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Accounting for the energies and entropies of kinesin's catalytic cycle

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Abstract. When the processive motor protein kinesin walks along the biopolymer microtubule it can occasionally make a backward step. Recent single molecule experiments on moving kinesin have revealed that the forward-to-backward step ratio decreases exponentially with the load force. Carter and Cross (Nature 435, 308–312, 2005) found that this ratio tightly followed $802 \times \exp[-0.95F]$, where F is the load force in piconewtons. A straightforward analysis of a Brownian step leads to $L/(2k_BT)$ as the factor in front of the load force, where L is the 8 nm stepsize, k_B is the Boltzmann constant, and T is the temperature. The factor $L/(2k_BT)$ does indeed equal 0.95 pN^{-1}. The same analysis shows how the 802 prefactor derives from the power stroke energy G as $\exp[G/(2k_BT)]$. There are indications that the power stroke derives from the entropically driven coiling of the 30 amino acid neck linker that connects the two kinesin heads. This idea is examined and consequences are deduced.

PACS. 87.16.Nn Motor proteins – 05.40.-a Fluctuation phenomena, random processes, noise, and Brownian motion

1 Accidental backstepping at physiological ATP concentrations

Processive motor proteins are tiny engines that utilize the energy released in ATP hydrolysis to literally move in a hand-over-hand fashion along a biopolymer. Kinesin, for instance, is a dimer, consisting of two units of about 350 amino acids, that moves along microtubule. Kinesin helps maintain cell organization by pulling organelles and chemical-filled vesicles to and from different parts of the cell. Over the past decades increasingly sophisticated techniques have been developed to follow motor protein motion and manipulate it [1].

Recently, Nick Carter and Rob Cross reported how they were able to resolve the accidental backsteps of kinesin [2] as they were, at the same time, also pulling back on the moving motor protein with a load force F (see Fig. 1). These researchers found that the ratio of the forward step probability and the backward step probability decreases as

$$P_f/P_b = 802 \times \mathrm{e}^{-0.95F}, \tag{1}$$

where F denotes the load force in piconewtons. The observed stopping force of 7 pN is implicit in this formula; it is where $P_f = P_b$. The same relationship (1) appears to be valid at saturating, physiological ATP concentration (about 1 mM), as well as at a much lower non-saturating ATP concentration (10 μM). Similar numbers were found in [3]. Below I will first show that detailed structural knowledge of the involved molecules is not necessary to derive this relation. The entire formula, including the values of the parameters, follows from some very basic features of the stepping mechanism in a Brownian regime.

Figure 1 shows how a Brownian step proceeds. With the front head firmly attached, the back head detaches. A reorientation of the neck linker over a distance $L = 8$ nm, sometimes referred to as a "power stroke," brings the detached head to the vicinity of the next forward binding site. Subsequently, it is random Brownian motion that makes the detached head eventually hit the next forward binding site [4,5]. No energy is dissipated on this diffusional route. The power stroke, however, involves a forced 8 nm displacement. In vivo, friction is the force that is overcome in the course of this displacement. This friction is made up of internal friction of the motor protein as it goes through its conformational changes [6] and of friction that occurs through the interaction with the liquid medium. It is only when the cargo gets occasionally "stuck" in a crowded cell, that the motor has to pull against an actual load. For over a decade in vitro experiments have been performed in which the motor protein is made to pull a silica bead [7]. With an optical tweezer it is then possible to pull back and make the protein work against a load F.

The power stroke in Figure 1 is driven by an energy difference G between the forward orientation and the

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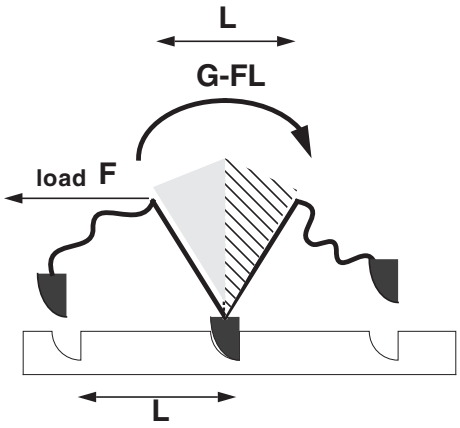

Fig. 1. A step of the processive motor protein kinesin. After the detachment of the rear head, the attached head carries out a power stroke and reorients to bring the detached head close to the forward binding site. It is the anisotropy of the biopolymer track that determines the direction of motion. An energy G, which derives from ATP hydrolysis, drives the reorientation. If the motor protein is pulling against a load F, then an energy $G - FL$ is available to force the reorientation, where L denotes the steplength. We associate the probability for an accidental backstep with the probability for the attached head to be in the grey area. A simple Boltzmann distribution gives a very accurate prediction for the ensuing backstep probability.

backward orientation. Each kinesin step involves the hydrolysis of one ATP and this is what the G derives from. The energy difference that drives the power stroke is diminished by FL if the motor protein is also working uphill against a load F that derives from a silica bead and an optical tweezer. The presence of Brownian motion implies that there is always a probability that random molecular collisions will push the attached head against the ATP driven reorientation. For a system with two energy levels, where one level is E higher than the other, it is the Boltzmann factor, $\exp[-E/(k_BT)]$, that gives the ratio of the probabilities to be in the higher versus the lower energy state. Here T represents the temperature in Kelvin and $k_B = 1.4 \times 10^{-23}$ J/K is the Boltzmann constant. In relation to Figure 1, it is obvious that, after the appropriate relaxation, the probability to be closer to the back binding site (i.e. to be in the grey area) versus the probability to be closer to the forward binding site (i.e. to be in the striped area) also follows a Boltzmann relation. After all, for each position in the striped area there is a position in the grey area that has an energy that is $(G - FL)/2$ higher. It is reasonable to assume that the forward step probability and the backward step probability are the same as the forward binding probability and the backward binding probability of the detached head. We take these probabilities to be P_f and P_b, respectively. We thus find [8]:

$$P_f/P_b = \mathrm{e}^{\frac{1}{2}\frac{G}{k_BT}} \ \ \mathrm{e}^{-\frac{1}{2}\frac{L}{k_BT}F}. \qquad (2)$$

At $T = 300$ K, we have $L/(2k_BT) = 0.95 \times 10^{12}$ N^{-1}. With F expressed in piconewtons, $L/(2k_BT)$ turns out to be exactly the 0.95 that Carter and Cross recorded! It is remarkable that the 0.95 in the exponent is not a consequence of the complicated subtleties in the structure of the kinesin and the microtubule, but, instead, simply follows from the obvious length of a microtubule period and from the natural unit of thermal noise. The factor 1/2 in the exponents in equation (2) is what makes the boundary between the striped area and the grey area run exactly through the middle of the power stroke in Figure 1. Because of the obvious symmetry this is the simplest and most likely configuration for the boundary. The 802 that Carter and Cross observed for the prefactor should derive from the $\exp[G/(2k_BT)]$ term and implies that $G = 13\ k_BT$-units. The free energy released by ATP hydrolysis is, under physiological conditions, about $22\ k_BT$-units. A $13\ k_BT$ power stroke implies an efficiency of the conversion of chemical energy into mechanical work of about 60%. Also other motor proteins (like myosin) and ion pumps (like F_0F_1-ATPase) have, in physiological conditions, been found to operate at an efficiency of about 50% (see Ref. [1], p255).

2 How the energy of ATP hydrolysis is expended

At physiological conditions the hydrolysis of one ATP releases about $22\ k_BT$-units of energy. With $13\ k_BT$ going into the power stroke, the remaining $9\ k_BT$ can be utilized to drive ATP binding, release of ADP and P, and attachment and detachment of kinesin heads to the microtubule. In a recent comprehensive review [9], some of the rates of these chemical transitions are reported. For the ATP binding step the ratio of the unbinding and the binding rate is $K_d^{\mathrm{ATP}} \approx 75\mu$M. With the physiological ATP concentration of 1 mM, this leads to $G_{b,\mathrm{ATP}} = \ln([\mathrm{ATP}]/K_d^{\mathrm{ATP}}) \approx 2.6\ k_BT$-units. For the release of the inorganic phosphate group, reference [9] gives the estimates $k_b \approx 0.25\ \mu\mathrm{M}^{-1}\mathrm{s}^{-1}$ for the phosphate binding rate constant and $k_d \approx 250\ \mathrm{s}^{-1}$ for the phosphate dissociation rate constant. For these numbers we find that there is no energy difference driving phosphate release at the physiological [P] = 1 mM. The ADP release, with $K_d^{\mathrm{ADP}} \approx 120\ \mu$M and [ADP] = 10 μM, appears to be driven by $G_{d,\mathrm{ADP}} = \ln(K_d^{\mathrm{ADP}}/[\mathrm{ADP}]) \approx 2.5\ k_BT$-units. In [9] evidence is furthermore presented that the kinesin head's binding to and detachment from microtubule is driven by energies of about one k_BT-unit. All in all, the energies that drive the different steps in kinesin's stepping cycle do properly add up to the roughly $22\ k_BT$ units that are released by the ATP hydrolysis.

As was mentioned before, Carter and Cross found that the numbers in equation (1) remain unaffected when the ATP concentration is decreased hundredfold. This while, on the other hand, the moving speed of the motor protein is observed to significantly decrease with such a decrease of ATP concentration. It is, of course, perfectly well possible to change the speed of the motor protein while preserving equations (1) and (2). Decreasing the ATP concentration

does not affect the G of the power stroke. It does, however, affect the energy that drives ATP binding and, thus, the time it takes for a new ATP to bind at the start of the catalytic cycle.

3 An entropic force drives the power stroke?

Over the past decade significant research effort has focused on trying to figure out to what extent the different conformational changes are entropy versus enthalpy driven [10,11]. Rice et al. have presented evidence [10] that the binding of ATP involves the concurrent docking of a neck linker to the two kinesin heads (see Fig. 2). The enthalpy change associated with ATP binding is presumably large, but the entropic cost of uncoiling the neck linker is such that the net free energy difference driving the ATP binding is only a few k_BT units. In reference [10] it is, furthermore, shown that the coiling that follows the undocking provides enough energy to account for the power stroke.

The neck linker consists of 30 amino acids [12]. There are many ways for the neck linker to be configured as a roughly spherical and random coil, whereas there is only one way to be docked. Left to itself in a Brownian environment, the neck linker would therefore "prefer" to be randomly coiled. The difference in available configurations is expressed in a lower entropy for the docked state. Through $G = T\Delta S$, where T represents the temperature in Kelvin, the entropy difference ΔS is translated into a free energy difference G [13]. The free energy associated with the polypeptide going from a fixed and docked configuration to a random coil is

$$G = \frac{3}{2} k_B T N. \tag{3}$$

Here N represents the number of Kuhn segments in the chain. In a simplified view, a polymer segment shorter than the Kuhn length l_K is considered rigid. Longer polymers are conceived of as chains where each subsequent straight segment of length l_K is oriented randomly relative to the previous one. Random coiling of polypeptides has been studied for a long time [14] and it is been found to be of importance in a variety of systems [15]. Many texts use the persistence length which equals half the Kuhn length [13]. For a polypeptide, one amino acid covers about 0.33 nm and the Kuhn length is about four amino acids. We thus have $N = 7.5$ for the neck linker. With (3) we then find a coiling energy of about 11 k_BT. This is indeed close to the 13 k_BT that we found earlier for the power stroke energy. The average end-to-end distance of the randomly coiled neck linker is about $l_K\sqrt{N}$ [13]. Comparing this with the length $l_K N$ for a fully extended neck linker, we find a difference of roughly 7 nm. This is in good correspondence with kinesin's 8 nm stepsize. Dividing the power stroke energy of 11 k_BT by this 7 nm, we retrieve the average force of 7 pN that drives the power stroke.

The entropic mechanism appears to account for the observations. However, the theoretical 11 k_BT represents an overestimate as it does not take the random coil's self avoidance into account. The random coil, furthermore, cannot freely "explore" the entire configuration space as it is wedged in between the two kinesin heads. So the entropic forces in the neck linker can not completely explain the 13 k_BT power stroke.

It is by running the same experiment at different temperatures that one can establish whether the involved forces are entropic or not. Equation (3) tells us that the magnitude of an entropic force increases linearly with T.

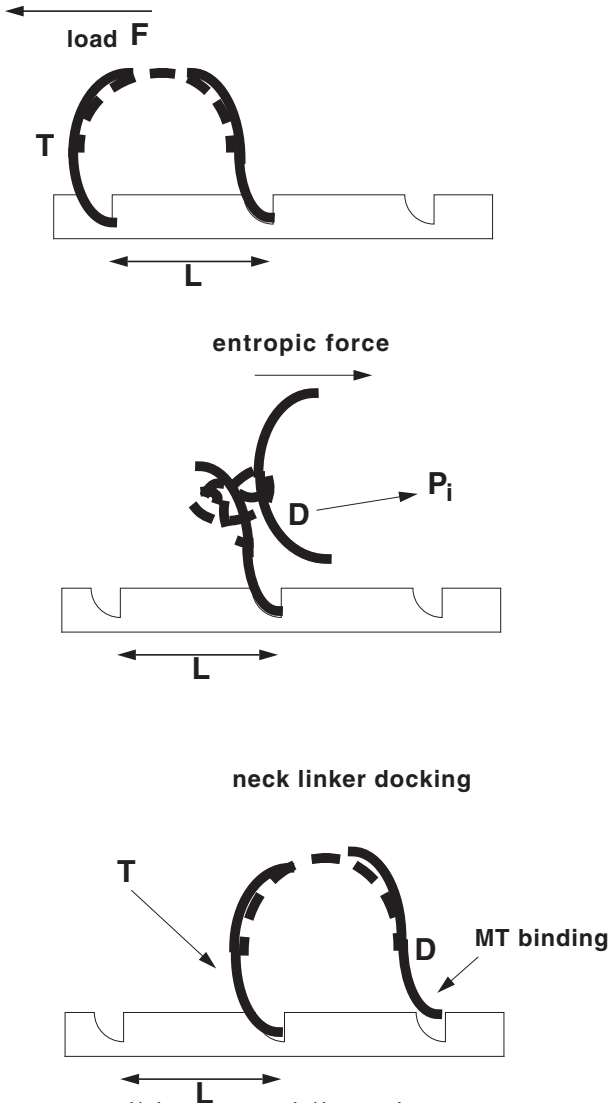

Fig. 2. Recently gathered data suggest that the force behind the power stroke derives from the entropic coiling of the neck linker that connects the two heads. After the binding of an ATP to the rear head, the neck linker, which is 30 amino acids long, detaches and moves out of a state in which it is stretched and docked against the two heads of the motor protein. The stretched configuration is unique, but there are many coiled configurations and coiling is, ultimately, an effect of Brownian motion [10]. In the text it is shown that entropic effects can quantitatively account for observed forces and power.

If the aforementioned entropic coiling were to underlie the power stroke, then, by substituting equation (3) into equation (2), we have

$$P_f/P_b = \exp\left[\frac{3}{4}N - \frac{1}{2}\frac{L}{k_BT}F\right], \qquad (4)$$

where N is again the number of Kuhn segments in the strand. The implications of this expression are clear. At the stopping force we have $P_f/P_b = 1$ and an exponent in equation (4) that is therefore equal to zero. So the stopping force is expected to go up with a factor λ when T is increased by a factor λ. If the power stroke mechanism were non-entropic, there would be a k_BT in the denominator of the first term of the exponent in equation (4). That would lead to a stopping force that is independent of temperature.

Kawaguchi and Ishiwata [16] measured the stopping force at different temperatures between 15 and 35 degrees Celsius. They found the stopping force to not vary with temperature within their margin of error. However, going from 15 °C to 35 °C means that the absolute temperature is only varied by about 7%. The margin of error in the results of Kawaguchi and Ishiwata is such that a stopping force that increases by 7% between 15 °C to 35 °C fits the data equally well. Given our model, more accurate measurements of the stopping force or the forward-to-backward step ratio are needed to determine whether the power stroke is indeed entropically driven.

4 Discussion

Models of motor protein operation have generally been divided into "power stroke models" and "Brownian ratchet models." Power stroke models conceive of the motor protein as going through a sequence of conformational changes. At least one of these changes would involve the generation of a mechanical force and the dissipation of power. In these models Brownian motion only appears as a nuisance that may possibly interfere with the deterministic procedure. In Brownian ratchet models the presence of Brownian motion is central and the action of the motor protein consists of blocking such motion in one direction and allowing it in the opposite direction. In a recent review of the matter it was stated that "power stroke" versus "Brownian ratchet" is basically a false dichotomy and that any credible model should include Brownian motion and directionality as well as conformational states and power generation [17]. The model presented in this paper illustrates the point. The entropic coiling can be thought of as a conformational change, but, at the same time, it is driven by Brownian motion and it would not occur in a deterministic $T = 0$ environment.

The model for chemo-mechanical energy transduction presented in this article is simple and intuitive. The concept of an overdamped Brownian stepper includes few adjustable parameters, leads to a consistent accounting for the energy of ATP hydrolysis, and makes some measured data derivable as implications of other measured data. In this article some further verifiable consequences of the stepper model and the coiling based power stroke are derived. Many researchers have modeled the motor protein's action as a sequence of chemical reactions. Such an approach, however, yields few falsifiable implications as the rates of the involved chemical transitions can be chosen to fit any data. Furthermore, processes like the coiling of a polymer do not have an identifiable activation barrier and are therefore are not adequately described in terms of states and rates. Such processes require a higher level description, i.e., a description involving a kind of collective dynamics of large and complex molecular aggregates [18]. Traditionally the notion of entropy is applied mostly in setups with a very large number of identical molecules. It is remarkable that the concept of entropy can also be applied to a short sequence of different amino acids to successfully explain the eventual behavior of one protein.

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