PHYSICS IN MOLECULAR BIOLOGY

KIM SNEPPEN & GIOVANNI ZOCCHI

CAMBRIDGE
UNIVERSITY PRESS

6

Proteins in action: molecular motors

Kim Sneppen

Molecular motors: energy from ATP

Proteins can be grouped into a few broad categories with respect to their function. Some are regulatory, some are enzymes, some are structural, and some proteins do mechanical work. It is this latter group that we now discuss. Molecular motors include kinesin, myosin, dynein, the motors connected to DNA replication, to gene transcription and to translation. The motors are mostly driven by ATP hydrolysis: ATP$\rightarrow$ ADP + P, a process with $\Delta G \approx 13$ kcal/mol for typical conditions in the cell. Exactly how the free energy difference from ATP hydrolysis is converted into directed motion and mechanical work is a most interesting question, which is not resolved. In many cases the conformational changes of the protein are known in considerable detail from structural studies. The sequence of events associating conformational changes and substrate binding and release is also known. Nonetheless, the actual physical mechanism by which the motor works is not obvious. Thermal noise and diffusion certainly play a role, making this "soft" machine qualitatively different from a macroscopic motor. In the next section we elaborate on these ideas through some models.

The most studied motors include myosin and kinesin, which move along the polymers that define the cytoskeleton. Kinesin walks on microtubules (Fig. 6.1), whereas myosin walks on polymerized actin. Microtubules and actin fibers are long (μm) polymers where the monomer units are proteins. Microtubules are very stiff; actin fibers more flexible. Kinesin motors work independently of each other, and are associated with the transport of material (vesicles) inside the eukaryotic cell. A myosin protein works in coordination with other myosin proteins, and is associated with muscle movements. Myosin and kinesin are structurally related; they both have a head, which directs the action, and they both go through an ATP burning cycle, which involves binding to the substrate (actin or microtubule), a forced power stroke, and a detachment phase for each cycle of the motion. Figure 6.1 illustrates

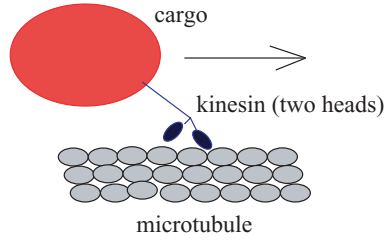

Figure 6.1. Kinesin walking on a microtubule. Kinesin forms dimers, consisting of two globular heads, a stretched stalk about 80 nm long, and a tail. The head and the tail domains contain the microtubule- and the cargo-binding sites, respectively. Microtubules are 25 nm thick and about 5–20 μm long hollow cylindrical fibers that are formed by tubulin dimers. Microtubules are polar; there are kinesins moving from + to −, and there are kinesins designed to move the opposite way. Each tubulin dimer is 8 nm long, and the kinesin moves in steps of 8 nm along the surface of microtubules.

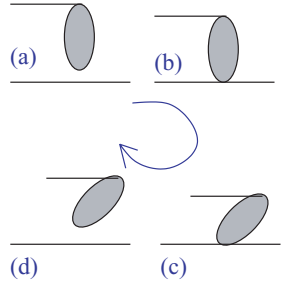

Figure 6.2. Schematic view of some states during one cycle of a motor protein that walks on a substrate. The protein may, for example, be kinesin, in which case the substrate is a microtubule. Or it may be myosin walking on actin. The stroke state (b) → (c) is associated with release of the products P and possibly also ADP from the motor. State (c) is called the rigor state, because this is the state a motor without ATP ends in. The release of the head from substrate (c) → (d) is associated to binding of ATP. For myosin, the energetics of all involved transitions are tabulated by Smith & Geeves (1995a,b). ATP bind weakly to attached rotated myosin (c) but strongly to de-attached myosin (d,a) with $\Delta G = -16$ kcal/mol, and myosin with ATP/ADP bind to actin with $\Delta G = -6$ kcal/mol (b). ATP → ADP + P releases ∼ 9 kcal/mol inside the myosin head (a) or (b), and when P and ADP is released from the attached and rotated myosin there is a further gain of about 7–8 kcal/mol, coupled to the power stroke (b) → (c) (see also Rayment, 1996).

kinesin motion along a microtubule. Figure 6.2 gives a cartoon visualization of the steps involved in the movements of the individual heads.

Contemporary experiments on kinesin show that the motion takes place in discrete 8 nm steps (corresponding to the spacing of tubulin dimers along the microtubule). There is one ATP hydrolysis per step, and the efficiency is large (maybe >50%) at low load. The stall force (maximum force the motor can move against) is

about 5–6 pN (Meyhofer & Howard, 1995). The maximum speed of a kinesin motor is about 1–2 μm/s, which means that one step of 8 nm in total takes about 0.005 s. The speed depends on ATP concentration when this is low. In vitro, maximum speed is reached above ∼1 mM concentration of ATP.

On a more detailed level we now examine the energy source, namely the ATP→ ADP + P reaction. In the cell ATP is found at a concentration of 1 mM. Thus each potential reaction point in the cell will be bombarded with $\sim 10^5$ ATP molecules per second (see the Questions on p. 131). Thus the limit imposed by the ATP capturing rate is very high when compared with the overall time it takes kinesin to move one step. Thus it can be ignored: the motor typically works under conditions with ample energy supply. Energetically, the reaction

$$\mathrm{ATP} \rightleftharpoons \mathrm{ADP} + \mathrm{P} \tag{6.1}$$

is characterized by the dissociation constant

$$K = \left(\frac{[\mathrm{ADP}][\mathrm{P}]}{[\mathrm{ATP}]} \right)_{\mathrm{eq}} = [\mathrm{M}] \cdot \mathrm{e}^{\Delta G_0 / k_\mathrm{B} T} \tag{6.2}$$

where the concentrations are at equilibrium and $\Delta G_0 = 7.3$ kcal/mol is the *standard free energy change of the reaction*. This means that ΔG_0 is defined as the free energy of ATP relative to ADP + P when all reactants are present at 1 M concentration ([M]). Having all reactants at 1 M is not equilibrium; in fact if [ADP] = [P] = 1 M, the equilibrium [ATP] concentration

$$[\mathrm{ATP}]_{\mathrm{eq}} = \mathrm{e}^{-\Delta G_0 / k_\mathrm{B} T} \mathrm{M}^{-1} [\mathrm{ADP}][\mathrm{P}] = \mathrm{e}^{-7.3/0.617} \mathrm{M} \sim 10^{-5} \mathrm{M} \tag{6.3}$$

is very low. This reflects the fact that [ATP] is in an unlikely high-energy state. This high-energy state of ATP is exactly what makes it a good way to store energy in the living cell.

The equilibrium concentration of [ATP] is the one where it is not possible to extract energy from the hydrolysis. In the cell, concentrations are not at equilibrium, and it is therefore possible to extract work from the reaction in Eq. (6.1). The in vivo free energy that ATP has stored relative to ADP+P in the living cell, $\Delta G = G(\mathrm{ATP}) - G(\mathrm{ADP} + \mathrm{P})$, is (see derivation in Question 6 on p. 131):

$$\Delta G_{\text{in vivo}} = k_\mathrm{B} T \ln \left(\frac{([\mathrm{ADP}][\mathrm{P}]/[\mathrm{ATP}])_{\mathrm{eq}}}{([\mathrm{ADP}][\mathrm{P}]/[\mathrm{ATP}])_{\text{in vivo}}} \right) \tag{6.4}$$

$$= \Delta G_0 - k_\mathrm{B} T \ \ln ([\mathrm{ADP}][\mathrm{P}]/[\mathrm{ATP}])_{\text{in vivo}} \tag{6.5}$$

$$= 7.3 \text{ kcal/mol} - k_\mathrm{B} T \ln(0.000\,02) \approx 13 \text{ kcal/mol} \tag{6.6}$$

where, in the last equality, we insert the actual in vivo concentrations: [ATP] $\sim$1 mM, [ADP] $\sim$20 μM, and [P] $\sim$1 mM. Equation (6.5) simply states that the free energy release due to hydrolysis is equal to what it would be at 1 M concentrations of all reactants, ΔG_0, plus a contribution due to the entropy gain of diluting/concentrating the reactants to the actual in vivo concentrations. Thus to a large extent it is the high concentration of ATP relative to ADP and P concentrations that brings us up to the hydrolysis energy of 13 kcal/mol in the living cell.

For the motor myosin (or kinesin) the ATP hydrolysis takes place in a small pocket deeply buried inside the protein. This pocket has a size of about 1 nm. Confining the reactants to this 1 nm^3 cavity in fact corresponds to concentrations of order 1 M. Thus the last of the two terms in Eq. (6.6) means that about half the in vivo free energy of hydrolysis is associated to the entropy gained by moving the ADP and P far away from this reaction volume after the hydrolysis. Figure 6.2 illustrates the cycle of steps that the motor goes through during capture, hydrolyses and release of fuel.

Questions

(1) Consider ATP $\rightarrow$ ADP + P with 7.3 kcal/mol energy released in the cavity of 1 nm^3 inside the myosin head. If we ignore thermal conductivity, and other ways to store energy chemically, what is the maximum temperature increase one may have in this small volume?

(2) A kinesin molecule takes a step of 8 nm and can sustain a load of 6 pN. What work does can it do per ATP cycle? What is its efficiency in percent?

(3) Convert 1 k_BT to pN $\cdot$ nm units.

(4) A myosin motor has a power stroke of 3–5 pN. How many myosin molecules are needed to carry a man of 80 kg? The speed that myosin can induce is of order 0.5–4 μm/s, dependent on the myosin. How does one obtain macrosocopic speeds of, say, 1 m/s? How many myosins should be used to move a human body of 80 kg 1 m/s vertically? How much do these weigh, when each myosin molecule is 500 000 Da? Muscles contain about 7–10% myosin, so what is the lowest muscle mass that can sustain us?

(5) Find, by dimensional analysis, a formula for the rate at which diffusing molecules visit a given region of space, depending on the concentration c, diffusion constant D, and size s of the target region. Show that a small molecule like ATP, at mM concentrations, visits a reaction center $\sim 10^5$ times per second.

(6) We here consider an alternative derivation of Eq. (6.5), using the formalism derived in the λ-phage chapter. Argue that statistical weight of an ATP in the in vivo cell is $Z_{\rm ATP} = [{\rm ADP}][{\rm P}]\, {\rm e}^{-\Delta G_0/k_BT}$, and that the statistical weight of the de-hydrolyzed state ADP+P is $Z_{\rm ADP+P} = [{\rm ATP}]$. Show that the maximum work that one can obtain from the hydrolysis, $W_{\rm max} = k_BT \ln(Z_{\rm ADP+P}/Z_{\rm ATP})$, is given by Eq. (6.5).