Motor Protein Mechanics: A Stochastic Model with Minimal Mechanochemical Coupling

Thomas Duke and Stanislas Leibler
Departments of Physics and of Molecular Biology, Princeton University, Princeton, New Jersey 08544 USA

ABSTRACT A stochastic model for the action of motor proteins such as kinesin is presented. The mechanical components of the enzyme are 1) two identical head domains that bind to discrete sites on a microtubule and that are capable of undergoing a conformational change; and 2) an elastic element that connects each head to the rest of the molecule. We investigate the situation in which the strain dependence of the chemical reaction rates is minimal and the heads have independent biochemical cycles. The enzyme advances stochastically along a filament when one head detaches and diffuses to a new binding site, while the other head remains bound to the microtubule. We also investigate the case in which the chemical cycles of the heads are correlated so that the molecule shifts each head alternately. The predictions of the model are found to be in agreement with experimentally measured force-velocity relationships for kinesin—both when the force is applied externally and when the enzyme is loaded by a viscous drag. For reasonable values of the parameters, this agreement is quantitative. The molecular stepping characteristics observed in recent motility assays are also reproduced. A number of experiments are suggested that would provide a more stringent test of the model and help determine whether this simple picture is an appropriate description of motor proteins or whether models that include strain-dependent reaction rates or more complicated types of cooperation of the two heads need be considered.

INTRODUCTION

Motility assays (Scholey, 1993) are one of the main tools for studying the functioning of molecular motor enzymes such as kinesin, myosin, and dynein. Purified motors are examined as they move on a single complementary protein fiber (an f-actin or a microtubule) in the presence of a controlled amount of ATP. This simplification of the motile system to its basic minimum permits quantitative measurement of the biochemical and mechanical activity of the enzymes. Data provided by motility assays—such as the dependence of translocation velocity on ATP concentration and on the number of active enzymes, or the dependence of ATPase rate on ATP concentration—provide constraints on physical models of motor enzymes and the underlying process by which chemical energy is transduced into mechanical work. The various sets of data have to be explained within the same theoretical framework using a minimal number of phenomenological parameters.

The most recent set of such quantitative data has come from motility assays of kinesin motors moving along microtubules. Two groups have measured the dependence of the velocity of a single enzyme on the load (force) resisting the movement (Hunt et al., 1994; Meyhofer and Howard, 1995; Svoboda and Block, 1994). The techniques employed for these measurements were different in the two experiments: in one case the force was imposed externally by the action of an optical tweezer (Svoboda and Block, 1994) or a microneedle (Meyhofer and Howard, 1995); in the other the load was due to a viscous drag acting on the moving fiber (Hunt et al., 1994). In both cases the measured force-velocity curve could be approximated by a simple linear function. This may be contrasted with classical results in muscle systems that indicate an approximately hyperbolic force-velocity dependence (Hill, 1938). How can a linear force-velocity law be explained by a theoretical model? Does it introduce any constraints on existing theories? Can it be explained within the standard tight-coupling picture of motor action? These are some of the questions raised by the most recently performed motility assays.

The linear force-velocity dependence has, in fact, been predicted by a simple stochastic model for a general class of motor enzymes considered by Leibler and Huse (1993). A crucial assumption of this model is the strain independence of biochemical rate constants. By this we mean that all rate constants characterizing transitions between different biochemical states are strain-independent. This can be achieved when one of the transitions (e.g., the one leading to the working state) is irreversible, as was assumed in the analysis of the model. If, on the other hand, all of the transitions are reversible, then the general thermodynamic condition of detailed balance implies the strain dependence of at least one of the transitions. In the case of only one transition being strain-dependent, the functional form of this dependence is determined by the detailed-balance condition. We call such a case the "minimal strain dependence" situation.

One should stress, though, that in general any of the transitions could be strain-dependent. The rates of attachment and detachment of motors to and from the fiber, the rate of ATP hydrolysis, and the rates of release of hydrolysis products (P_i and ADP) may all depend on the strain acting on the motor head. In such a case, the modeling becomes

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Address reprint requests to Dr. Thomas Duke, TCM, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, England. Tel.: 44-1223-337377; Fax: 44-1223-337356; E-mail: td18@cam.ac.uk.

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extremely difficult without direct measurements of the functional form of these strain dependencies. To put it bluntly, the quantities measured in motility assays (such as the force-velocity curve) do not adequately constrain the space of possible models. It is a very easy exercise (and quite an empty one) to choose the strain-dependence functionals so that one perfectly fits all motility data. A "minimally strain-dependent" model is more rigorous, however, because the single functional is fixed by basic physical principles and cannot be chosen at will. Although there is clearly no guarantee that real motor proteins are so straightforward, it is worthwhile to investigate the degree to which this most simple model is compatible with the experimental data.

In addition to the linear force-velocity curve, the Leibler-Huse model, with no strain dependence of the rate constants, correctly described other functional dependences measured in motility assays, namely the [ATP] dependence of the velocity and of the ATPase rate, as well as the dependence of the velocity, on the number of active motors. It also introduced a natural classification scheme for various motors, depending on which of the biochemical rates is fastest; the extreme cases of the model correspond naturally to "porter" enzymes (working alone) and "rower" enzymes (working in groups). The model, however, was built on three simplifying assumptions: 1) the motors were supposed to bind at any location along the fiber, rather than at discrete binding sites; 2) the elasticity of the motor enzyme was assumed to be equivalent to a simple harmonic spring; 3) only the action of a single head of a motor enzyme was considered, so that in the detached state the motor was free to diffuse or be pulled away by the external force.

In this paper we would like to explore further the mechanisms of action of motor enzymes. In particular we want to determine whether one can replace the above assumptions by more realistic ones and still obtain agreement with the results of motility assays. In view of the above discussion, we shall do this in the framework of a minimally strain-dependent model; otherwise the constraints provided by the motility assay data are simply too weak. We introduce a model that is a generalization of the Leibler-Huse model to the case of 1) minimal strain dependence of the biochemical rate constants; 2) two motor heads; 3) nonlinear elasticity of the protein molecules; 4) discrete binding sites, equally spaced along the fiber. We also deal with the main problem posed by recent motility assays, namely the force-velocity dependence. We consider the two cases encountered in the experiments: the load coming either from an external force or from a viscous drag acting on the fiber. For both of these cases we explore the force-velocity relation for different values of the parameters of the model. We also address the question of discrete stepping of the motor. We examine whether the model predicts any characteristic features of the stepping. This is particularly interesting in the view of recent experiments in which the individual steps of the kinesin enzymes have been observed with nanometer resolution. In developing our model we have been motivated by recent experiments with kinesin. Many of the results should hold, however, for other "porter" enzymes. Later we discuss possible molecular interpretations of our phenomenological model, and we compare our results with other existing theoretical models. In addition to determining whether one can consistently explain the existing results of motility assays with a simple model and minimal number of phenomenological parameters, our goal is to find out which experiments could be performed to further constrain the modeling. Finally, in the Appendix we give the details of the numerical methods used to solve our model.

MODEL

Enzyme structure

In terms of its basic architecture, a motor enzyme can be viewed as consisting of a pair of identical globular "head" domains attached to a rodlike "shaft" (see Fig. 1). The heads are capable of hydrolyzing ATP and have an affinity for binding sites spaced evenly along a microtubule. This af-

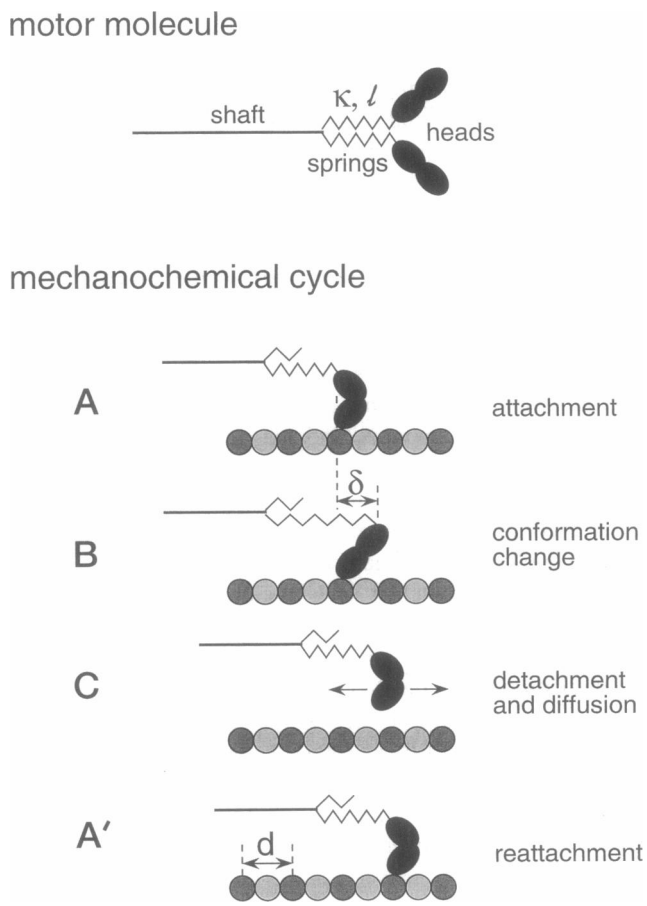

FIGURE 1 Mechanical action of a motor protein assumed in the model. The microtubule lattice is represented by a row of beads; binding sites (dark beads) are evenly spaced a distance $d = 8$ nm apart. Only one head domain is shown as it goes through its mechanical cycle. The active head attaches to the microtubule (A), undergoes a conformational change (B), then detaches (C) and diffuses until it rebinds to the microtubule (A'). During this cycle, the head may have translated along the microtubule.

finity is modulated by the hydrolysis cycle, so that each head alternates between bound and unbound states. Following the picture of Huxley and Simmons (1971), our simplified model for kinesin action comprises just two mechanical components. First, each head is able to undergo a large conformational change, switching between a "relaxed" state and one in which it is "cocked." Second, each head is linked to the shaft by an elastic element or "spring" (in reality, this elastic spring may at least partly overlap with the head domain; see Possible Molecular Interpretations for Kinesin, below). The shaft itself is considered to be rigid and to play no part in the mechanical action other than directly communicating the tension in the springs to the load, which is assumed to be carried by a "tail" domain located at the end of the shaft.

Biochemical cycle

The supposed mechanochemical cycle that a head undergoes is illustrated in Fig. 1. The relaxed head binds to one of the binding sites on the microtubule in a stereospecific manner (A). It subsequently undergoes a rapid transition to its cocked state (B), using energy derived from hydrolysis. Eventually, the head detaches from the microtubule and relaxes (C). It diffuses, limited in its movement by the spring tethering it to the rest of the molecule, until it rebinds to the microtubule, thereby returning to A and completing the cycle. All reactions are reversible, but it is assumed that the reverse transitions are slower than the forward ones in the absence of strain. When strain is present, we assume that it affects only one transition: the conformational change A↔B. The rates of the other transitions remain constant. A↔B is the most natural choice of the location of strain dependence within the cycle, because it is the conformational change that causes the strain. Furthermore, we make four assumptions about the rates of different events in the cycle: 1) detachment B→C is the rate-limiting step, so that a head spends the overwhelming majority of the time in state B; 2) the kinesin shaft does not have time to respond to the conformational change before transition A→B is completed (as a result, the change in conformation induces an alteration of the tension in the spring); 3) rebinding of the head C→A is reaction-limited, rather than diffusion-limited; 4) the reverse transition C←A, although slower than C→A, is faster than B→C (in which case a highly strained head that fails to make the transition A→B will rapidly detach and rebind, possibly at a different location where it is less strained).

We shall investigate two antithetical assumptions about the degree of cooperativity between the two heads.

Model 1: independent heads

In the first case, we suppose that the two motor heads go through their chemical cycles entirely independently. Because each head spends most time in state B and detachment from the microtubule is brief, the kinesin molecule is nearly always bound to the filament by at least one head. The molecule moves along the microtubule by displacing one head while holding on with the second one (Fig. 2). Because the chemical cycles are independent, it shifts the heads in an uncoordinated fashion, rather than one after the other in a regular way.

Model 2: coordinated heads

In the second case, we suppose that the chemical cycles of the two heads are tightly correlated. There is a one-to-one correspondence between the biochemical cycles of the two heads, but the cycles are out of step, so that detachment of one head is invariably accompanied by attachment of the other. One way that such coordination may arise is if the transition A→B of one head is impeded when the other head is attached in state B. Such a situation would be consistent with the assumed strain dependence of the transition A↔B if there were, for example, a strong steric interaction between a pair of heads bound in state B. Only a single head is attached to the microtubule for the majority of the time, and the enzyme moves by shifting the two heads alternately, in a coordinated way (Fig. 2).

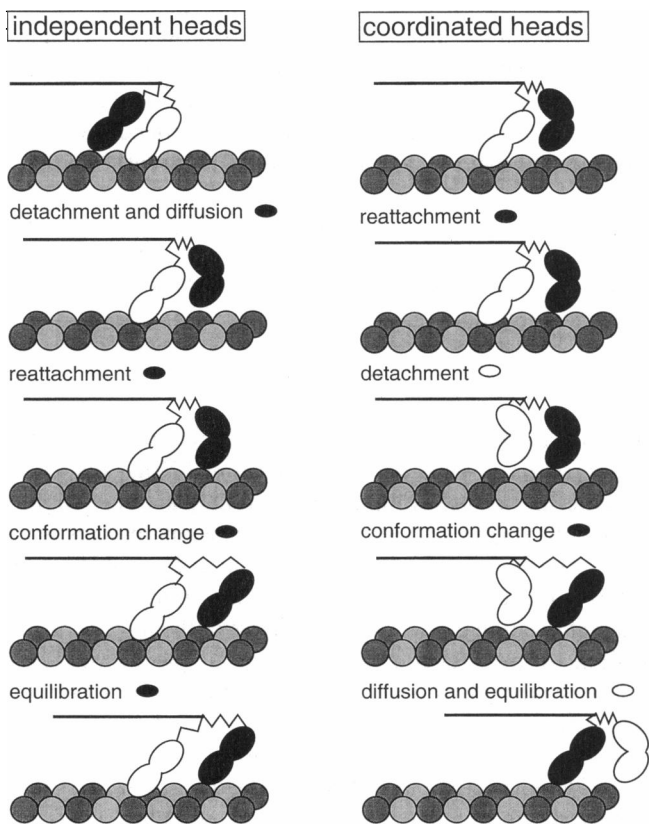

FIGURE 2 Motion of a motor molecule for 1) the independent head model and 2) the coordinated head model. The two heads are colored black and white; a symbol of the corresponding color indicates which head is involved at each step.

Method of translocation

How does the enzyme translocate in a prefered direction? When one of the heads detaches from the filament, its diffusion is restricted because it is tethered to the second head by the pair of springs. Its spatial distribution is consequently centered on the location of the basal terminus of the other head. Because the attached head is (according to our assumption 1) above) in state B, this location is in advance of the site to which the kinesin is currently bound (Fig. 2). Consequently, when the detached head rebinds to the filament, it is more likely to do so at a site ahead of the other head than at one behind it. This produces a polarity of movement that is statistical in nature—during each cycle there is a greater probability of a head shifting forward between microtubule binding sites than backward. The origin of the directionality lies in the specific conformational change of the heads and their stereospecific binding to the microtubule.

Phemomenological parameters

The mechanical model contains three variable phenomenological parameters that all have well-defined physical meaning and could, in principle, be measured by independent experiment. They are the size δ of the conformational change that the head undergoes, and two parameters κ and l that characterize the elasticity of the springs. It is natural to suppose that the springs are Hookean with spring constant κ when slightly stretched and that they have a maximum extension l when a large force is applied. We shall assume that they obey a force-extension law of the form

$$x/l = L(3f/\kappa l), \tag{1}$$

where f is the spring tension, x the extension, and L the Langevin function

$$L(y) = \coth(y) - 1/y. \tag{2}$$

The precise form of this function is unimportant—slightly different forms yield very similar results. What matters is that the springs behave linearly when they are only slightly stretched ($f(x) \approx \kappa x$, $x \ll l$), but the tension diverges when their extension approaches a finite value l. The values of the two parameters l and κ are much more important for the characterization of the elastic elements of the motor enzyme than the form of function and can, in principle, be measured.

We make no a priori assumptions about the values of the variable parameters. Rather, it is our aim to determine what possible range of values is compatible with the experimental data. However, the physical nature of motor enzymes does dictate rough orders of magnitude for two these variables. The size of the protein suggests that δ is a few nanometers and l at most a few tens of nanometers. The value of the spring constant κ might vary widely, on the other hand, depending on the nature of the elastic element.

The overall length and time scales are set by two fixed parameters, the values of which have been established from experimental studies. The distance between binding sites along the microtuble axis is $d \approx 8$ nm (Harrison et al., 1993). The average turnover time t_{cycle} for the kinesin mechanochemical cycle is governed by the Michaelis-Menten relation (Leibler and Huse, 1993), which may be written in the form

$$t_{cycle} = k_{cat}^{-1}(1 + K_m/[ATP]) \tag{3}$$

where the turnover rate at saturating ATP concentration k_{cat} has been estimated to lie in the range $10–100\ s^{-1}$ (Hackney, 1994a) for kinesin. Our assumption of minimal strain dependence, together with the supposition that transition A→B is not rate-limiting, implies that t_{cycle} is load-independent.

The free energy change associated with each hydrolysis event is known to be $G_{hydrol} \approx 20k_BT$, and this drives the reversible cycle in the foward direction. A fraction of the energy change occurs during dissociation and rebinding and ensures that the reverse rates of the transitions B↔C and C↔A are slower than the forward rates. The remaining free energy change is associated with the conformational change A→B. We shall use the estimate $G_{A\to B} \approx 10k_BT$. The assumption that only this transition is strain-dependent, together with the principle of detailed balance, fixes the ratio of forward to reverse transition rates to be

$$r_{A\to B}/r_{A\leftarrow B} = \exp\{(G_{A\to B} - W)/k_BT\} \tag{4}$$

where W is the change of strain energy induced in the spring when the head changes conformation, A→B. By way of illustration, Eq. 4 might be accommodated by a constant and rapid forward rate, $r_{A\to B}$, and a backward rate, $r_{A\leftarrow B}$, which is very slow at zero load, but which increases exponentially with the strain.

FORCE-VELOCITY RELATIONS

Two different motility assays have recently been performed to determine the velocity of translocation of an individual kinesin molecule as a function of load. One involved an external force, the other a viscous load. Svoboda and Block (1994) used an optical trap to exert a force on a bead being carried by a kinesin molecule and measured the speed of the enzyme as it traveled along an immobilized microtubule. Hunt et al. (1994) challenged a kinesin molecule anchored to a glass surface to propel microtubules of various lengths through a viscous solution and measured the speed at which the microtubules advanced. The two experiments yielded rather similar force-velocity relations. In both cases the velocity decreased almost linearly with increasing load, dropping to zero when the opposing force reached a value of about 5 pN.

Force-velocity relation for independent head model

We have determined the behavior of the model with independent heads in both of these assays, calculating the relation between force and velocity by numerical simulation. Details of the simulation procedure are given in the Appendix. Setting the distance between microtubule binding sites to be $d = 8$ nm, the free energy $G_{A\to B} = 10k_BT$, and $k_BT = 4$ pN nm, we determined the force-velocity curves for a variety of values of the parameters κ, l, and δ. Results for the external force assay are displayed in Fig. 3 *a* and those for the viscous load assay in Fig. 3 *b*.

Linear force-velocity relation at low loads

In all cases the force-velocity relation is approximately linear in the quadrant of positive velocity and positive load, as observed experimentally. Moreover, the two assays pro-

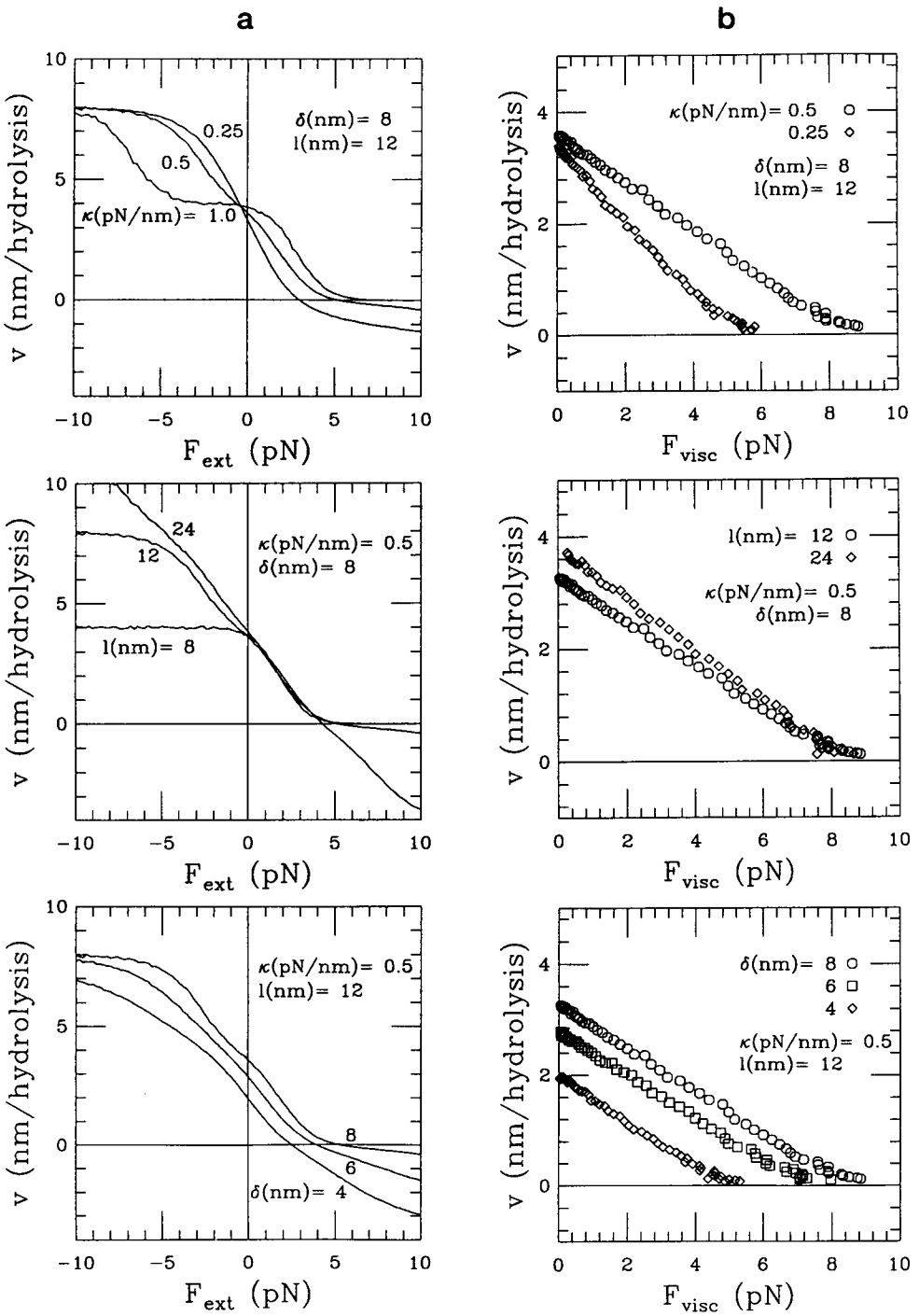

FIGURE 3 Force-velocity relations for (*a*) the external force assay and (*b*) the viscous load assay for the independent head model. Velocity is measured as the distance traveled per hydrolysis event. The curves are for varying spring constant κ (*top row*), spring length l (*middle row*), and size of conformational change δ (*bottom row*), as the other parameters are held constant.

duce closely similar relations that depend on the phenomenological parameters in the same qualitative manner. A noticeable difference, however, is that the load F_{halt} that stops the forward motion of the motor is larger (by a factor of about 2) for a viscous load than for an external force.

Nonlinear force-velocity relation at high loads

In the assay that uses an external force, we have extended the simulation to investigate what happens when the load is increased beyond F_{halt} and what occurs when a force is applied in the forward direction (negative F_{ext})—two regimens that have not been studied experimentally. We find that the motor can be driven backward in the former case and forward at a speed higher than the zero-load velocity v_0 in the latter. When these two regimens are included, the entire force-velocity curve is sigmoidal, with the velocity saturating at large positive and negative forces. The magnitudes of the saturating speeds grow with increasing spring length l but vary little as the other parameters are changed. By contrast, the velocity at zero-load v_0 increases with the size of the conformational change δ but is insensitive to the properties of the elastic elements (unless the spring length is very short, $l \leq d$, in which case v_0 falls). The maximum load against which a motor can advance F_{halt} increases as the conformation change δ is made bigger, or as as the springs are made stiffer (larger κ). Only for very stiff springs ($\kappa \approx$ 1 pN/nm) does the discreteness of the binding sites become noticable. In this case the thermal energy is insufficient to stretch the spring over a distance d, and a nonsigmoidal force-velocity relationship results.

Force-velocity relation for coordinated head model

The force-velocity relation for this cooperative model is shown in Fig. 4. It has a form similar to that of the relation for the model with independent heads, but differs in two significant details. First, the value of the stalling force F_{halt} is the same for both an external and a viscous force. Second, the velocity at zero load v_0 is twice that observed in the model with independent heads.

Similar force-velocity relations for viscous and external loads

The close correspondence of the forms of the force-velocity relations for the two cases 1) constant external force and 2) viscous load deserves some comment, for the physical cause of the decrease in speed with increasing load differs in each case.

Consider first the external force assay. When one head is detached and the other is in state C (Fig. 1), a constant force F_{ext} applied to the tail induces a tension (of magnitude equal to F_{ext}) in the spring linking the enzyme to the microtubule. The shaft is pulled back behind the basal terminus of the attached head, and the unbound head is tugged along with it so that its center of diffusion is offset. As a result, the probability of the free head reattaching at a site ahead of the other head is reduced and the average speed of the motor drops. Consider now the assay in which the motor protein drives microtubules through a viscous solution. The conformational change of a head induces a strain in the associated spring, and it is the subsequent relaxation of this strain that drives the motion of the microtubule. A viscous drag hampers this process, so that the springs do not have time to relax before one of the heads detaches again. Consequently, the distance that the filament moves each cycle is reduced.

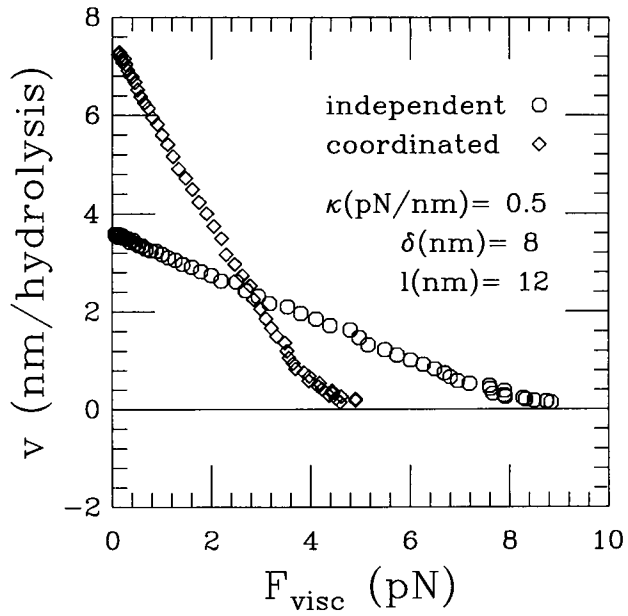

FIGURE 4 Comparison of force-velocity relation in the viscous force assay for a kinesin enzyme with coordinated heads (◇) and independent heads (○).

When acting against a mechanical force, a motor steps by a discrete amount with each cycle, but the probability of stepping forward decreases continuously with increasing force. When a motor propels a viscous load, the displacement of a microtubule during each cycle continuously decreases as the viscous load increases. Because the mechanisms that lead to a reduction in translocation speed are completely different, we do not expect to obtain the same force-velocity curve in the two assays.

Value of stopping force: external load

When the average distance that the shaft is pulled back by the force attains δ, the forward bias conferred by the conformational change is negated and the motor's net movement is halted. In effect, the motor walks on the spot, hydrolyzing ATP in a futile manner. The value of the force F_{halt} that stalls the molecule can readily be estimated in the case of small κ and large l (in which the springs remain closely linear): it is simply the force that stretches a spring

to equilibrium length δ, so $F_{\text{halt}} \approx \kappa\delta$. This agrees with the values obtained in the simulation. It is worth remarking that the strain dependence of the reaction rate affects the form of the force-velocity curve only in the case of stiff springs $\kappa >$ 1 pN/nm. Then stalling occurs rather by inhibition of the conformational change, owing to the fact that the attendant work required to stretch the spring would exceed $G_{A\to B}$. It is the strain dependence of the conformation change, then, that limits the maximum force against which a motor can progress when $\kappa > 1$ pN/nm. The limited energy of hydrolysis means that the stalling force cannot be made indefinitely large simply by increasing the spring stiffness. The experimentally observed value $F_{\text{halt}} \approx 5$ pN for kinesin is close to the maximum value that can be obtained in our minimally strain-dependent model.

Value of stopping force: viscous load

The apparent intercept F_{halt} of the viscous load-velocity curve can be estimated for the case of closely linear springs. When the viscous drag is very large, the equilibration time of the springs is much longer than the cycle time and consequently a spring will typically remain strained by an amount close to δ during the entire cycle. In the model with independent heads, both heads are attached, and because the two springs act in parallel, the total force that they exert on the microtubule is $F \approx 2\kappa\delta$. In the coordinated head model, only one head is attached and the force the spring exerts is $F \approx \kappa\delta$. If the drag coefficient of the filament is ζ, its mean velocity during a cycle is $v = F/\zeta$. Thus the effective viscous load $F_{\text{visc}} \equiv \zeta v$ is equal to F, and we obtain the estimate $F_{\text{halt}} \approx 2\kappa\delta$ for the independent head model and $F_{\text{halt}} \approx \kappa\delta$ for the model with coordinated heads. In the former case, the viscous load required to stop the motor is twice the magnitude of the external force that will halt it. Physically, this is because both springs are involved in force production as a motor molecule moves a viscous load; an external force, by contrast, acts on only one spring when the enzyme, holding on with one head, attempts to move the other head along a microtubule.

Experimentally, a value $F_{\text{halt}} \approx 5$ pN was reported for kinesin for both the external force (Meyhofer and Howard, 1995; Svoboda and Block, 1994) and the viscous assay (Hunt et al., 1994). In light of the above discussion, this appears to favor the assumption that the motor heads have coordinated, rather than independent biochemical cycles. A firm conclusion cannot be drawn, however, because there are uncertainties in the experimental data. For example, the direction of the external force applied by the optical tweezers is not well known. Nor can we rule out the possibility that the viscous medium chemically affects the motor's action.

Value of velocity at zero load

For the independent head model, the velocity at zero load corresponds to an average displacement of the enzyme through approximately $\delta/2$ per hydrolysis event. This may be interpreted in the following way. Because the chemical cycles of the heads are independent, for any two successive hydrolysis events there is a 50% probability that alternate heads detach and a 50% probability that one of the heads detaches twice in succession. In the former case, the molecule advances an average distance that is approximately equal to the strain δ in the attached head. In the latter situation, the molecule repeats the previous step and so does not move on average. Thus the mean displacement per hydrolysis event is close to $\delta/2$. For the model with coordinated heads, the heads move alternately, so that the molecule tends to advance at every hydrolysis event. In this case, the velocity at zero load corresponds to an average displacement of the enzyme through approximately δ per hydrolysis event.

MOLECULAR STEPPING

In their motility assay, Svoboda and Block (1994) were able to detect the discrete motion of a kinesin molecule. At high loads, the bead attached to the enzyme was observed to pause at locations separated by multiples of 8 nm and make rapid "steps" between these locations. At lower loads, discrete stepping was much less evident. Our model also displays these features. The steps arise because of the discrete separation between locations where the enzyme prefers to dwell. As such, they reflect the underlying microtubule lattice spacing d, rather than the size δ of the conformation change of the motor head.

Steps visible at high load

As shown in Fig. 5 *a*, when a high external force is applied to the tail, the motion reflects the discreteness of the microtubule binding sites: the molecule spends the most time at positions separated by an interval d. Evidence is provided both by the trace of the enzyme's displacement and, more clearly, by the autocorrelation function of the trace. Examination of the motion indicates that the molecule actually moves between these locations by making two steps of size $d/2$ in quick succession. The reason is that a high external force stretches the springs almost to their full extension. To accommodate this, the molecule is most likely to be in a conformation in which both heads are level with one another. Only with small probability are Brownian forces able to advance one of the heads to the next site, causing the shaft (and any load attached to the tail) to move forward through a displacement of approximately $d/2$. Many hydrolysis cycles will typically pass before such an event occurs. Subsequently, the pull exerted by the spring of the advance head makes it much easier for the other to move up to join it. Thus a second movement of the shaft through $d/2$ is rapidly accomplished.

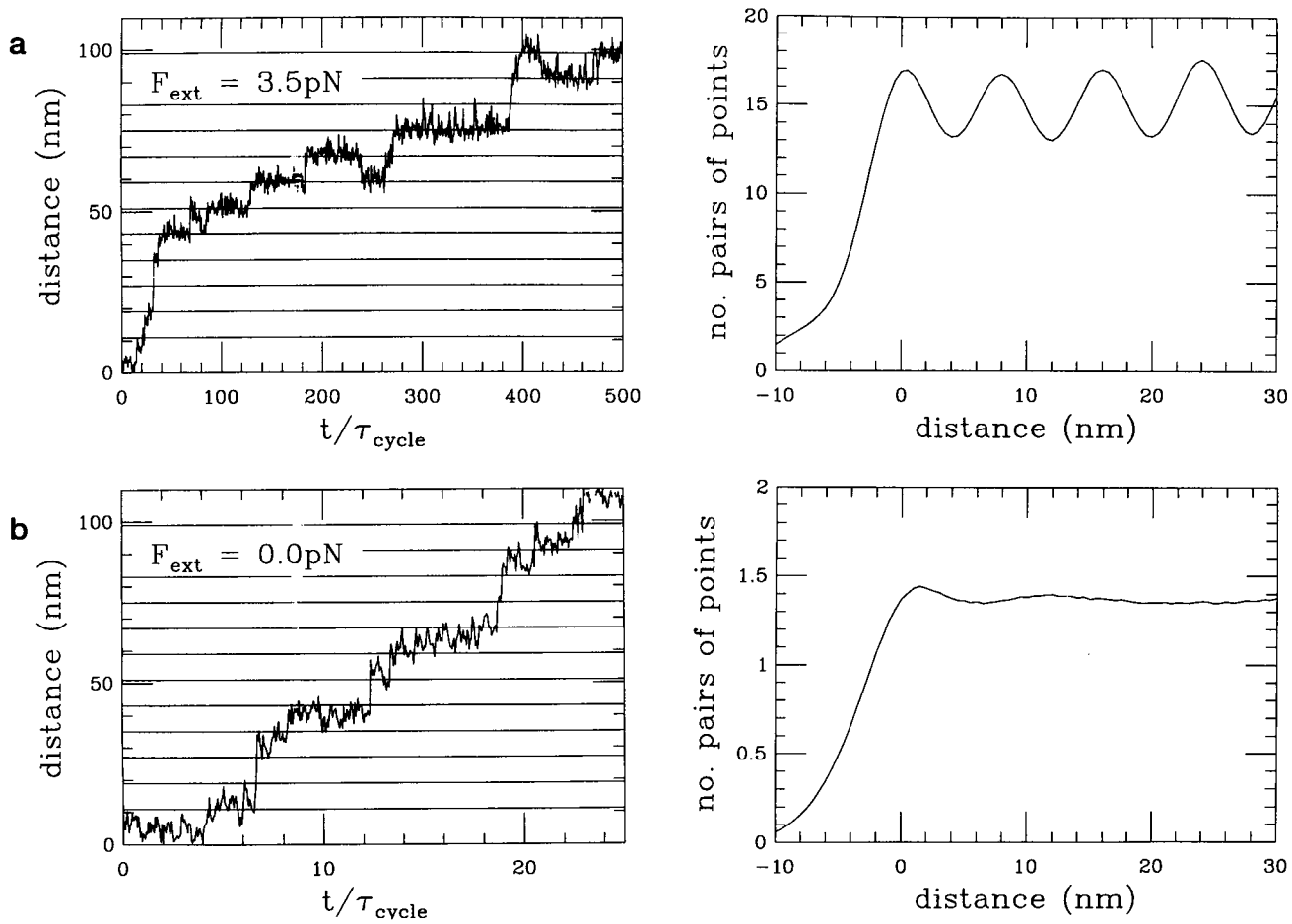

FIGURE 5 (*Left*) Trace of motor enzyme's progess for (*a*) a large external force, close in value to the force required to halt the enzyme, and (*b*) zero external force. Horizontal lines mark intervals equal to the distance between binding sites d = 8 nm. (*Right*) Corresponding autocorrelation function of the motor displacement, calculated from a data set comprising 20,000 hydrolysis events.

Steps invisible at low load

At low loads the motion of the kinesin shaft betrays little evidence of the discreteness of the microtubule binding sites (Fig. 5 *b*). The reasons are twofold and are related to the fact that the elastic elements are fairly slack. In the first instance, even with both heads bound to the microtubule, the kinesin shaft is still able to wander by Brownian motion over a rather large range. This movement blurs the location of the load relative to the discretely positioned heads. Second, during each chemical cycle a head is able to shift through any one of a range of distances; it may jump forward one, two, or three sites, backward one or two, or return to the same site it left. At low loads, the kinesin molecule advances in steps of variable size $md/2$, where m is a small positive or negative integer.

Stepping statistics

Svoboda et al. (1995) have also analyzed the fluctuations in the displacement of a kinesin enzyme as it moves along a microtubule. They found that the variance in displacement increases linearly with time, but at a rate lower than they expected by supposing that the motor makes steps of fixed length 8 nm at a constant rate (i.e., Poisson distributed in time). With a Poisson process, one expects that the ratio R of the variance in displacement to the mean displacement is equal to the step length. Svoboda et al. (1995) measured $R = 4.7 \pm 2.0$ nm, significantly smaller than the supposed step length 8.0 nm, and argued that this result implies that molecular stepping is not a Poisson process. The discussion in the preceding paragraph, however, suggests an alternative explanation of this result. In our model, steps of 4 nm can occur as well as those of 8 nm, so one would expect a value of R of less than 8 nm, even though the two heads of the kinesin enzyme have uncorrelated chemical cycles and the time sequence of steps has Poisson statistics. In fact, we find that the value of R depends on the values of the model parameters, but can indeed be smaller than 8 nm in the model with independent heads (see Fig. 6, in which $R = 6.8 \pm 0.2$ nm). This demonstrates that caution must be exercised when interpreting the results of Svoboda et al. The small value of the variance does not provide definitive

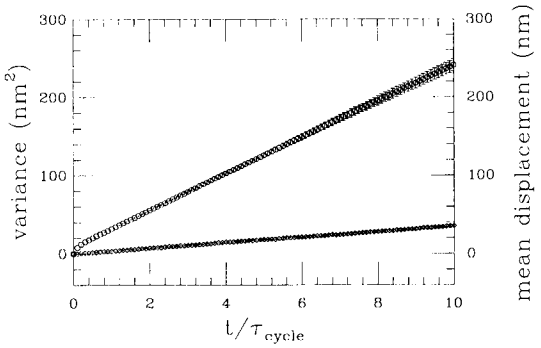

FIGURE 6 Variance in the displacement (○) and mean displacement (◇) as a function of time under conditions of zero external load (for parameter values $\kappa = 0.75$ pN/nm, $l = 8$ nm, $d = 8$ nm; independent head model). The ratio R of the variance and mean is $R = 6.8 \pm 0.2$ nm. Averages were calculated from a data set comprising 20,000 hydrolysis events.

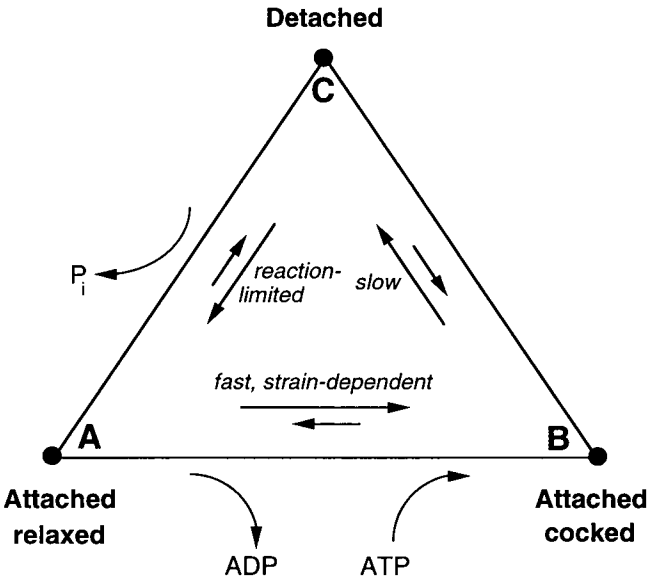

FIGURE 7 Example of a chemical reaction scheme that is consistent with the assumptions made in the model about the mechanochemical cycle.

evidence on whether there are correlations in the chemical cycles of the two heads.

POSSIBLE MOLECULAR INTERPRETATIONS FOR KINESIN

The model that we have investigated makes a number of assumptions about the biochemistry and the physical structure of the kinesin enzyme. Agreement with the experimental data constrains the values of the phenomenological parameters to lie in particular ranges: $\delta = 4-8$ nm, $\kappa = 0.4-1.0$ pN/nm, $l > 6$ nm. In this section, we show that both the assumptions and the parameter values are consistent with current knowledge of the motor protein and indicate a range of possible microscopic interpretations of our phenomenological model.

Biochemical cycle

A variety of chemical schemes are in accord with our suppositions about the mechanochemical cycle. One example is shown in Fig. 7. Here, the kinesin detaches as a K·ADP·P_i complex, then subsequently releases P_i and rebinds to the microtuble as K·ADP. Binding of ATP occurs while the kinesin is attached to the microtubule, and the conformational change of the head may be stimulated either by this, or by the hydrolysis of ATP. This sequence of events is suggested by recent kinetic studies (Gilbert et al., 1995), but many other chemical schemes are consistent with the model.

Independence/coordination of the head domains

Recent data by Hackney (1994b) suggest a correlation between the heads. When microtubules were added to kinesin dimers with bound ADP, they provoked the fast (1 s) release of only half of the ADP molecules; the second half was released at a much slower rate (100 s). The implication is that the two heads are not equivalent—only one can be bound to the microtubule at a time, perhaps because of steric effects. This result seems to support the model in which the cycles of the heads are coordinated. Some caution is required though, because Hackney's result was obtained in the absence of ATP. The correlation that is seen may be caused by the fact that the kinesin is unable to complete its chemical cycle; in the presence of ATP the correlation may become negligible.

Enzyme structure

Regarding the physical structure of the enzyme, the model makes two assumptions: that the kinesin head can undergo a large conformational change, and that there is an elastic region linking each head to the shaft of the molecule. Currently, no direct evidence of a conformational change in the kinesin head has been obtained. Structural studies of a different motor protein, the myosin enzyme, do indicate that a change in conformation on the order of a few nanometers could be accommodated by its globular head domains (Rayment et al., 1993). The presence of an elastic region linking each head to the shaft is also conjectural. Such an elastic element could be accommodated either directly in the head domain (aa 1–337) or in the dimerization domain (aa 338–380; in this case the action of the spring could be the dissociation and association of the two coils). Because kinesins shortened to their first 400 aa seem to move along microtubules in the usual fashion (Gelles, private communication), we expect that the elastic element is contained within this region. If the spring is of entropic origin, the spring constant would be $\kappa = 3k_BT/lb$, where b is the Kuhn length of the chain (so, for example, a simple polypeptide strand with $b = 4$ nm and $l = 8$ nm would provide $\kappa = 0.4$

pN/nm). Alternatively, the spring may be enthalpic, its strength depending on the detailed structure of the protein. By comparing the stiffness of the motor with that of a glass fiber, Meyhofer and Howard (1995) have estimated that $\kappa >$ 0.15 pN/nm, consistent with the range of values appropriate for our model.

Binding to the microtubule

In the model, we supposed that each head binds to sites evenly spaced on a linear lattice running along the microtubule. In reality, the microtubule contains 13 parallel protofilaments. Our model is consistent with a number of different patterns of movement on the microtubule surface. For example, the two heads might walk on adjacent protofilaments, each keeping to its own track. Alternatively, a head might be able to diffuse to an adjacent protofilament each time it detaches, so that the kinesin molecule diffuses around the circumference of the microtubule as it advances. Experiments by Ray et al. (1993) indicate that a kinesin enzyme can track the protofilament axis over a long range, and these results favor the first pattern of motion. It is also conceivable that the two heads walk on the same protofilament. A slight modification of our model would be required to accommodate this possibility (to exclude the occupancy of one site by both heads), but we would not expect this to substantially alter the model's behavior. Another possibility is that the enzyme walks only along the microtubule "seam," where two adjacent protofilaments are offset longitudinally so that the kinesin-binding sites on one lie midway between those on the other. Again, the model can be adapted to handle this pattern of motion. In this case, the enzyme typically advances when the trailing head detaches and diffuses past the other head to the next binding site on the microtubule. Consequently, the molecule moves predominantly in 4-nm steps. As a result, a low value of the ratio R of the variance in displacement to the mean distance traveled arises naturally (see discussion under Molecular Stepping, above). In this case, one would expect to detect peaks at 4-nm intervals in the two-point correlation of the motor's displacement if sufficiently good statistics could be obtained.

COMPARISON WITH OTHER MODELS

This study shows that a minimally strain-dependent model of motor enzymes that incorporates two equivalent head domains that attach to discrete binding sites on the microtubule resolves the two main problems exhibited by the simplified stochastic model proposed by Leibler and Huse (1993). The first of these is that a velocity that decreases linearly with load demands a displacement per cycle of the motor that decreases continuously with increasing force. This was accommodated in the Leibler-Huse model by assuming a continuum of binding sites along the microtubule. In this paper, we have shown that the force-velocity relationship remains approximately linear if the binding sites are discrete, because the *probability* of making a forward step at each hydrolysis event varies almost linearly with the load. Second, the Leibler-Huse model predicted that the maximum external force against which a motor can progress depends on the viscosity of the solution, because the stalling of the motor is caused by slippage while it is detached from the filament and the amount of slippage is proportional to the force and inversely proportional to the viscosity. Meyhofer and Howard (1995) have found, however, that the stalling force of kinesin is independent of viscosity. We have shown that this discrepancy is resolved if two heads work together, so that the motor enzyme always holds on with at least one head. Then the probability of stepping forward depends only on the applied force and the elastic properties of the motor, and not on the viscosity of the solution.

Alternative models

Although the model that we have investigated is consistent with the experimentally measured force-velocity curves, it is important to note that a linear force-velocity relation in the positive quadrant does not severely constrain the field of candidate models. The fact that both viscous and external forces produce similar relations, on the other hand, does make certain classes of model improbable. For example, models that propose that motor enzymes advance by using the chemical cycle to rectify Brownian motion (Astumian and Bier, 1994; Magnasco, 1993; Prost et al., 1994) do predict, with an appropriate choice of parameters, an approximately linear force-velocity relation. But in these models the translocation speed depends sensitively on the diffusion coefficient, so they would predict very different curves for the external force assay, in which the motor is diffusing, and for the viscous assay, in which the microtubule is diffusing. A problem also exists with models that include an "escapement mechanism" (Huxley, 1981), which permits completion of the hydrolysis cycle only when the strain is completely relaxed. In these models, the motor translocates a fixed distance with each cycle, but the cycle time increases at higher loads, so that the velocity drops (Hunt et al., 1994). Although such a mechanism would predict a linear force-velocity relation for the viscous assay, it fails to explain the similar curve that is obtained when an external force is applied.

Alternative models that include strain-dependent rates or more complicated correlations in the chemical cycles of the two heads could evidently be constructed to fit the available experimental data. For example, Peskin and Oster (1995) have recently advanced a model of kinesin in which the hydrolysis cycles of the two heads are coordinated. They propose that the motor progresses as a result of the hindmost head detaching from the microtubule more rapidly than the leading one, a feature that can conveniently be arranged if the biochemical rate constants are strain dependent. From a

theoretical standpoint, such models have the disadvantage that current motility data do not impose sufficient constraints on the functionals describing the strain dependencies. Although it is possible to choose a function that fits the data, there is currently little prospect of performing an experiment to test whether the biochemistry of real kinesin molecules depends on the strain in the supposed way.

SUGGESTED EXPERIMENTS

The experimentally measured force-velocity curve corresponds closely to the optimal performance that can be achieved by our minimally strain-dependent motor (optimal in the sense that the stalling force is maximized). Agreement with the experimental data constrains the values of the phenomenological parameters to lie in particular ranges: $\delta = 4-8$ nm, $\kappa = 0.4-1.0$ pN/nm, $l > 6$ nm. Several experiments can be conceived that would establish independent estimates of the values of these parameters, or test other predictions, thereby providing a more stringent test of the model.

Force-velocity relation

Current force-velocity data have been collected only in the positive quadrant (i.e., positive resisting force and positive translocation velocity). Using the external force assay, it would be possible to measure the velocity response to a force applied in the forward direction by observing a motor as it walks into an optical trap. Similarly, one could examine the behavior of a motor subjected to a resistive force greater in magnitude than F_{halt} by allowing the motor to walk out of a trap until it stalls and then suddenly increasing the trap power. Our model predicts that the behavior in these regimes depends principally on the nonlinearity of the springs (i.e., the value of the maximum extension l).

Elasticity of the kinesin molecule

Direct measurement and characterization of the elasticity of a kinesin molecule bound in rigor to a microtuble may be possible using current optical trap technology. This would provide independent estimates of the parameters κ and l.

Hydrolysis rate

At zero load, the velocity obtained in our model is $v_0 \approx 3.5$ nm per hydrolysis for the independent head model and $v_0 \approx 7$ nm per hydrolysis for the model with coordinated heads. Comparison with the experimentally measured kinesin velocity at zero load and saturating [ATP], $v_0 \approx 0.7$ μm/s, predicts that the hydrolysis rate is approximately 100 s^{-1} or 50 s^{-1} per kinesin head, depending on the model. Furthermore, the strain independence of the rate-limiting step implies that the hydrolysis rate should be load-independent. Although technically difficult to accomplish (Funatsu et al., 1995), a direct measurement of the hydrolysis rate, and particularly its strain dependence, would provide one of the most stringent tests of candidate models.

Duty ratio

In the model, we neglected the possibility that the two heads can be detached simultaneously. If the heads act independently, the probability that this occurs during a cycle is equal to the ratio of the average detachment and attachment times of a single head. Expressed in terms of the "duty ratio" f of the motor, the probability is $(1 - f)/f$. Our model supposes that the enzyme advances by holding on with one head while moving the other. If an external force is applied to the motor, it is reasonable to suppose that the molecule would be ripped off the filament in the event that both heads let go. Thus we expect that a motor molecule operating according to our independent head model would be pulled off the filament after mean time $t_{\text{cycle}} f/(1 - f)$. The failure of kinesin under external load has been observed experimentally—Svoboda and Block (1994) reported that the enzyme typically walked for approximately 1 s before falling off the microtubule. Comparison of this time and our prediction yield an estimate of the duty ratio, $f \approx 0.99$.

One-headed kinesin

We cannot readily predict the behavior of one-headed kinesin in the external force assay. It would depend critically on the length of time for which the head is detached, and this is not explicitly included in the model. Most likely, though, the kinesin molecule would diffuse away from the microtubule before rebinding, so that little or no motility would be observed (as indeed appears to be the case in a recent experimental investigation (Berliner et al., 1995) involving many single-headed kinesin molecules attached to a bead). Using the viscous load assay, however, the investigation of the force-velocity relationship of one-headed kinesin would be perfectly feasible. In this case, the kinesin is stuck to a surface and cannot move away, and because the microtubules diffuse only slowly, they always remain in range of the kinesin head. The predicted force-velocity relation is the same as that of the cooperative two-headed model shown in Fig. 6. Bearing in mind that the overall hydrolysis rate is halved, this leads to the following predictions about the comparative performance of one- and two-headed species in the viscous force assay: 1) independent head model: one-headed kinesin moves at the same zero-load velocity but supports half the maximum force; 2) coordinated head model: one-headed kinesin moves at half the zero-load velocity and supports the same maximum load. Experimental measurements on one-headed kinesin would be particularly valuable for testing the hypothesis that each head goes through its chemical cycle independently.

SUMMARY

To summarize, we have presented a simple theoretical model for the action of motor proteins in which the strain dependence of the enzyme kinetics and the cooperativity of the heads are both minimal. The behavior of the model agrees in many respects with recent experimental results for kinesin, although we caution that the existing experimental data do not provide enough constraints to exclude alternative strain-dependent models or models with more complicated correlations between the heads. The concurring features of our model and experimental data include

- dependencies of the velocity and ATPase rate on the ATP concentration and the number of motors (Leibler and Huse, 1993);
- linear force-velocity characteristics of an individual motor for both external and viscous loads (for a natural choice of parameters this agreement is quantitative);
- molecular stepping characteristics.
- The present model also makes predictions about the behavior of motor proteins in situations that have not yet been investigated in practice, and suggests a number of experiments that could be carried out to provide a more stringent test of its validity.

APPENDIX: SIMULATION PROCEDURE

Kinesin, subject to an external force, translocating along a microtubule

The assumption that the time scales for reaction and diffusion are well separated means that during each stage of the cycle the motor is equilibrated. The model may therefore be simulated using standard Monte Carlo procedures to calculate equilibrium probability distributions. The action of the motor is modeled as a series of five discrete events corresponding to 1) detachment, 2) diffusion, 3) reattachment, 4) conformational change of a head, and finally, 5) reequilibration of the motor. Each complete sequence counts as a single step of the simulation and models a single mechanochemical cycle of one of the heads. The five events are performed as follows:

1. *Head detachment.* One of the heads, chosen at random, is detached from the microtubule (transition B→C). Label the terminus of this head H1 and label the terminus of the head that remains bound H2. Label the point at which both springs are joined to the shaft J. Let z measure displacement along the microtubule.

2. *Diffusion.* Thermal diffusion of both the free head and the shaft, subject to the forces in the springs, is modeled by moving H1 and J according to a Monte Carlo algorithm. Trial moves of H1 through a small displacement Δz of randomly chosen sign are accepted according to the Metropolis probability

$$p = \begin{cases} 1, & \Delta E \le 0, \\ \exp(-\Delta E/kT), & \Delta E > 0, \end{cases} \qquad \text{(A.1)}$$

where $\Delta E = (f_1 - F)\Delta z$ is the energy change associated with the trial move. Here, $f_1 = f(z(\mathrm{H1}) - z(\mathrm{J}))$, given by Eqs. 1 and 2, is the tension in the spring that links H1 to J, and F is the external force pulling back on the shaft. We set $|\Delta z| = d/24$. Similarly, trial moves of J are accepted with probability given by Eq. A.1, with $\Delta E = (-f_1 - f_2 - F)\Delta z$, where $f_2 = f(z(\mathrm{H2}) - z(\mathrm{J}))$ is the tension in the spring that links H2 to J. Because Eq. A.1 obeys detailed balance, this procedure permits the determination of the equilibrium probability distribution of z(H1) over the entire range [z(H2) − 2l, z(H2) + 2l] allowed by the tether composed of the two springs linking H1 to H2.

3. *Head reattachment.* H1 is rebound to the microtubule at one of the binding sites [$z = md$, m integer] within the above range (transition C→A). According to the assumption that reattachment is reaction-limited, the choice of site is weighted by the relative equilibrium probability of H1 being located there.

4. *Conformational change of head.* The conformational change of the head is attempted. z(H1) is incremented by δ and the corresponding work done in stretching the spring, $W = \sum_0^\delta (f_1 - F)\Delta z$, is calculated. The conformational change is deemed to take place successfully (transition A→B) with probability $p_B = 1/(1 + \exp\{-(G_{A\to B} - W)/kT\})$. This is derived from Eq. 4, which indicates that the ratio of probabilities of the head being in states A and B is $p_A/p_B = \exp\{-(G_{A\to B} - W)/kT\}$. If the conformational change does not take place, the head detaches (transition C←A) and the algorithm returns to step 2.

5. *Reequilibration of motor.* The shaft is reequilibrated with respect to the new (fixed) locations of the heads by moving J diffusively as in event 2.

One full simulation step corresponds to duration t_{step} in real time, equal to the waiting time for one of the heads to detach. Consequently, t_{step} is a random variable with an exponential distribution and mean value $t_{cycle}/2$. The velocity of translocation of the enzyme may be estimated as

$$v = \langle z_{step}\rangle/\langle t_{step}\rangle = 2\langle z_{step}\rangle/t_{cycle}, \qquad \text{(A.2)}$$

where $\langle z_{step}\rangle$ is the measured mean displacement of the point J per simulation step.

Kinesin propelling a microtubule subject to a viscous drag

In this case the viscous drag on the microtubule impedes the reequilibration of the motor molecule, and to model correctly the physics of a dynamic simulation must be used. Events 1–4 are performed as above, with the exception that J is not moved during step 2, because the shaft is attached to the surface and cannot diffuse. Event 5 is replaced by event 5′ as follows:

5′. *Incomplete reequilibration of motor.* The microtubule diffuses for time t_{step} under the influence of Brownian impulses and the forces exerted by the kinesin springs. The microtubule's movement is modeled as a stochastic process in which the pair of kinesin heads H1 and H2 take (small) steps of fixed magnitude Δz with variable time step Δt and haul the microtubule along with them. The heads move in the positive sense with probability p_+ and in the negative sense with probability p_-. Let ζ be the longitudinal friction coefficient of the microtubule. Writing $t_0 = \zeta\Delta z^2/kT$ and $\epsilon = -(f_1 + f_2)\Delta z/kT$, where $f_1 = f(z(\mathrm{H1}) - z(\mathrm{J}))$ and $f_2 = f(z(\mathrm{H2}) - z(\mathrm{J}))$ are the tensions in the springs pulling on the mictotubule, the dynamics is correctly described by the choice

$$\Delta t = t_0 \tanh(\epsilon/2)/\epsilon \qquad \text{(A.3)}$$

$$p_\pm = 1/(1 + \exp(\mp\epsilon)) \qquad \text{(A.4)}$$

The stochastic process is continued until the total elapsed time $\Sigma\Delta t$ attains the duration t_{step} of the simulation step.

In the simulation of a motor propelling a microtubule, the effective viscous force acting on the microtubule $F_{visc} = \zeta v$ may be calculated from the estimate of the microtubule velocity v (Eq. A.2) provided by the simulation

$$F_{visc} = 2k_BT\langle z_{step}\rangle/\Delta z^2(t_0/t_{cycle}) \qquad \text{(A.5)}$$

Thus a range of viscous forces may be simulated simply by setting the ratio t_0/t_{cycle} to a range of different values.

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