A Chemically Reversible Brownian Motor: Application to Kinesin and Ncd

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ABSTRACT Kinesin and nonclaret disjunctional protein (ncd) are two microtubule-based molecular motors that use energy from ATP hydrolysis to drive motion in opposite directions. They are structurally very similar and bind with similar orientations on microtubule. What is the origin of the different directionality? Is it some subtle feature of the structure of the motor domains, not apparent in x-ray diffraction studies, or possibly some difference near the neck regions far from the microtubule binding site? Perhaps because the motors function as dimers, the explanation involves differences in the strength of the interaction between the two motor monomers themselves. Here we present another possibility, based on a Brownian ratchet, in which the direction of motion of the motor is controlled by the chemical mechanism of ATP hydrolysis and is an inherent property of a single head. In contrast to conventional power stroke models, dissociation of the individual heads is not obligatory in the chemomechanical cycle, and the steps during which motion and force generation occurs are best described as one-dimensional thermally activated transitions that take place while both heads are attached to the microtubule. We show that our model is consistent with experiments on kinesin in which the velocity is measured as a function of external force and with the observed stiochiometry of one ATP/8-nm step at low load. Further, the model provides a way of understanding recent experiments on the ATP dependence of the variance (randomness) of the distance moved in a given time.

INTRODUCTION

Kinesin and nonclaret disjunctional protein (ncd) are two members of the kinesin superfamily of microtubule (MT)-based molecular motors. Powered by ATP hydrolysis, these two molecules move in opposite directions along an MT. They are, however, structurally very similar (Kull et al., 1996; Sablin et al., 1996), and bind with similar orientations on MT, eliminating the possibility that the origin of the opposite-directed motion comes about because the motors bind facing opposite directions (Hirose et al., 1996). The mystery is depended by a recent elegant experiment in which a chimera was formed by attaching the motor domain of ncd to the neck region of *Neurospora* kinesin (Henningsen and Schliwa, 1997; Case et al., 1997). Surprisingly, the resulting motor catalyzed "+" end-directed motion characteristic of kinesin from which the neck (and not the motor) region was taken. In addition to structural studies, there has been an explosion of work on the mechanical behavior of kinesin, leading to a consensus in the field that, with saturating ATP, the velocity of a single kinesin dimer moving processively on MT is between 0.5 and 1 μm/sec, and that the force (either elastic [Svoboda and Block, 1994; Coppin et al., 1997; Meyerhofer and Howard, 1995] or viscous [Hunt et al., 1994]) needed to stop the forward progress is $\sim$5 pN. Further, single-molecule studies of kinesin motion have shown that the motor moves in single steps of about 8 nm/step (Svoboda et al., 1993), corresponding well with the lattice spacing $d \approx 8$ nm of tubulin monomers along the axis of MT. Recently, it has been established that, in the absence of a load, the stoichiometry is one ATP/8-nm step of the motor (Schnitzer and Block, 1997; Hua et al., 1997). The challenge to theory is to reconcile all of these facts into a single model.

Here we present a model, based on a Brownian ratchet (Huxley, 1957; Hänggi and Bartussek, 1996; Astumian, 1997; Jülicher et al., 1997) where the direction of motion is controlled by the chemical mechanism of ATP hydrolysis. Some other models (Millonas and Dykman, 1994; Doering et al., 1994) have shown that changing the frequency of an external fluctuating force acting on a particle moving in a ratchet potential can cause a reversal in the direction of the induced flow. These fluctuating-force ratchets (Magnasco, 1993), although possibly technologically relevant (Lee et al., 1999), are inconsistent with transport driven by a chemical reaction such as ATP hydrolysis, because the fluctuating driving force is long range, and chemical reactions only act locally. Chauwin et al. (1995) and Bier and Astumian (1996) have proposed models for flux reversal based on a ratchet where the macroscopic force is constant but the local potential fluctuates (a so-called flashing ratchet), which is consistent with the action of a chemical reaction. In those studies, no attempt was made, however, to directly link the fluctuating potential with the chemistry of ATP hydrolysis. Our present model, where the fluctuations between different potential configurations is explicitly driven by the chemical reaction of ATP hydrolysis, is motivated by the realization that changes of a few amino acid residues, not manifest in the tertiary structure of the molecule, can significantly influence the chemical mechanism of catalysis. Also, the neck region of the molecule, which can determine the direction of motion of a chimera, is more likely to influence events at the site of ATP hydrolysis than the binding to, and interaction with, the MT.

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The central point of our paper is that a relatively simple model is sufficient to describe a reversible motor. A key assumption is that the ATP bound state has a large one-dimensional diffusion coefficient for lateral motion along the MT backbone, although this state has a very small dissociation constant, allowing the motor to retain energetic contact with its polymeric track while undergoing motion (Astumian and Bier, 1994; Prost et al., 1994; Astumian and Bier, 1996). After this theoretical prediction, experimentalists have indeed confirmed that a single-headed kinesin construct can move processively (Okada and Hirokawa, 1999; http://www.sciencemag.org/feature/data/985876.shl), and is well described by the simplest picture of an isothermal Brownian motor (Astumian, 1997). For a very nice simulation see the website http://monet.physik.unibas.ch/~elmer/bm/#why.

In contrast to the standard hand-over-hand mechanism, our model does not require either head of the motor to dissociate at any time during a mechanochemical cycle. The steps in which motion and force production occur are pictured as thermally activated transitions over an energy barrier on a one-dimensional potential between molecular states, each of which is close to thermal equilibrium even in the presence of large (5–10 pN) external forces. The system is thus appropriately modeled by chemical kinetics and no power stroke (i.e., a viscoelastic relaxation from a nonequilibrium conformation) is involved. This mechanism is fundamentally similar to that used to describe the coupling of ATP hydrolysis to drive uphill transport of ions by ion pump (Lauger, 1990; Astumian and Derenyi, 1998).

One preduction of our model is that a single-headed kinesin or ncd should display some processivity. The number of steps per encounter between a motor and MT may be small because the binding between the motor and MT is weak in the ADP bound state, and the rate for dissociation of the motor is significant compared to the rate of release of ADP.

To compare our model with experimental results for the effect of external force on the velocity of dimeric kinesin (little data is available for ncd), we provide an extension to a two-headed model, and incorporate alternating site kinetics for the ATP hydrolysis because this seems to be well established experimentally. In this extended picture, the mechanical motion is still described in terms of thermal activation on a one-dimensional potential. The presence of the second head significantly stabilizes the overall interaction between the kinesin and MT so that highly processive motion is possible. In addition to reproducing quantitative aspects of the effect of an external force on the velocity of the motor, and the stoichiometry of one ATP/step at zero load, our picture is consistent with four key observations: 1) a force applied in the direction of motion increases the velocity of the motor but the effect saturates (Coppin et al., 1997); 2) although the motor seems to be completely coupled at zero load, experiments show that at low ATP concentration the motion is more random even than predicted based on a single rate limiting step (Schnitzer and Block, 1997); 3) increasing significantly the strength of the coiled–coil interaction between the two necks of a kinesin dimer does not abolish processive motion (Romberg et al., 1998); 4) the motion driven by single-headed kinesin seems to be consistent with a small duty cycle motor, whereas that driven by dimeric kinesin is consistent with a large duty cycle motor (Young et al., 1998; Hancock and Howard, 1998).

A CHEMICALLY REVERSIBLE BROWNIAN MOTOR

To approach the mechanism by which chemical energy is used to drive unidirectional motion, imagine a particle moving along a filament to which it is associated through Van der Waals, hydrophobic, and other electrostatic interactions. The particle is an enzyme and the filament is a long chain of identical protein molecules. Although motion away from the filament is constrained, the particle can still move laterally along the filament by minute conformational changes arising from Brownian motion and accompanied by the making and breaking of many weak interactions. Typically, there are a few sites of contact within a periodic unit of the filament where the enzyme is localized with high probability (binding sites), but occasionally a transition over an energy barrier to an adjacent binding site on the left or right will occur.

The energy of the motor along the filament, $U(x)$, can be drawn as a curve representing the projection of the complex conformational and physical trajectory of the motor molecule onto a single coordinate. This is analogous to collapsing a multidimensional chemical reaction onto a single "reaction coordinate." Consider the model shown in Fig. 1 *a*, which describes the energy profile for movement of a single motor head along an MT in each of four different chemical states. Transitions between chemical states of the motor are shown on the *y*-axis. For simplicity, we have taken a relatively symmetric case, where the energy profile for the $E^{ADP\cdot Pi}$ and E^{ADP} are mirror images of each other, but this is certainly not necessary. The essential feature is that the sense of the anisotropy is opposite for two chemical states, and that there are at least two energy minima in these states. The higher energy minimum (H) acts as a switching station, where a chemical rate for release of ADP or inorganic phosphate (Pi) is compared to a mechanical rate for transition to the stable L position.

In the E state, where nucleotide phosphate is not bound, the motor is tightly pinned to one binding site on the MT. When ATP binds, the activation energy for lateral movement is decreased and transitions to the monomer on the left or right are fairly fast, but the motor is still tightly associated to MT. This makes the prediction that the one-dimensional diffusion coefficient will increase upon binding ATP to the motor even though the motor remains tightly bound to the MT.

Hydrolysis of ATP at the active site changes the interaction between the motor and track such that there are two ways the motor can bind in the $E^{ADP\cdot Pi}$ state—a relatively

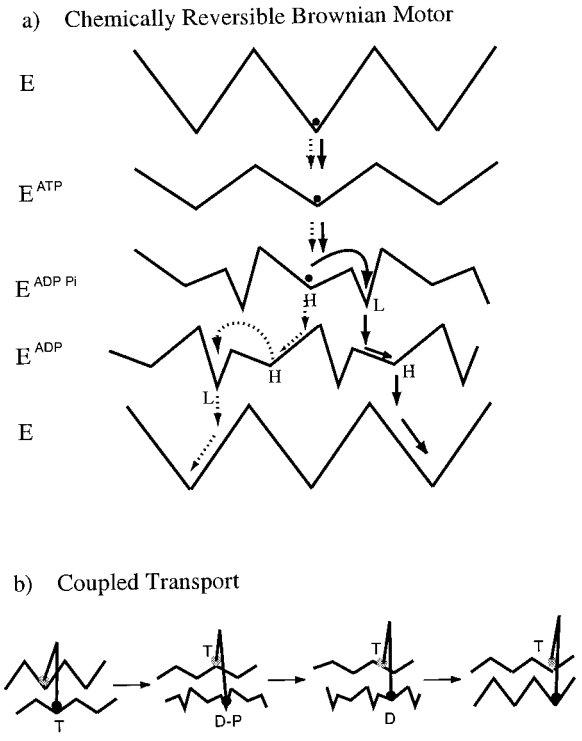

FIGURE 1 Ratchet mechanism for chemically reversible motion. (*a*) The protein concomitantly cycles through its chemical states while catalyzing ATP hydrolysis (on the *y*-coordinate) and translocates through space along a MT (possibly varying its conformation in the process) as plotted on the *x*-coordinate. (*b*) Illustration of how this mechanism would work with a two-headed motor. We show only the case for coupled motion directed to the right. Initially, either head can bind ATP (T) and the interaction of that head with the MT is weakened. This is followed by hydrolysis of ATP at the active site changing the interaction with MT, and inducing binding of ATP to the other head. As the catalytic and mechanical cycle of the first head proceeds, the second head follows along. Finally, ADP dissociates from the first head and a new cycle begins by hydrolyzing the ATP in the second head.

high energy H position and a lower energy L position. The barriers between the H and L positions are asymmetric—transition from the H to the L position on the right is much faster than transition to the L position on the left. Dissociation of Pi again changes the interaction between the motor and the MT such that the binding positions on the one-dimensional coordinate are shifted in the E^{ADP} state, and the barriers are interchanged such that a transition from the H to the L position on the left is much more rapid than a transition to the L position on the right. Release of ADP completes a chemical cycle of ATP hydrolysis, returning the motor to the tightly pinned E state.

One simple possibility for controlling the direction of motion in this model is by the relative rates for release of Pi and ADP (Astumian and Derenyi, 1998). This is similar to recent models for physical ratchets where a position-independent modulation of the potential, coupled with spatial anisotropy, allows directed motion (Astumian and Bier, 1994; Prost et al., 1994; Bier and Astumian, 1996). If release of Pi is slow and release of ADP fast compared to the H→L transition, the motor will most likely make a transition to the L position while Pi is bound, but will release ADP while in the transient H position, following the trajectory outlined by the solid arrows. On the other hand, if release of Pi is fast and release of ADP is slow compared to the H→L transition, the motor will most likely release Pi in the transient H position, but will make a transition to the L position before release of ADP, following the trajectory outlined by the dashed arrows. Sadly, this elegant mechanism alone is not sufficient to explain the mechanical data—it predicts that application of a modest external force opposing the ATP-driven motion should cause the motor to begin stepping backward, and this is not seen. Experimentally, a force of 5 pN is sufficient to halt kinesin, but the motor remains fixed and does not undergo significant backward motion even when challenged by forces as large as 12 pN (Coppin et al., 1997).

A second possibility, on which we focus here, is that the direction is controlled by the specificities for release of ADP and Pi from the H and L positions. This is closely related to A. F. Huxley's model for muscle contraction, where the rate constants for the chemical transitions are anisotropic along the reaction coordinate but the potential itself can be symmetric (Huxley, 1957). Once again, this closely parallels ideas taken from the coupling mechanisms of ion pumps (Jencks, 1989).

Consider that the L position of the $E^{ADP\cdot Pi}$ state is specific for release of Pi and that the H position of the E^{ADP} state is specific for release of ADP (*solid arrows*). First, ATP binds to the motor, decreasing the interaction energy holding the motor to a fixed site. Most likely, ATP is hydrolyzed before a transition to the left or right occurs. Because the H position is not specific for release of Pi, a transition to the L position on the right-most likely occurs, triggering release of Pi. The motor then rapidly equilibrates in the H position in which it finds itself. Now, ADP release most probably occurs from the ADP-specific H position, completing a chemical cycle. Rapid equilibration in the tight binding site completes a mechanical cycle of movement one period to the right of the starting point.

If the H position of the $E^{ADP\cdot Pi}$ state is specific for release of Pi, and the L position of the E^{ADP} state is specific for release of ADP, the direction is reversed (*dotted arrows*). ATP hydrolysis is followed by release of Pi from the Pi-specific H position. Then, because the H position is not specific for release of ADP, a transition over the low barrier to the L position on the left is quite likely. The L position is specific for ADP release, thus completing one chemical cycle of ATP hydrolysis, and the motor equilibrates in the tight binding site one period to the left of where it started, completing a mechanical cycle.

KINETIC MECHANISM FOR A SINGLE-HEADED MOTOR

If the local equilibration within a state is fast compared to any chemical transitions and to relaxation between the H

and L positions, we can rewrite the model in terms of chemical kinetics (Astumian and Bier, 1996) (Fig. 2). For simplicity, we assume that ATP hydrolysis is irreversible. With this assumption, the steady-state rate of ATP hydrolysis is $J_{ATP} = k_{hyd}\, P(E^{ATP})$, where $P(E^{ATP})$ is the steady-state probability for the motor to be in the weakly constrained ATP bound state E^{ATP}. We assume that the transition over the high barrier in the E, $E^{ADP\cdot Pi}$, and E^{ADP} states is essentially precluded. The constant s parametrizes the specificity difference for Pi and ADP release for the H and L positions. When $s \gg 1$, the L position is highly specific for release of Pi, and the H position is highly specific for release of ADP, and vice versa when $s \ll 1$. The parameter K is the equilibrium constant for transition from the H to the L position, and α and β are the rate constants for the translocation and chemical transitions, respectively.

An externally applied homogeneous force F can be visualized as superimposing a net tilt on each of the energy profiles in Fig. 1 ($U(x) \rightarrow U(x) + Fx$, where the origin is arbitrary). The energy difference between neighboring binding sites in both the E and E^{ATP} states is then Fd. If we assume that the physical distance between the H and L position is $d/2$, and that the barrier is halfway between them, the energies of the H and L positions change relative to each other by $Fd/2$ due to the force, and the effect of the external force on the transition rates can be parametrized by $f = e^{-Fd/(4k_BT)}$. In our model, the effect of an external force appears only in the lateral transitions between the H and L positions, and the diffusive step (*dashed arrows*) in the weakly pinned ATP bound state. The force dependencies of the chemical steps required by thermodynamics are subsumed in the rate constants for binding ADP and Pi. Far from equilibrium, we can assume that Pi and ADP release are irreversible, and that these binding steps do not occur. This reflects a minimal mechanochemical coupling (Duke and Leibler, 1996).

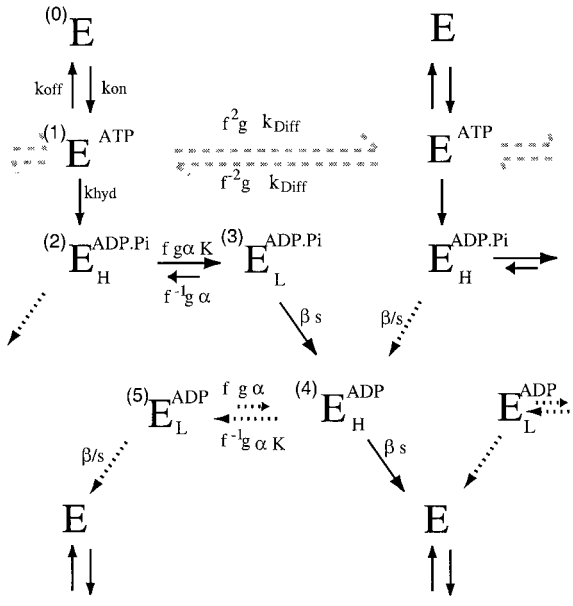

FIGURE 2 (*a*) Kinetic mechanism for a chemically reversible ratchet. k_{on} is a bimolecular rate constant, which, when multiplied by the concentration of ATP ([ATP]), gives the on rate for ATP, k_{off} and k_{hyd} are unimolecular rate constants and represent the off rate and hydrolysis rate for ATP, respectively, and k_{diff} is the rate for a transition to the binding site on the monomer to the left or right while in the weakly attached ATP bound state. K is the equilibrium constant for the H to the L transition. α and β are rate constants that set the relative time scales for the mechanical and chemical transitions, respectively, and g and f parametrize the effect of external viscous and elastic forces, respectively.

Rate constants for processes involving motion through space depend inversely on the friction. In general, the friction is the sum of an internal friction (Leibler and Huse, 1993; Vale et al., 1989) and the external friction due to the medium. This can be written $\eta_{eff} = \eta_{int}(1 + \eta_{ext}/\eta_{int})$. The effect of the internal friction is absorbed into the rate constants, so the effect of varying the external viscosity is obtained by multiplying the rate constants by $g = (1 + \eta_{ext}/\eta_{int})^{-1}$. This apportionment of the external force and viscosity, while by no means unique, seems to be the simplest possibility.

The kinetic equations for the model can be easily worked out in terms of the time scales of the individual steps to obtain the net rate of ATP hydrolysis, and the velocity of the motor along the MT. The stoichiometry (i.e., the number of mechanical steps per ATP hydrolyzed) at zero load is shown as a function of s in Fig. 3 *a*. The sign changes at $s = 1$, reflecting the change in direction of the motor. For sufficiently large s the stoichiometry approaches unity and ATP hydrolysis is described by the closed Markov chain

$$(0) \rightarrow (1) \rightarrow (2) \rightarrow (3) \rightarrow (4) \rightarrow (0),$$

where the numbers refer to those in Fig. 2. The rate of ATP hydrolysis can then be written

$$J_{ATP} = \left[\sum_{i=0}^{i=4} \tau_i\right]^{-1}, \qquad (1)$$

where $\tau_0 = (k_{hyd} + k_{off})/k_{hyd} \times (k_{on}[ATP])^{-1}$, $\tau_1 = k_{hyd}^{-1}$, $\tau_2 = (fg\alpha K)^{-1}$, $\tau_3 = (s\beta)^{-1}$, and $\tau_4 = (1 + Kf^{-2})(s\beta)^{-1}$. Eq. (1) can be rearranged to Michaelis–Menten form,

$$J_{ATP} = \frac{k_{cat} \times [ATP]}{K_M + [ATP]}, \qquad (2)$$

with

$$k_{cat} = 1 \Big/ \left(\frac{1}{k_{hyd}} + \frac{1}{fg\alpha K} + \frac{2 + Kf^{-2}}{s\beta}\right) \qquad (3)$$

and

$$K_M = \frac{(k_{hyd} + k_{off})}{k_{hyd}} \times \frac{k_{cat}}{k_{on}}.$$

For $s \ll 1$, the equations are the same except with the transformation $f \rightarrow f^{-1}$. For large s, the stoichiometry is $+1$ step for each ATP hydrolyzed, so the ATP-driven mechan-

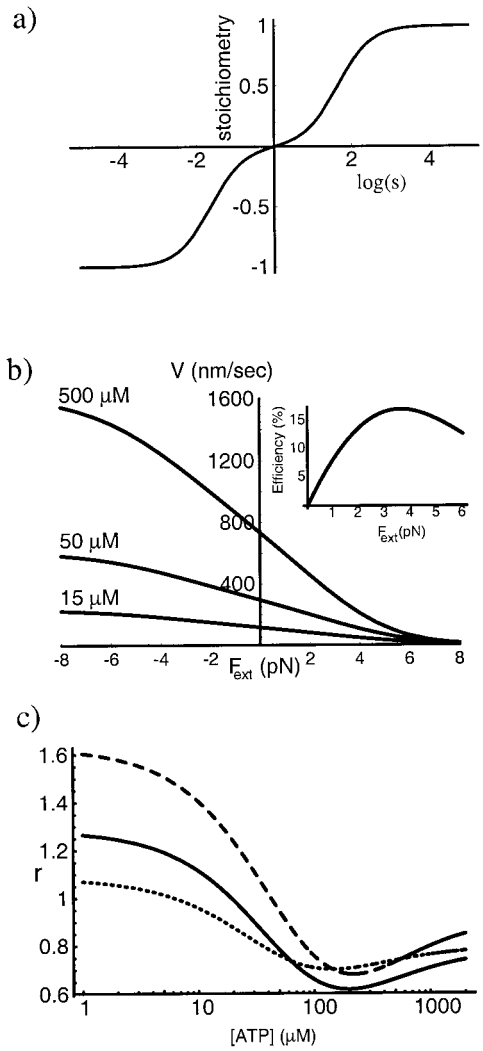

FIGURE 3 (*a*) Stoichiometry versus the log of the specificity at zero load. The basic shape of the curve is independent of the values of the kinetic constants. (*b*) Plot of velocity versus external elastic force at three ATP concentrations, with $s = 10^5$, $K = 1000$, $\alpha = 10$/sec, $\beta = 1$/sec, $k_{\text{diff}} = 25$/sec, $k_{\text{hyd}} = 125$/sec, $k_{\text{on}} = 2\ \mu\text{M}^{-1}\text{sec}^{-1}$, and $k_{\text{off}} = 100$/sec. The inset shows a plot of the thermodynamic efficiency versus external force. (*c*) Plot of the randomness as a function of ATP concentration for zero load (*solid curve*), a force of 3 pN opposing ATP catalyzed motion (*dotted curve*), and a force of 3 pN in the direction of ATP catalyzed motion (*dashed curve*). We used the same parameters as in (*b*), with $r_\infty = 0.5$. This reflects two approximately equal rate-controlling steps in the chemical cycle at large [ATP]. In our model with the parameters used, these are ATP hydrolysis $k_{\text{hyd}} = 125$/sec and ADP release, with an effective off rate $\beta s/K \approx 100$/sec.

ical velocity is $v_{\text{ATP}} = dJ_{\text{ATP}}$, where d is the step size (8 nm for kinesin). However, in the weakly pinned ATP bound state, an applied force can cause slip via the transition indicated by the dashed line in the kinetic mechanism shown in Fig. 2. For a single head, or two independent heads, the term $dk_{\text{diff}}g(f^2 - f^{-2})\text{P}(\text{E}^{\text{ATP}})$ would have to be added to v_{ATP} to obtain the net velocity, predicting that a force applied in the direction of ATP-catalyzed motion would increase the observed velocity without bound. Coppin et al. (1997) carried out such an experiment and found that, although a force applied in the direction of motion does in fact increase the velocity of the motor, the effect saturates. This can be explained by a cooperative two-headed model (Hackney, 1994; Peskin and Oster, 1995) where only one head can bind ATP at a time, schematically shown in Fig. 1 *b* and Fig. 4.

COOPERATIVE TWO-HEADED MOTOR

In our two-headed model (see Fig. 4), we consider that the heads can either be together (the minimum energy configuration, where the heads occupy neighboring subunits) or apart (where the heads occupy subunits that are displaced relative to each other). We assume that ATP hydrolysis at the active site of one head cooperatively induces binding of ATP to the other but that ATP hydrolysis at the second head cannot proceed until ADP dissociates on the first head. This ensures alternating site kinetics for the ATP hydrolysis, which is well established experimentally (Gilbert et al., 1998). In this case, there are three possibilities following ATP binding to the first head. 1) ATP hydrolysis occurs while the heads are together (Fig. 4, *middle column*), inducing binding of ATP to the second head. The first head completes its mechanical and chemical cycle, hydrolyzing one ATP and moving the motor one period to the right. 2) The first head might diffuse a period to the right before ATP hydrolysis at the active site occurs and induces ATP to bind to the second head, which then rapidly moves to a position adjacent to the first head (Fig. 4, *right column*). At this point, the motor is one period to the right of its starting position. Completion of the mechanical and chemical cycle of the first head results in movement an additional period to the right. Thus, the motor will have moved two steps while

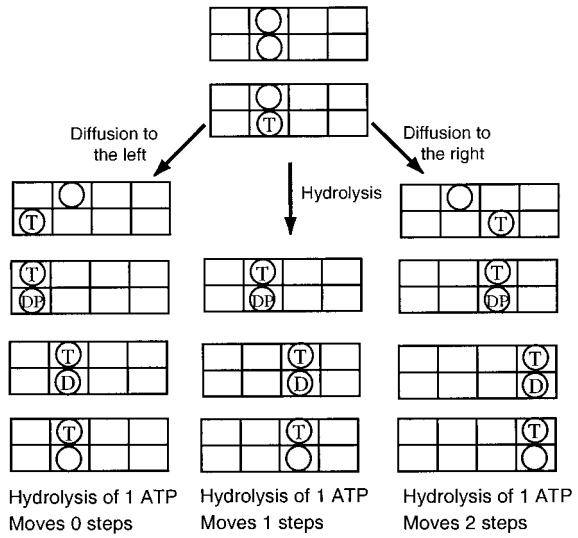

FIGURE 4 Pattern of kinesin stepping during normal coupled cycle (*middle column*), when a diffusive step to the left occurs before hydrolysis at the active site (*left column*), and when a diffusive step to the right occurs before hydrolysis at the active site (*right column*).

hydrolyzing only one ATP. 3) The first head might diffuse a period to the left before ATP hydrolysis at the active site occurs (Fig. 4, *left column*). Hydrolysis induces ATP to bind to the second head and rapidly move to a position adjacent to the first head. At this point, the motor is one period to the left of its starting position. Completion of the mechanical and chemical cycle of the first head results in movement one period to the right, back to the starting position. Thus, the motor will have moved zero steps while hydrolyzing one ATP. In the absence of an applied force, possibilities 2 and 3 are equally likely and do not contribute to the net rate. These possibilities are consistent with the observations that occasionally a motor may step back and then forward, but almost never takes two steps backward in a row (Schnitzer and Block, 1997; Coppin et al., 1997).

An external force biases the diffusive steps, making one more likely than the other. The effect on the net velocity can easily be calculated in terms of the splitting probabilities at the branch point E^{ATP},

$$P_2 = \frac{k_{diff} g f^2}{k_{diff} g (f^2 + f^{-2}) + k_{hyd}},$$
$$P_0 = \frac{k_{diff} g f^{-2}}{k_{diff} g (f^2 + f^{-2}) + k_{hyd}}. \quad (4)$$

These probabilities are the fraction of molecules that, having bound ATP, diffuse to the right or left before hydrolyzing ATP and are thus the fraction of events in which the motor moves two steps for one ATP and zero steps for one ATP, respectively. The net velocity can be written as

$$v_{net} = L J_{ATP} (1 + P_2 - P_0), \quad (5)$$

where $(1 + P_2 - P_0)$ is the average number of steps per ATP. Fig. 3 *b* shows a plot of the velocity versus external force at various ATP concentrations calculated using Eqs. 1, 3, and 4. With the parameters used, the Michaelis–Menten constants at zero force are $K_M = 60\ \mu M$ and $k_{cat} = 100$/sec, in good agreement with experiment (Schnitzer and Block, 1997; Hua et al., 1997). The velocity is a nearly linear function of the applied elastic force, and the extrapolated intercept (stopping force), above which no further forward progress can be observed, is ~5 pN and independent of ATP concentration, consistent with experimental results (Svoboda and Block, 1994). This stopping force is limited by the free energy available from ATP hydrolysis. The actual intercept, where the velocity crosses zero and becomes negative, can be arbitrarily large, limited only by the largest kinetic barrier to motion found in any chemical state. This is consistent with the results of Coppin et al. (1997), who found that, even at forces as high as 12 pN, the molecule does not step backward. The inset of Fig. 3 *b* shows the thermodynamic efficiency versus the external elastic force, which reaches a maximal value of ~15%. With much smaller k_{diff} the efficiency can approach 100% but the fit to experiment in this case is not nearly as good.

Hunt et al. (1994) measured the viscosity dependence of kinesin motility using a gliding assay, where the kinesin molecules were attached to a surface, and the motion of MT was observed. They plotted the velocity versus the product of velocity and viscosity (i.e., versus the viscous drag force) and found an apparent intercept of ≈4 pN. Interpreting these data requires some assumptions about the coupling between the surface and kinesin. If the coupling is elastic (spring-like), the kinesin will walk along the MT stretching the spring, and the amount of stretch increases with the solution viscosity. In this case, the viscous drag of the MT acts much like an external elastic force (which we parametrized by f in our model)—the spring retards forward transitions and favors backward transitions (Derényi and Vicsek, 1996). In contrast, if the coupling is rigid, the viscous drag of the MT is transduced directly as an effective viscous drag force on the kinesin motor. Forward and backward transitions are equally retarded. This case is parametrized by g in our model. If the internal viscosity would be characteristic of motion of a protein through water ($\eta_{int} \approx 4 \times 10^{-6}$ pN s/nm) a plot of velocity versus viscous drag force would yield an apparent intercept much smaller (Duke and Leibler, 1996) than that observed by Hunt et al. (1994). With an internal viscosity ten times greater than water $\eta_{int} = 5 \times 10^{-5}$ pN s/nm, however, one finds a linear relation between velocity and viscous drag force with an apparent intercept of 4 pN, independent of [ATP] (not shown). This internal viscosity is consistent with our model in which the motor moves laterally while still bound to the MT. The water at the interface is certainly more highly structured than in the bulk, and it is reasonable to assume that this leads to a higher internal viscosity that does not increase with increasing viscosity of the solution. As a result, the velocity of the motor is not particularly sensitive to changes in external viscosity even though diffusion and thermal activation of translocation steps are an integral part of the mechanism.

STOCHASTIC BEHAVIOR OF SINGLE-MOTOR STEPPING

Recently, several groups have studied the stepping motion of single motors (Svoboda et al., 1993; Vale et al., 1996; Higuchi et al., 1997). Because the individual transitions are stochastic, the displacement of a motor in a given time is characterized by an average value and a variance. If the stepping is controlled by a single rate-limiting process, the variance is large, but if a step is made up of many discrete subtransitions, each of which on average takes about the same time, the variance is much smaller. Svoboda et al. (1994) defined a randomness parameter r in terms of the variance in the displacement of the motor due to ATP hydrolysis, the average displacement, and the step size d evaluated in the limit of very long observation time.

For a completely coupled kinetic cycle where hydrolysis of one ATP always produces one mechanical step of fixed

length, the randomness r^* varies between 0 if many transitions of similar lifetime make up a single step (a clocklike mechanism), and 1 if there is a single rate-limiting process (a Poisson stepper). Thus, for any model, r^* depends on ATP concentration (Schnitzer and Block, 1997). This is very easy to see by reference to Eq. 1 for a Markov chain. At very low [ATP], τ_0 for ATP binding must be the single rate-limiting step in the reaction and r^* is unity. At intermediate ATP concentration the number of rate-controlling transitions is maximum because ATP binding (τ_0) and other relatively slow steps (τ_2 and τ_4 for the parameters used in Fig. 3) will have similar characteristic times, thus minimizing the randomness. Finally, at very high ATP concentration, ATP binding no longer plays any rate-controlling role, and the randomness approaches a value r_∞ characteristic of the number of [ATP] independent rate-limiting transitions in the mechanism (τ_2 and τ_4). Thus it is clear that the minimal model necessary to fit data where the randomness at high [ATP] is less than 1 must involve at least a 3-state Markov cycle.

If the pathway is not completely coupled, hydrolysis of ATP can sometimes produce more or less than one mechanical step as described above. This situation is somewhat more complicated, and the randomness can be larger than unity. For the kinetic model in Fig. 2, r is derived to be

$$r = \frac{1 - P_0 + 3P_2}{1 - P_0 + P_2} + (r^* - 1)(1 - P_0 + P_2), \qquad (6)$$

where

$$r^* = \frac{r_\infty + K_M^2/[ATP]^2}{(1 + K_M/[ATP])^2} \qquad (7)$$

is the randomness for the completely coupled cycle (i.e., when $k_{diff} \to 0$). A plot of r versus [ATP] is shown in Fig. 3 *c* for several values of applied force. The black line is that for zero force and is consistent with the experiments of Schnitzer and Block (1997). The dashed and dotted lines are for -3 pN and $+3$ pN applied force, respectively.

An important point to note is that, in the limit of very small k_{diff}, the model is very tightly coupled, and slowing of the motor is accompanied by a commensurate decrease in the rate of ATP hydrolysis, analogous to the Fenn effect in myosin (Fenn, 1924). In this limit the randomness cannot be greater than 1. Schnitzer and Block (1997) however found a randomness of $\approx$1.25 for kinesin at low ATP concentration. With larger k_{diff}, the motor is not completely coupled, and at low ATP the randomness can be greater than 1. Also, at large force, significant slip occurs and ATP hydrolysis continues even when the motor comes to a halt. As seen in Fig. 3 *c*, the randomness depends strongly on the applied force for $k_{diff} = 25$/sec. However, for $k_{diff} < 1$/sec (not shown), the randomness is far less sensitive to the applied force. Thus, measuring the randomness at several forces will allow direct determination of k_{diff} and discrimination between tightly and loosely coupled models.

PROCESSIVITY

Dimeric kinesin is highly processive, and can move for over a hundred steps before dissociating from MT. Monomeric kinesin (and apparently also ncd) is much less processive, moving at most 2–4 steps before dissociating. In experiments where the motors are adsorbed onto a surface, MT motion driven by monomeric kinesin is also qualitatively different from that of the dimeric wild type. The MT velocity increases almost linearly with increasing surface density of monomers, and is effectively zero in the limit that only one monomer interacts with the MT. This is similar to the behavior of myosin, and is consistent with a motor that is neither pulling nor offering appreciable resistance to motion a large fraction of the time, i.e., a small duty ratio (Howard, 1997). Dimeric kinesin, in contrast, catalyzes processive motion in the limit of very small surface density and the velocity quickly saturates with increasing surface density of motors. This is consistent with a high duty ratio motor that spends most of the time either pulling or immobile on the surface.

This behavior is most often interpreted in terms of a hand-over-hand mechanism for motion of dimers, where one head dissociates and swings forward while the other head remains attached. This swinging head then binds, allowing the other head to release and swing forward. The process continues, with the heads strictly alternating roles as swinging arm and anchor. Because one head is always firmly attached, the duty cycle is very high, and the velocity saturates at low motor surface density. Motion catalyzed by single-headed kinesin is pictured as occuring in a much more haphazard fashion, where an individual motor must release MT altogether before moving forward (Young et al., 1998). In the detached state, an individual motor offers no resistance to motion caused by other motor molecules, so the velocity increases with increasing surface density.

Our mechanism is entirely different. Neither head need dissociate at all during a chemomechanical cycle. However, in the ATP bound state (in which an individual head spends about 50% of the time) a monomer offers little resistance to lateral motion even though it is attached, but in the case of dimers, at least one of the heads is tightly pinned, reproducing the observed dependence of velocity on surface density of the motor. Dissociation is a side reaction and not an essential element of the chemomechanical cycle (see Fig. 5). This picture is analogous to the treatment of Young et al. (1994) for processivity of ATP-driven translocases such a DNA helicase. If dissociation is allowed mainly from the ADP bound state, the probability that a monomeric motor (Fig. 5 *a*) dissociates in a given ATP hydrolysis cycle is $P_{mon} = k_d/[k_d + \beta s/(1 + Kf^{-2})]$, where k_d is the rate constant for dissociation in the ADP bound state. The average number of steps per encounter with the MT is $N_{mon} = P_{mon}^{-1} - 1 = \beta s/[(1 + Kf^{-2})k_d]$. With $k_d = 100$/sec and the parameters used to obtain the fit shown in Fig. 4, $N_{mon} \approx 2$.

Dissociation of a dimer, in contrast, requires two sequential dissociation events (Fig. 5 *b*). After dissociation of the

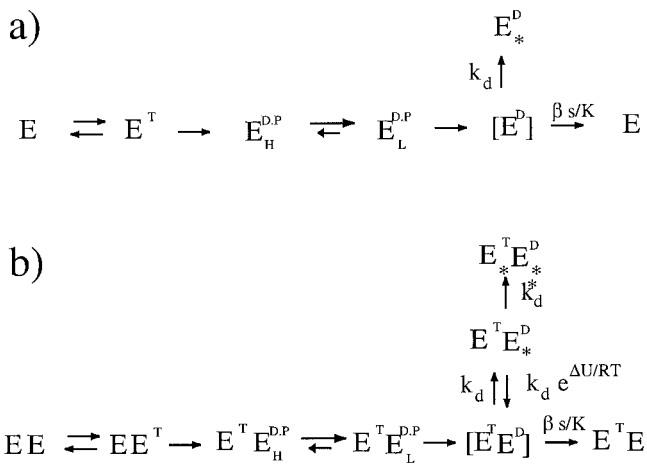

FIGURE 5 (*a*) Reaction along the predominant pathway for a monomer showing the side reaction of dissociation in the ADP bound state. (*b*) Reaction along the predominant pathway for a dimer showing the side reaction of dissociation in the ADP bound state. Here, two sequential steps are required—dissociation of one head followed by dissociation of the second head—before the dimer can be considered dissociated.

ADP bound head, the other head remains tightly bound. The effective rate constant for dissociation of this tightly bound head is likely much smaller than k_d and we label it k_d^*. While the one head is bound, the dissociated ADP bound head has a high local concentration (of order 1 M), and the recombination rate constant is $k_d \exp(\Delta U/k_B T)$, where ΔU is the binding energy. For this mechanism, the probability per cycle that the dimer dissociates can be calculated from

$$P_{dim} = P_{mon}\left[\frac{k_d^*}{k_d^* + k_d \exp(\Delta U/k_B T)} + \left(1 - \frac{k_d^*}{k_d^* + k_d \exp(\Delta U/k_B T)}\right) P_{dim}\right], \quad (8)$$

and thus the number of steps before dissociation is

$$N_{dim} = P_{dim}^{-1} - 1 = N_{mon}\frac{k_d^* + d_d \exp(\Delta U/k_B T)}{k_d^*}. \quad (9)$$

We see that, with very reasonable values for the binding energy of only 10–20 kJ/mol, a dimer can take a hundred steps per encounter even if the monomer takes only two with $k_d \geq k_d^*$.

DISCUSSION AND CONCLUSION

We have proposed a Brownian ratchet mechanism for motion of motor proteins in the kinesin family where the direction of motion is governed by the rates and specificities of different binding states for ADP and Pi release. It must be remembered that the ideas behind Brownian ratchets and fluctuation-driven transport do not represent a revolution in physics but simply a somewhat different way of looking at molecular motility that is often useful. Specifically, consideration of the ratchet in Fig. 1 with fluctuations between several configurations driven by ATP hydrolysis led us to realize that a subtle change in the chemical mechanism could dramatically manifest itself as a reversal in the preferred direction of the motor. Subsequent approximation led to the effectively four-state Markov model involving only chemical kinetic steps shown in Fig. 2.

Motion and force generation for this kinetic picture involves transitions between states that are close to thermal equilibrium even at very large driving force. ATP energy is used to change the relative affinities and barrier heights between neighboring binding sites. The timing and regulation is controlled by thermally activated steps from the H to L sites, and the H sites act as switching stations where the chemical rates are compared to the mechanical H→L transition rate. The H and L sites may represent either different physical locations along the microtubule or different conformations of the kinesin head. This simple model shows that Brownian ratchet mechanisms can have a stoichiometry very close to unity and offers a new way of thinking about how molecular motors work.

Our picture of how ATP hydrolysis causes directed motion is entirely different from the mechanical hand-over-hand model often used to interpret the observation that kinesin dimers can move many steps along MT without dissociating. The hand-over-hand model requires each head to successively detach from MT, swing forward, and reattach to MT. In contrast, our mechanism does not require dissociation as an obligatory step in the mechanochemical cycle, but does require relatively free lateral diffusion of a head while in the tightly associated ATP bound state. The dissociation in the ADP bound state observed experimentally is viewed as a side reaction.

How can kinesin move if neither head dissociates from MT during a chemomechanical cycle? To visualize this, it is necessary to realize that there are strong nonspecific electrostatic and van der Waals interactions that act to keep a kinesin molecule near MT even if the specific hydrogen and ionic bonds between kinesin and MT at the binding site are severed. The nonspecific interactions result in an effectively one-dimensional motion (diffusion) on a rough potential along the backbone of the MT. Transition from one binding site to the next is best described as a thermally activated process (like most chemical reactions), where energy to surmount the barriers along the way is reversibly borrowed from the environment. Dissociation to the bulk is of course possible, and much more likely to occur for a monomer than for a dimer.

There is a tendency to think in terms of an all or none binding—either a kinesin head is at its most stable binding site, or it is dissociated. Because structurally it is difficult to see how the two heads of a kinesin dimer could simultaneously occupy two binding sites 8 nm apart, it is commonly thought that only one head can be bound at a time. However, once we realize that the interaction energy profile experienced by a kinesin head moving along the MT axis is a continuum (possibly with deep wells at the putative binding sites, and that the energy near the MT even at its highest

point along the coordinate may be lower than the energy in the bulk, the paradox is resolved—kinesin takes up a position that minimizes its overall energy, with neither head precisely at the binding site, but with both heads still attached to MT.

This also helps us to understand how it is that increasing the coil–coiled interaction between the necks of kinesin does not destroy the processivity—in general the kinesin dimer moves as a unit, with one head in the ATP bound form that is tight-binding, but with a large one-dimensional diffusion coefficient so that, as the other head goes through its hydrolysis cycle in which motion occurs, dissociation is relatively improbable (allowing very large processivity) but lateral friction is not overwhelming.

There are three significant aspects that distinguish our model from more conventional models, each of which is experimentally testable:

1. Our model is a Brownian Ratchet (Huxley, 1957; Hänggi and Bartussek, 1996; Astumian, 1997; Jülicher et al., 1997; Astumian and Bier, 1994), and is not a power stroke mechanism. A major distinction between a power stroke and a thermally activated transition is the dependence on temperature. A power stroke is a deterministic viscoelastic relaxation of a nonequilibrium conformation of a protein, and hence depends only weakly on temperature. A thermally activated process, in contrast, requires thermal noise, and hence is strongly (exponentially) dependent on temperature. As techniques for studying individual steps of molecular motors develop, it will be possible to deconvolute the temperature dependence of chemical and mechanical steps and thus to unambiguously determine whether the specific transitions by which a motor molecule moves and exerts force are deterministic power-strokes or whether thermal activation is necessary.
2. Direction is controlled by the chemical mechanism of ATP hydrolysis and not by structure. The interactions between the motor and the MT are identical for opposite-directed motors in every chemical state. This is supported by the very similar structures of kinesin and ncd (Kull et al., 1996; Sablin et al., 1996), and is in broad agreement with studies on the kinetics of monomeric kinesin and ncd, where the ATP hydrolysis and ADP release steps are much slower in the latter than in the former. More detailed studies should be able to definitively determine whether the order of chemical and mechanical states is different for ncd and kinesin as predicted by our model. In Fig. 2, a significant motion or conformational change precedes Pi release for kinesin (*solid arrows*) but, for ncd, Pi release occurs before significant protein motion along the MT (*dashed arrows*).
3. Dissociation of the motor is not an essential step in the chemomechanical cycle. This predicts that a motor molecule may have an appreciable one-dimensional diffusion coefficient even if the overall binding to MT is very tight. Specifically for our model, we predict that binding ATP will increase the one-dimensional diffusion coefficient even though binding ATP does not significantly weaken the binding of kinesin with MT.

To directly compare our model with mechanical experiments on kinesin in which the effect of external force on the velocity of motion was studied, we introduced a cooperative two-headed model. In this model one head of kinesin at random binds ATP. Hydrolysis of ATP induces binding of ATP to the other head, reducing the activation barrier for transition to a neighboring binding site. As the first head continues through its catalytic cycle, moving a period to the right in Fig. 1 *b*, the second head is more or less dragged along for the ride. This model is able to explain how a randomness greater than unity is obtained, and predicts that a force opposing the ATP-driven motion will decrease the randomness at low [ATP] and increase it at high [ATP], and that a force acting in the direction of ATP-driven motion will increase the randomness at all [ATP].

An interesting prediction of the model is that, if the interaction between the heads were stiffened by substituting a different neck region, the motor could still work well, but the probability for diffusion to the left or right in the E^{ATP} state would be significantly reduced. This would cause a more complete coupling, resulting in a hyperbolic flow-force curve, and the randomness would be decreased. This should be testable using the construct of Romberg and Vale (Romberg et al., 1998).

We made several simplifying assumptions to allow us to express the chemical and mechanical rates in terms of only a few parameters not taken directly from experiment, K, k_{diff}, α, β, and s. Nevertheless, the model fits experimental data on kinesin for velocity as a function of external force and the observed stoichiometry and statistical behavior of single-molecule stepping extremely well. We anticipate that transient experiments on the biochemical mechanisms of ATP hydrolysis by kinesin and ncd (Gilbert et al., 1995; Ma and Taylor, 1997; Pechatnikova and Taylor, 1997), can be used to further constrain the rate constants.

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