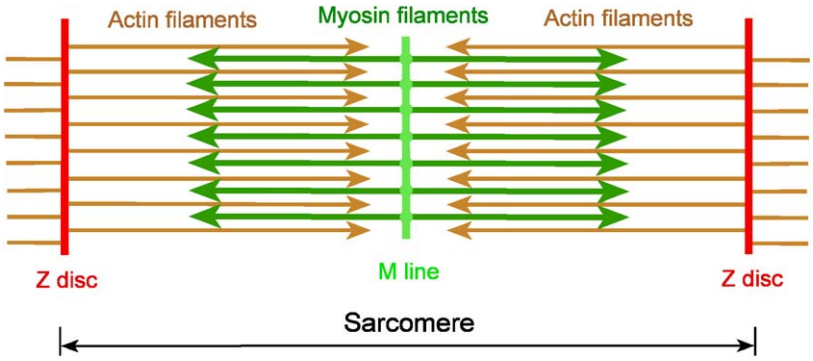

Fig. 4. Schematics of a single sarcomere. The *Z* discs, at each end of the sarcomere, are attachment sites for the plus ends of actin filaments; the *M* line, shown by light green, is the location of proteins that link the oppositely oriented myosin II filaments, shown by dark green, to one another. (For interpretation of the references to colour in this figure legend, the reader in referred to the web version of this article.)

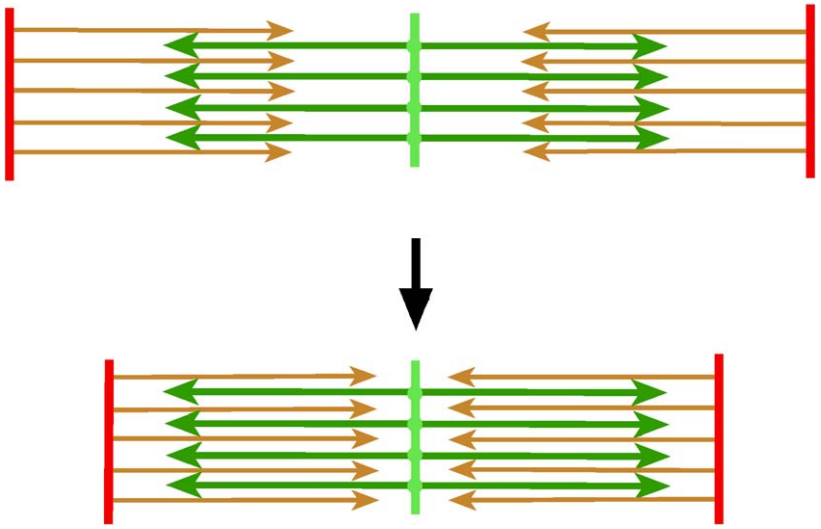

Fig. 5. The sliding-filament model of muscle contraction. The actin (brown) and myosin (green) filaments in a sarcomere slide past one another. The lengths of actin and myosin filaments are not changed during the muscle contraction. (For interpretation of the references to colour in this figure legend, the reader in referred to the web version of this article.)

toward the middle of the sarcomere, where they overlap with myosin filaments, formed from specific muscle isoforms of myosin II. Each myosin filament includes about 300 myosin molecules which heads interact with actin filaments.

Sarcomere shortening is caused by the myosin filaments sliding past the actin filaments, with no change in the length of either type of filament (Fig. 5). The sliding results in reducing the distance between minus ends of oppositely oriented actin filaments. Bipolar myosin filaments move toward the plus ends of two sets of actin filaments of opposite orientations, driven by independent myosin heads that are positioned to interact with each actin filament. There is no coordination among the movements of the myosin heads, so it is critical that they remain tightly bound to the actin filament for only a small fraction of each ATPase cycle so that they do not hold one another back. The synchronized shortening of the thousands of sarcomeres lying end-to-end in each myofibril results in rapid contraction of skeletal muscle. Of course, a sarcomere includes many accessory proteins which are important for its functioning (see [24], for example). These proteins are not important for further analysis, however, which will be concentrated on the sliding myosin filaments along actin filaments.

Muscle myosin (myosin II) consists of two identical halves. Each half includes one heavy chain and two much smaller light chains of two distinct types. Dimerization of the halves is stabilized by two long α-helices of the heavy chains which wrap around each other (Fig. 6). The two myosin heads, formed by both heavy and light chains, are the

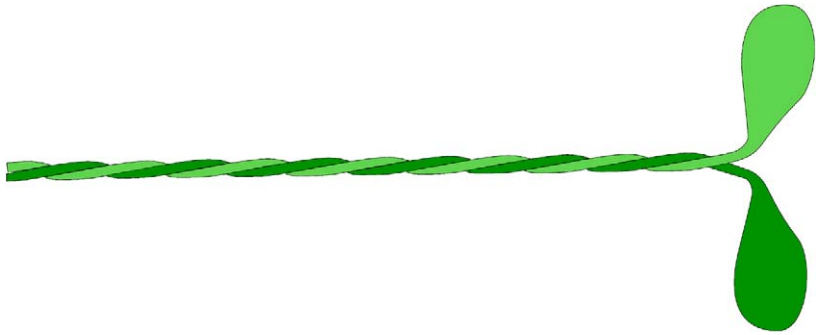

Fig. 6. Schematics of myosin II. The molecule consists of two identical halves, shown by light and dark green. Each half is formed by one heavy and two light chains. The two long α-helices wrap around each other to form a dimer. The heads of myosin II, which movement results in the muscle contraction, act independently from one another. (For interpretation of the references to colour in this figure legend, the reader in referred to the web version of this article.)

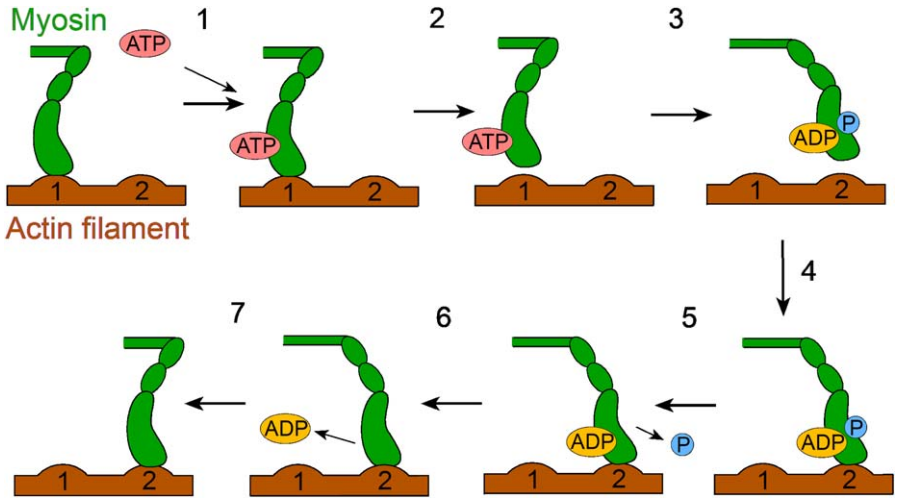

Fig. 7. Summary of steps of the myosin II cycle. The diagram outlines successive allosteric transitions of myosin II resulted from binding ATP (step 1), release from actin filament (step 2), ATP hydrolysis (step 3), weak binding to the next binding site on the actin filament (step 4), release of phosphate and strong binding with the actin filament site (step 5), release of ADP and another transition in the myosin (steps 6 and 7). The last conformational transition in the myosin results in a local shift between the myosin and actin filaments.

major moving parts of the molecule. Two heads of the dimer act independently one from another, so we can consider the sliding cycle for one head only.

One cycle of the myosin head movement, diagramed in Fig. 7, includes at least seven distinct steps [3,12,25,26], and all of them are associated with allosteric transitions.

1. Myosin begins its cycle tightly bound to the actin filament, with no associated nucleotide. The first step starts from ATP binding which causes the first allosteric transition in the myosin head, resulted in the weaker interaction between the myosin and the actin filament.
2. The myosin releases from the actin site. The release sets another allosteric transition in the myosin head.
3. The new conformation of the myosin head catalyses hydrolysis of bound ATP. Subsequently, bound ADP and phosphate cause the third allosteric transition of the myosin head, while it is detached from the actin filament. During this transition the head assumes a cocked conformation. Both ADP and phosphate remain tightly bound to the head. Due to the geometrical arrangement of the myosin head and actin filament, the cocked conformations has weak binding affinity for the next binding site on the actin filament (site 2 in Fig. 7), but not for the site left at step 2 (site 1).

4. The myosin head with bound ADP and phosphate binds the next site on the actin filament. The binding initiates the next allosteric transition.
5. The allosteric transition resulted from the actin binding causes release of phosphate and another allosteric transition. The binding between the myosin head and actin site becomes much stronger.
6. Strong binding with the actin site results in the next allosteric transition in the myosin head. ADP binding is weak in this new conformation of the head, and it releases the ADP. This sets the condition for the last allosteric conformational change in the myosin head.
7. The allosteric transition changes the conformation of the myosin head. This step is "the power stroke" that moves the myosin filament relative to the actin filament.

The allosteric transitions in the myosin head are caused by binding/releasing actin, ATP, ADP, phosphate. These association/dissociation reactions can go in both directions, depending on the related free energy changes. Clearly, for the efficient work of the motor, thermodynamic equilibrium for all of the above steps have to be shifted in the direction indicated in Fig. 7. Therefore, the corresponding free energy change for each step has to be negative. On the other hand, the conformational free energy of myosin at initial and final states of the cycle is clearly the same. This is possible because some or all conformational changes in myosin, associated with steps 3, 5 and 6, increase the free energy of myosin, if we consider the free energy of the corresponding conformations ignoring the bound ligands. The free energy changes go in the desired direction because they are coupled with ATP hydrolysis and release of ADP and phosphate, which are associated with the free energy decrease. Thus, the total free energy of the molecules, participating in the cycle, reduces at each step, while the free energy of myosin, considered separately, goes up and down during the cycle. Thus, the free energy of ATP hydrolysis does not provide a directional movement of the myosin head but instead is used to change the free energy landscape for the myosin conformational changes. The desired conformational changes are the result of a chaotic thermal motion which drives the myosin head to the next conformation of the cycle.

The precise free energy landscapes for myosin head bound with actin, ATP, ADP and phosphate are not known. There are strong indications, however, that the above diagram of the head cycle is in agreement with what we know about allosteric transitions in proteins. The sequence of the allosteric transitions coupled with ATP hydrolysis can explain, at least qualitatively, directed sliding of myosin heads along the actin filament. One more question arises here, however: can the model explain the high rate of the muscle contraction?

5. Speed of biological motors

It seems that the muscles contract very fast at macroscopic scale. The contraction results, however, from simultaneous action of many sarcomeres, and on the scale of sarcomeres the contraction is not so fast. The time of the myosin sliding cycle varies from 0.1 to 30 msec [24]. Can the allosteric transition in the myosin head yield such a speed? Experimental measurements of the rates of allosteric transitions, v, in other, non-motor proteins show that v has order of 10^4 sec^{-1} [19]. So, in general, the allosteric transitions can be sufficiently fast to yield the observed speed of muscle contraction.

At the recent years, however, an amazing progress has been achieved in study of biological motors at the molecular level. This progress became possible due to the development of experimental methods allowing quantitative measurements of events occurring with individual molecules or molecular systems. This booming field of biophysics was pioneered by Bustamante and co-workers who measured the force-extension dependence for individual DNA molecules [27]. These authors managed to apply a certain force to the molecule ends and measure the end-to-end distance, in the direction of the force. Further developments of this technique have made it possible to measure the rate of cycle of movement for individual motor molecules under various conditions. The studies of the cycle rate as a function of the load applied to the moving molecules have proved to be especially informative. To consider the issue in more details, let us start from a simple theoretical analysis. Fig. 8 shows the free energy landscape, $\Delta G(x)$, for an allosteric transition in a motor molecule, plotted along the motor displacement. Similar to any other molecular transformation, the landscape can have a barrier between initial and final states, δG. This barrier corresponds to a particular displacement along the movement coordinate, $x_1 + d$, so $\delta G = \Delta G(x_1 + d) - \Delta G(x_1)$. If a pulling load, F, directed against the movement ($F < 0$), is applied to a moving part of the molecule, the landscape is changed by the work against the pulling force. On this changed landscape, $\Delta G(x) - xF$, the barrier for the transition is $\delta G - Fd$

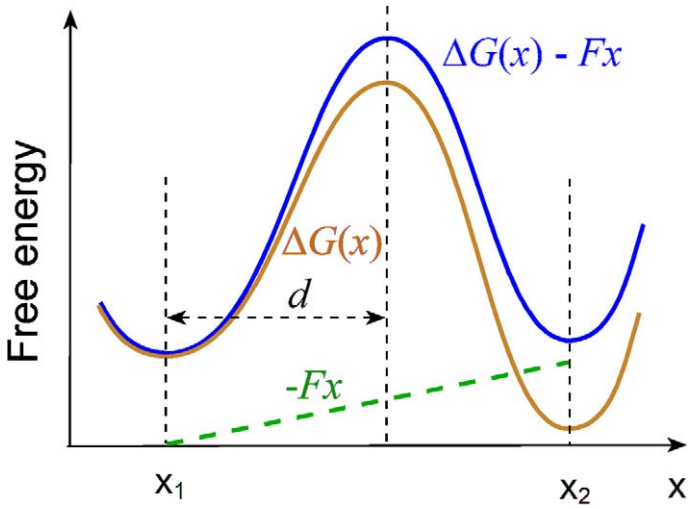

Fig. 8. The effect of load on the free energy profile for conformational transition associated with a motor displacement. In the absence of load, two states of the motor protein, 1 and 2, are separated by a barrier $\Delta G(x_1 + d) - \Delta G(x_1)$ (brown line). The load F requires the additional work $-Fx$ during the motor displacement (green line). As a result, under the load the potential barrier changes to $\Delta G(x_1 + d) - \Delta G(x_1) - Fd$, assuming that F is negative. The latter potential is shown by the blue line. (For interpretation of the references to colour in this figure legend, the reader in referred to the web version of this article.)

(see Fig. 8). The transition time, τ, should follow to Arrhenius equation,

$$\tau = \tau_0 \exp\bigl[(\delta G - Fd)/k_B T\bigr], \tag{5}$$

where τ_0 is a constant. Thus, the transition time grows exponentially with F.

The myosin-actin system is not the most convenient one to study the dependence of τ on F at the single-molecule level, since the myosin heads are attached to the actin filament only for a fraction of the cycle. Still, the researches managed to measure the dependence by applying the load immediately after a head attachment to the actin filament and measuring the time of conformational transition related with motor movement (the last step in Fig. 7) [28–31]. The quantitative data for the dependence of τ on F, obtained in [31], are in agreement with Eq. (5) and correspond to $d = 1.3$ nm.

Another motor system, kinesin moving along microtubule, is much more convenient for the single-molecule study since under normal conditions at least one of two kinesin heads is always attached to the microtubule. The kinesin-microtubule system is essential for various biological motile processes such as intracellular trafficking and cell division. Two heads of kinesin walk unidirectionally along microtubule by hand-over-hand mechanism, resembling a human walking (Fig. 9). The dependence of τ on F for kinesin has been a subject of intensive experimental investigation [32–39]. In the experimental setup of these studies a kinesin-coated bead, captured by the optical tweezers, is used as a probe for measuring kinesin displacement and applying a force to the motor molecule (Fig. 10). It was found that if the load exceeds 5 pN, backward steps of kinesin are observed with increasing probability [38,39]. This finding can be naturally interpreted in terms of rectified diffusion as a presence of certain bias for the forward step against the backward. The dependence of τ on F measured for kinesin fits well Eq. (5) [39]. The values of d, found in the latter study, are 2.5 and 0 nm, for forward and backward steps, respectively. Similar to myosin-actin system, these values are smaller than the size of kinesin step, 8 nm [37,40].

Recently, a study of the dependence of τ on F has been also performed for translocation of RNA polymerase along the DNA molecule [41]. In this case the experimental data fit well Eq. (5) with $d = 0.34$ nm, which corresponds to the whole length of the enzyme step. This means that the free energy barrier for the enzyme displacement, δG, is negligible.

Essentially, all the experimental data obtained so far can be quantitatively explained by the rectified diffusion mechanism. More and more often these experimental results are analyzed in term of this mechanism. Clearly, the single-molecule experiments have provided a strong support to the idea that the transformation of metabolic free energy into directed movement is based on rectification of the protein thermal diffusion.

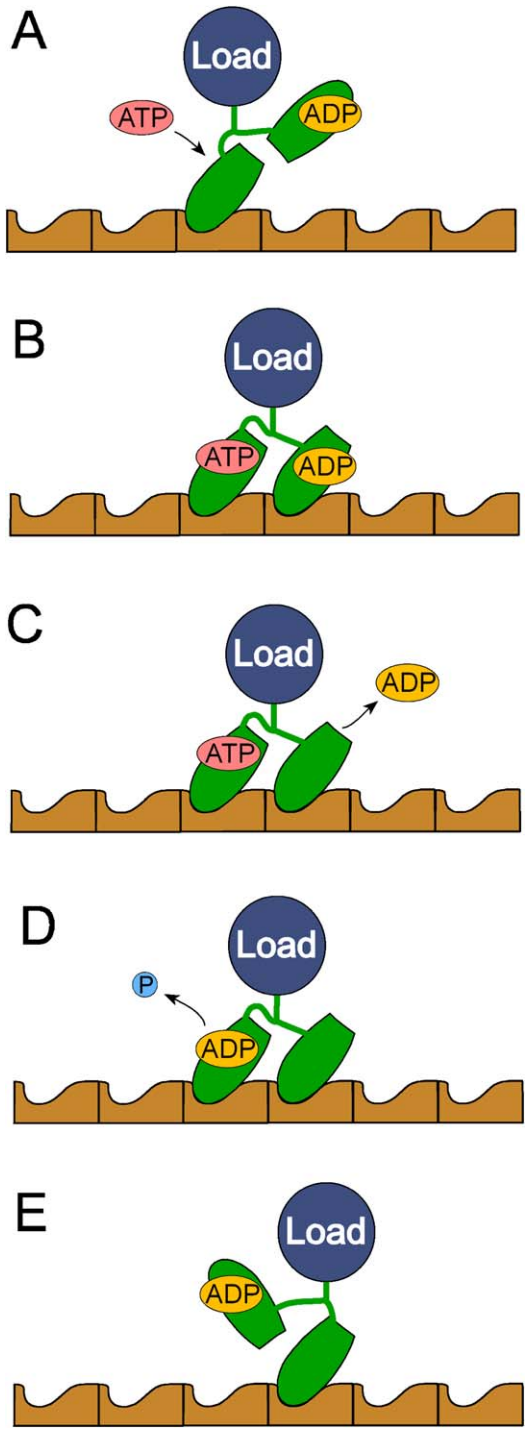

Fig. 9. The movement cycle of kinesin. (A) One head with bound ADP is detached from the microtubule and can diffuse to its next position. (B) The ATP binding to the attached head causes allosteric transition in the kinesin neck linker which favors the binding of the detached head. (C) After the head attachment, an allosteric transition causes dissociation of ADP from it, probably stabilizing the attachment. (D) Hydrolysis of bound ATP is followed by P dissociation. This causes an allosteric transition in the head to a new state with lower binding affinity to the microtubule site. (E) The latter head dissociates from its binding site, so the motor returns to state (A).

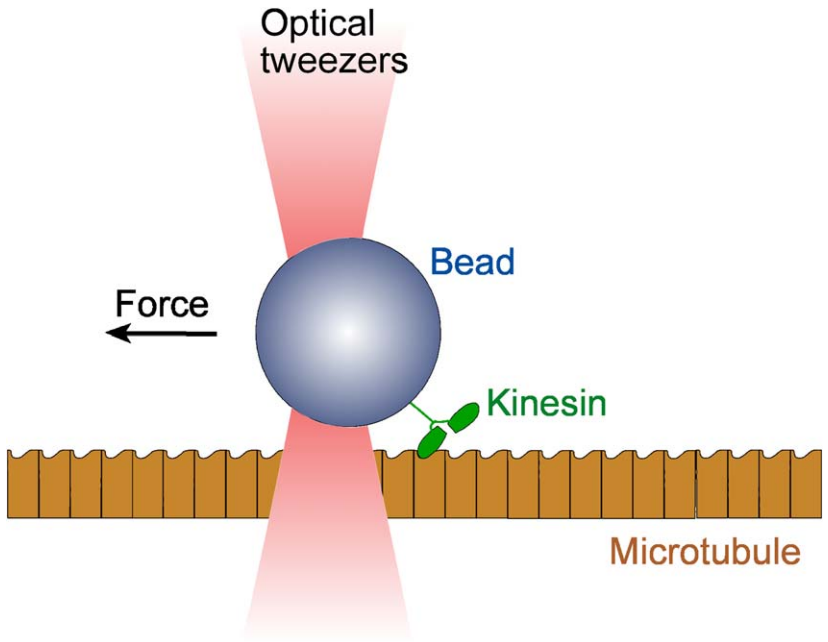

Fig. 10. Measuring the rate of kinesin displacement. The kinesin-coated bead is trapped by the optical tweezers and serves as a probe for measuring the kinesin movement. The microtubule is attached to a glass surface. Displacement of the bead in the optical trap creates a force which is transmitted to kinesin.

In the case of myosin-actin and kinesin-microtubule systems the directionality of the movement is specified by the polarity of the actin filament or microtubule. Sometimes, however, a directional movement can occur along a symmetrical filament. Double-stranded DNA represents an example of such filament, if we ignore its sequence. There are enzyme complexes moving along DNA in a particular direction which is set by initial assembly of such complexes at specific DNA sites. Recently, a different example of a very unusual directional movement along DNA attracted attention of researches, translocase FtsK [42–44]. The translocase binds to DNA at a random point and starts movement in a randomly chosen direction. It is capable to change this direction, however, passing through some specific DNA sequences [44]. This means that the protein can be in two states which correspond to two directions of the movement. Binding FtsK with the specific DNA sequences can cause the allosteric switch from one state to another, changing the direction of the movement.

6. Movement in enzymes

Many enzymes pass through a few different states during their catalytic cycles. The transitions between the states follow well-defined sequences, and therefore the action of such enzymes strongly resembles unidirectional movement of molecular motors. To illustrate the analogy, let us consider the catalytic act of type II DNA topoisomerases. These enzymes catalyze passing of one segment of the double helix through another (see review [45], for example). They introduce a transient double-stranded break in one DNA segment, capture another segment of the same or another DNA molecule and transport it through the break (Fig. 11). The break is resealed after the second segment passing. By this way the enzymes can change the topology of circular DNA molecules: create knots and catenaes from DNA circles as well as unknot and unlink them, and also relax DNA supercoiling. It is important for our subject that each successive steps of the catalytic cycle can be considered as allosteric transitions driven by the rectified thermal diffusion. The first such transition is caused by binding the first DNA segment. In the new state of the enzyme the gate for a second segment is open. Entrance of the second segment and binding with a certain site of the enzyme causes the catalytic act of breaking the first segment. The following allosteric transition results in closing the entrance gate and opening the exit gate for the second segment. The interaction of the second segment with the new state of the enzyme is weakened, the segment dissociates from the enzyme and diffuses through the exit gate. The release of the second segment from the enzyme causes the next allosteric transition. In this new state the enzyme reseals and releases the

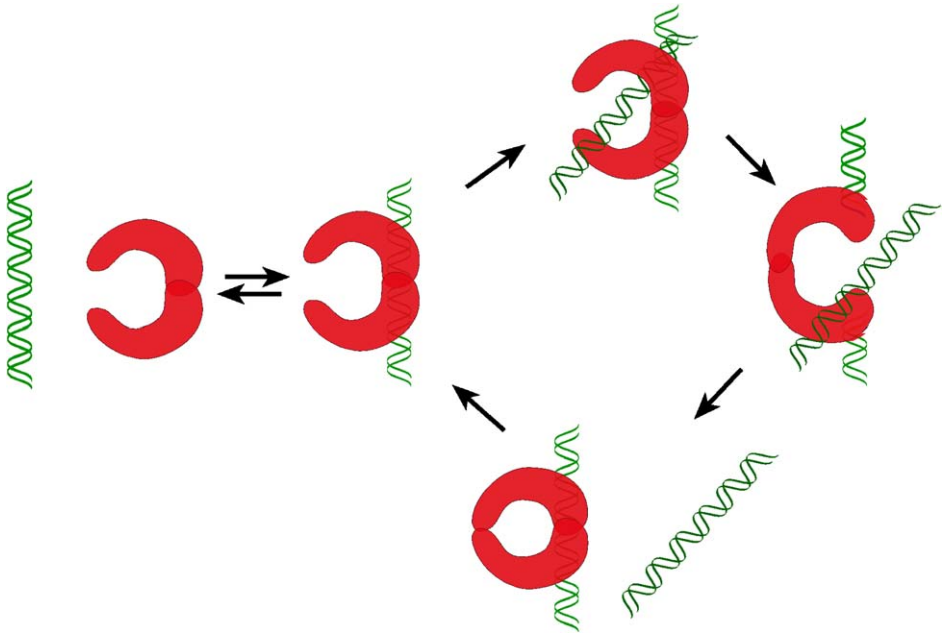

Fig. 11. Diagram of DNA topoisomerase cycle. The enzyme catalyzes passing of one DNA segment through another. The successive steps of enzyme cycle can be considered as a unidirectional movement (shown by arrows). Details of the enzyme action are described in the text.

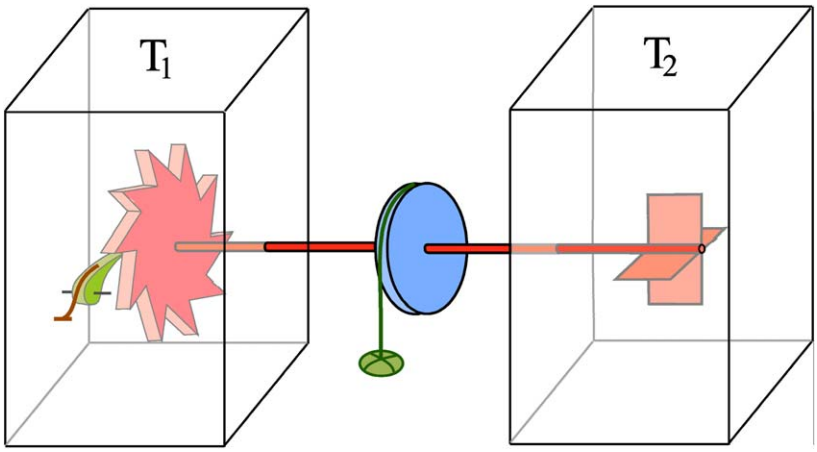

Fig. 12. The ratchet and pawl machine. There are two boxes with a vane in one and a wheel that can only rotate in one direction, a ratchet and pawl, in the other. The vane and ratchet have a common axle. The two boxes are filled with gas molecules at temperatures T_1 and T_2, respectively. Because of the bombardment of gas molecules on the vane, the vane oscillates and jiggles. Although the oscillations occur in both directions, the ratchet and pawl allow rotation in one direction only.

first segment. Although the described action of the enzyme should not increase the DNA free energy and returns to its initial state at the end of the cycle, going through these conformational transitions in one direction requires a free energy consumption. This energy is brought by the ATP hydrolysis which is coupled with the enzyme catalytic cycle. We see that the enzymatic catalysis and the movement of motor proteins have striking similarities.

7. Thermal ratchet

In his famous Lectures on Physics, Richard Feynman considered a hypothetical device which was dubbed the "Brownian ratchet" or the "Thermal ratchet" [5]. The device, which supposed to be of the molecular scale, is shown in Fig. 12. At first glance, the device is capable to extract energy of thermal motion of gas molecules in the right box,

since the ratchet can rotate in one direction only, lifting the load. We know, however, that such device is impossible since it contradicts to the Second Law of Thermodynamics. Indeed, very careful analysis, performed by Feynman [5], shows that the device can only work if the temperature in the right box is higher than in the left one.

The machine has attracted a lot of attention (see Ref. [46] for review). In particular, it was suggested that the principle can be used in biological molecular motors [6]. Vale and Oosawa assumed that ATP hydrolysis creates a temporary temperature difference, which is required for the machine. It is not a practical idea, however, since very fast energy dissipation. The idea hardly survived, but the term "Brownian ratchet" has become very popular. In biological literature the term has been used in much broader sense in recent years, referring to any use of thermal motion in molecular motors. The examples include extreme cases when authors believe that the machine can work without temperature difference, in other words, as the perpetuum mobile [47].

Although Feynman designed and analyzed the thermal ratchet simply as an illustration of Carnot's principle, the suggested machine became, probably, the first one capable to harness thermal motion and convert it to directed movement. It not just inspired many theoretical studies, but become a prototype of many manmade microscopic devices. However, the requirement of the temperature difference makes very problematic any biological applications of the ratchet principle.

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