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How occasional backstepping can speed up a processive motor protein

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ABSTRACT

Fueled by the hydrolysis of ATP, the motor protein kinesin literally walks on two legs along the biopolymer microtubule. The number of accidental backsteps that kinesin takes appears to be much larger than what one would expect given the amount of free energy that ATP hydrolysis makes available. This indicates that backsteps are not simply the forward stepping cycle run backwards. We propose here a simple effective model that consistently includes the backstep transition. Using this model, we show how more backstepping increases the entropy of the final state, and probably also the activation state, thus reducing their free energy. This free energy reduction of the activation state (related to backstepping) speeds up the catalytic cycle of the kinesin, making both forward and backward steps more frequent. As a consequence, maximal net forward speed is achieved at nonzero backstep percentage. In addition, the optimal backstep percentage coincides with the backstep percentage measured for kinesin. This result suggests that, through natural selection, kinesin could have evolved to maximal speed.

1. Introduction

Processive motor proteins are among the tiniest engines known to man. These proteins utilize the energy of ATP hydrolysis to literally walk along a biopolymer (Howard, 2001). In a living cell they help maintain organization by transporting cargo, like organelles or vesicles filled with chemicals.

Already one and a half decade ago the stepping of the processive motor protein kinesin was made visible on the nanometer scale with optical tweezers (Howard, 2001). Early communications (Visscher et al., 1999; Schnitzer et al., 2000) reported that 5–10% of all steps of kinesin were backward. But smaller fractions were described later on as methods and materials improved and better resolutions were achieved; Nishiyama et al. (2002) gave 1/220 and Carter and Cross (2005) gave 1/802. Theoreticians have always been interested in backstep fractions as they can help verify stochastic models.

In this article we will show how, in the Brownian environment of the motor protein, a "well-tuned" backstep fraction can actually help the motor speed up. We will show how the backstep fraction that leads to the highest net speed can be evaluated and how the resulting expression contains no freely adjustable parameters. Finally, we will see how the experimentally established backstep fraction of kinesin is close to our predicted optimal backstep fraction.

2. An appropriate model for kinesin

The operation of an ion pump is generally modelled with a cycle as depicted in Fig. 1. At equilibrium the product of the forward rates, $k_{12} \times k_{23} \times \cdots \times k_{n1}$, equals the product of the backward rates, $k_{21} \times k_{32} \times \cdots \times k_{1n}$, and no net cycling occurs. To drive the protein through the sequence of states, $S_1, S_2, ..., S_n$, a driving energy is necessary (Hill, 1968). Such energy comes available if one of the steps involves the binding of ATP and if the protein, in subsequent steps, catalyzes the hydrolysis of the bound ATP. Eventually the remaining ADP and an inorganic phosphate have to be released so as to complete the cycle and to put the protein again in a state in which it can bind a new ATP. Under physiological conditions the hydrolysis of ATP makes $G_{\mathrm{ATP}} = 22\,k_BT$ units of free energy available. In the course of a cycle of a membrane pump like Na,K-ATPase, part of G_{ATP} is utilized to bind, transport and release on the other side of the membrane three sodium ions and two potassium ions. The transport is generally against the electrochemical potential of the involved ions. Consistent with the model of Fig. 1, it is found that with a sufficiently low ATP–ADP potential and a high electrochemical potential for sodium and potassium the operation of the pump can be reversed (Läuger, 1991).

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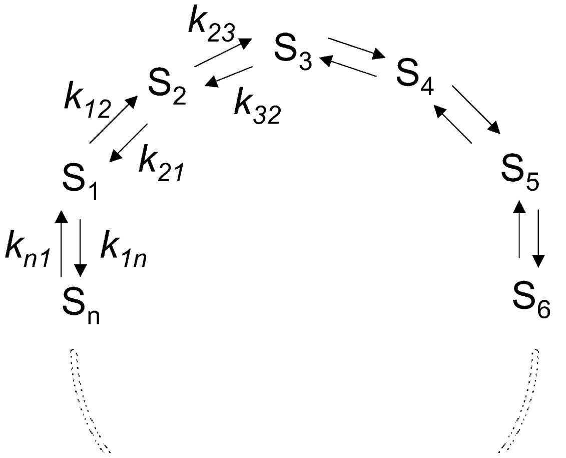

Fig. 1. An abstract conception of kinesin's catalytic cycle. The cycle involves the binding and hydrolysis of ATP, the actual mechanical stepping, and the release of ADP and inorganic phosphate. Each reaction is driven by an energy difference $G_{i,i+1}$ and the ratio of the forward and backward transition rate is an exponential function of $G_{i,i+1}$.

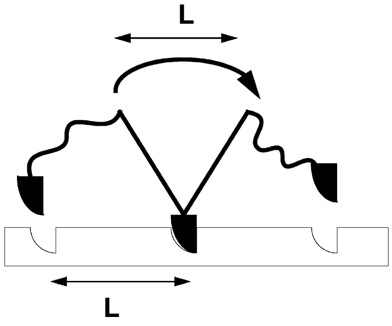

Fig. 2. The Brownian step of a processive motor protein. After detachment of the trailing leg, the attached leg reorients and brings the detached leg to the vicinity of the next forward binding site. After that, random diffusive motion will usually make the detached leg hit and attach to the forward binding site. Next the trailing leg detaches and a forward step is thus completed. However, there is a nonzero probability that the detached leg hits and attaches to the backward binding site. We assume that such backward site binding is what triggers the backstep. The probabilities p_f and p_b for forward and backward binding depend on the energy behind the reorientation, the applied load force, and, as the process occurs in the Brownian regime, on k_BT, the natural unit of thermal energy.

The stepping kinesin appears to operate in a fundamentally different way. Tight coupling, i.e. an 8 nm step for every hydrolyzed ATP and a hydrolyzed ATP for every 8 nm step, has been observed for kinesin (Schnitzer and Block, 1997; Hua et al., 1997). Without a mechanical load it is just the G_{ATP} that is driving the cycle in Fig. 1. Every mechanical step should correspond to one revolution around the catalytic cycle. If a backstep would correspond to the cycle being run in the direction against the ATP hydrolysis, then we should have $p_b/p_f = \exp[-G_{\mathrm{ATP}}]$ for the ratio of the backstep probability and the forward step probability. However, $\exp[-22]$ turns out 7 orders of magnitude smaller than the measured backstep fractions that were mentioned before. Furthermore, a model like in Fig. 1 leads to a stopping force, i.e. the load at which the kinesin comes to a standstill, that is determined by $G_{\mathrm{ATP}} = F_{\mathrm{st}}L$, where L is the steplength. If $G_{\mathrm{ATP}} = F_{\mathrm{st}}L$, then the two forces, chemical and mechanical, that are driving the cycle in opposite directions cancel each other out. But, with $G_{\mathrm{ATP}} = 22$ and $L = 8$ nm, the equation $G_{\mathrm{ATP}} = F_{\mathrm{st}}L$ predicts a stopping force F_{st} that is about twice as large as the measured 7 pN (Visscher et al., 1999; Schnitzer et al., 2000; Carter and Cross, 2005). Most importantly, it appears that kinesin still hydrolyzes ATP when it is pulled back with the stopping force and even when it is made to walk backwards with a load larger than the stopping force (Carter and Cross, 2005; Molloy and Schmitz, 2005). All these observations make a model as depicted in Fig. 1 untenable.

We are thus led to a different model for the stepping motor protein (Bier, 2003). After the detachment of the back leg, the attached leg reorients and brings the detached leg to the vicinity of the next forward binding site (see Fig. 2). Brownian motion is supposed to eventually make the detached leg hit the next forward site. Attachment there can then occur. The trailing leg next detaches, thus completing a forward step. It is the energy G that drives the reorientation of the attached leg and so biases the Brownian step towards the forward site. That reorientation is only one transition in the entire hydrolysis cycle of the kinesin. Therefore, the reorientation energy G is smaller than G_{ATP}. For the backward binding probability p_b and the forward binding probability p_f we can thus have $p_b/p_f \gg \exp[-G_{\mathrm{ATP}}]$ (Bier, 2007). Assuming that binding to the backward site is followed by a backstep, the model of Fig. 2 can actually lead to an accurate accounting for the backstep rates (Bier, 2003; Bier, 2007). Ultimately, the scheme depicted in Fig. 3 is a more appropriate model for the Brownian stepper than the kinesin's catalytic cycle of Fig. 1 alone. In the scheme in Fig. 3 at a particular point in the ATP hydrolysis cycle a kind of coin-toss takes place and the forward–backward determination occurs. The corresponding mechanical steps run in a dimension that is perpendicular to the plane of the chemical cycle.

3. Thermodynamics of backstepping and speed optimization

The question that needs answering at this point is: why has natural selection led to a backstep probability that is many orders of magnitude larger than the bare minimum of $\exp[-22]$ that thermodynamics requires? As was mentioned before, kinesin pulls organelles or chemical-filled vesicles across a eukaryotic cell. The reason that eukaryotic cells have such an active transport system in the first place is that they are, unlike prokaryotic cells, too large to rely on diffusion for their transport needs. Kinesin's stepping speed ultimately determines how fast a eukaryotic cell can react to environmental stimuli. There should be selectional advantage in engaging a kinesin that runs faster.

Our claim is that the entropy increase due to the forward versus backward "choice" can provide an answer to the question. The free energy $k_BT\ln 2$ that is associated with a doubling of the avail-

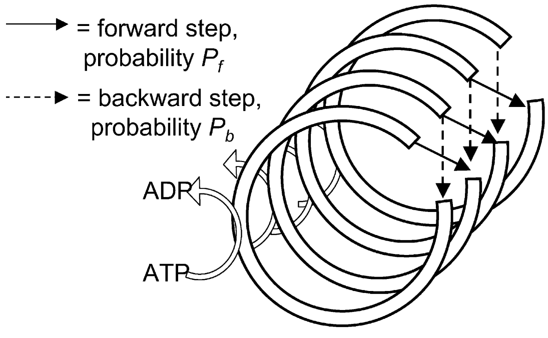

Fig. 3. Kinesin still hydrolyzes ATP when it is pulled back with a force larger than the stopping force. A setup like Fig. 1, with the ATP–ADP potential and the mechanical load pushing a single cycle in opposite directions, is therefore not the appropriate model and needs modification. Here we let the mechanical dimension run perpendicularly to the plane of the chemical cycle. ATP hydrolysis drives the chemical cycle in a clockwise direction. At the mechanical junction (cf. Fig. 2) a forward versus backward "decision" is made.