Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11782–11786, December 1994
Biophysics

Fluctuation analysis of motor protein movement and single enzyme kinetics

(kinesin/microtubule/thermal ratchet/Markov process/optical tweezers)

KAREL SVOBODA*†‡, PARTHA P. MITRA§, AND STEVEN M. BLOCK*¶

*Rowland Institute for Science, 100 Edwin Land Boulevard, Cambridge, MA 02142; †Graduate Committee on Biophysics, Harvard University, Cambridge, MA 02138; §AT&T Bell Laboratories, 600 Mountain Avenue, Murray Hill, NJ 07074; and ¶Department of Molecular Biology, Princeton University, Princeton, NJ 08544

Communicated by Howard C. Berg, August 15, 1994 (received for review June 7, 1994)

ABSTRACT **We studied fluctuations in the displacement of silica beads driven by single molecules of the motor protein kinesin, moving under low mechanical loads at saturating ATP concentrations. The variance in position was significantly smaller than expected for the case of stepwise movement along a regular lattice of positions with exponentially distributed intervals. The small variance suggests that two or more sequential processes with comparable reaction rates dominate the biochemical cycle. The low value is inconsistent with certain recently proposed thermal ratchet models for motor movement as well as with scenarios where the hydrolysis of a single ATP molecule leads to a cluster of several steps. Fluctuation analysis is a potentially powerful tool for studying kinetic behavior whenever the output of a single enzyme can be monitored.**

Steady-state measurements yield only limited types of information about enzymatic reactions. Individual reaction steps are buried in lumped parameters, such as k_{cat}, the turnover rate at saturating substrate concentration, and K_m, the apparent Michaelis–Menten constant. To overcome this limitation, transient kinetics may be employed (1). However, time resolution can be limiting, and large quantities of purified enzyme are required. Recently, it has become possible to measure the output of individual proteins. The discrete currents passed by single membrane channels, for example, can be measured using patch-clamp techniques (2). The movement of single motor molecules can be followed *in vitro* using optical techniques (3–6). For the case of the kinesin-microtubule (MT) system, single molecules can be observed for extended periods of time (7–9). Inside cells, kinesin proteins shuttle organelles and vesicles toward the plus ends of MTs, for example, during anterograde axonal transport (10). *In vitro*, single kinesin molecules can pull small silica beads through distances of several micrometers (8), moving with 8-nm-sized steps (9), along straight paths parallel to single protofilaments of the MT lattice (11).

Knowledge of the distribution of time intervals for enzyme turnover can be used to place severe constraints on the underlying kinetic scheme. In the case of ion channels, direct construction of the distribution of open and shut intervals is often possible (12). This is due to a remarkable amplification scheme: open channels pass $\approx 10^6$ ions per second, dissipating energy equivalent to the hydrolysis of thousands of ATP molecules during each current pulse. In contrast, molecular motors such as kinesin or myosin consume only ≈ 1 ATP per step, and the resulting low signal-to-noise ratios make direct measurements of such distributions difficult (9). Here, we show how displacement fluctuations can provide information that is largely equivalent to the direct measurement of step interval distributions. In contrast to direct measurements, however, fluctuation analysis is considerably more forgiving with respect to noise. We then apply our analysis to kinesin movement along MTs.

METHODS

Bead Assay. Details of the preparation can be found in ref. 13. Kinesin was purified from squid optic lobe (10, 13) and MTs were prepared from bovine brain (14). Experiments were done at room temperature (22–23.5°C). The assay buffer contained 80 mM Pipes, 4 mM $MgCl_2$, 1 mM EGTA, 50 mM KCl, 1 mM dithiothreitol, 1 mg of filtered casein per ml, 20 μM taxol, 1 μg of phosphocreatine kinase per ml, 2 mM phosphocreatine, and 2 mM ATP (pH 6.9). For some experiments, the last three reagents were replaced by 1 mM 5′-adenylyl imidodiphosphate (AMP-PNP). Homogeneous silica beads (diameter = 0.52 μm, gift of E. Matijevic) were incubated with kinesin (13). The lower coverglass of the flow chambers was pretreated with 4-aminobutyldimethylmethoxysilane (Huls) to produce a surface that binds MTs. Taxol-stabilized MTs were bound to this surface, and kinesin-coated beads were introduced by flow.

Displacement Measurements. A diffusing bead was captured with optical tweezers and held against a MT for a few seconds. At the extremely low kinesin concentrations used, the fraction of beads that subsequently moved was <0.5. The probability that movement was caused by two or more motors was estimated at <2%; hence movement was probably due to single motors (13). Bead displacements up to 200 nm from the trap center were measured with subnanometer resolution (at 1 kHz) using optical trapping interferometry (9). At low loads, beads powered by single molecules generally moved through the trapping zone before spontaneously releasing from the MT substrate. Such beads were frequently recaptured after escaping from the trap, so that several 200-nm-long runs in succession along the MT could be measured for individual molecules. The trap acts like a spring of constant stiffness α_{tr} acting on the bead (15). As a kinesin motor draws the bead toward the edge of the trap, the load on the bead increases and the bead-to-MT linkage (the kinesin tether) becomes stretched: $\approx$16% of the motor movement is absorbed in this linkage compliance. Kinesin velocity was corrected for this stretching, as described (13). The optical loads used here were low ($\alpha_{tr} = 7.4 \pm 0.2 \times 10^{-3}$ pN/nm), such that motor velocity was independent of position in the trap.

Data Analysis. The data sampling interval was $\Delta = 1$ ms. We estimated the mean kinesin motor velocity, v, by fitting

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MT, microtubule; AMP-PNP, 5′-adenylyl imidodiphosphate.

‡To whom reprint requests should be addressed at the present address: Department of Biological Computation, AT&T Bell Laboratories, 600 Mountain Avenue, Murray Hill, NJ 07074.

straight lines to traces, $x(t)$, derived from single runs and averaging over all the runs of an experiment (35 runs for each of two experiments). The mean-square displacement deviation from the mean [the variance, var(t)] for each run of duration $l\Delta$ was calculated from $\text{var}(t = j\Delta) = \Sigma_{i=0}^{l-j}(x(j\Delta + i\Delta) - x(i\Delta) - vj\Delta)^2/(l - j + 1)$. We then averaged var($t$) over all runs of an experiment. For increasing values of $t = j\Delta$, a decreasing number of independent estimates of var(t) could be averaged, and therefore sampling errors increased for larger t (16) (Fig. 1*B*). To measure the randomness, r, we computed the slope of the variance using a linear fit computed over the time interval 3–50 ms. To estimate the significance of r, we computed the expected standard deviation of r, σ_r, for a simulated Poisson stepper ($\sigma_r = 0.17$, 20 steps per run, 70 runs) and then computed the probability, p, that the measured r is compatible with such a process. Since for non-Poissonian steppers σ_r is relatively smaller, the error estimate given here should be considered conservative.

THEORETICAL RESULTS

Kinesin movement has been shown to be almost entirely unidirectional and spatially regular under conditions of either high load or low ATP, where individual steps can be seen (9). Given the recent finding that kinesin moves along paths parallel to single protofilaments of the MT lattice (11), and attaches preferentially to β-subunits of tubulin dimers that are spaced every 8 nm along such protofilaments (17), it seems likely that kinesin movement remains spatially regular even under conditions of low load, where individual steps cannot be discerned. We assume, therefore, that fluctuations in displacement derive from randomness in the step intervals, and not from irregularities in the fundamental step size. This randomness arises from two independent sources: (*i*) fluctuations in the timing of the chemical cycles that produce steps and (*ii*) fluctuations due to the finite probability, p_s, that a completed cycle actually produces a step. We seek to derive an expression for randomness due to both of these contributions and compare it with our experimental data. Assume, for the moment, that every biochemical cycle leads to a single stepwise displacement (this assumption will be relaxed shortly). The displacement will then be given by

$$x(t) = dN(t) + \eta(t), \quad [1]$$

where d is the step size, $N(t)$ is an integer corresponding to the number of motor turnovers during time t, and $\eta(t)$ is the Brownian displacement of the bead, characterized by $\langle\eta\rangle = 0$ and $\langle\eta^2\rangle = kT/(\alpha_{tr} + \alpha_{mot})$, by the Equipartition Theorem (18), where α_{mot} is the stiffness of the bead-to-MT linkage and α_{tr} is the stiffness of the optical trap (13). $N(t)$ increments at stochastic times.

If a single biochemical step is rate-limiting, the distribution of turnover intervals, $\rho_0(\tau)$, will be exponential (19), $\rho_0(\tau) = \tau_0^{-1}\exp(-\tau/\tau_0)$, where $\tau_0 = 1/k_{cat}$ is the mean turnover interval. In this situation, the probability of turning over N times in time t is given by the Poisson distribution, $P(N, t) = ((t/\tau_0)^N/N!)\exp(-t/\tau_0)$. For such a "Poisson enzyme," the mean displacement is given by

$$\langle x(t)\rangle = (d/\tau_0)t \quad [2]$$

and the variance in the displacement is

$$\langle(x(t) - \langle x(t)\rangle)^2\rangle = (d^2/\tau_0)t + 2\langle\eta^2\rangle. \quad [3]$$

Both the mean displacement and its variance rise linearly with time. The ratio of the slope of the variance versus time to the mean velocity, d/τ_0, gives the step size, d. For a Poisson enzyme, determination of the mean velocity and the variance are therefore sufficient to compute the step size. We simulated Poisson enzymes on a computer, with $\tau_0 = 10$ ms and $d = 1$, in the presence of varying amounts of Gaussian white noise (Fig. 1*A*). Inspection of the traces shows how even in the relatively favorable case where the noise is comparable to the step size, i.e., where $\sqrt{\langle\eta^2\rangle} = 1$, the distribution of turnover intervals cannot be discerned directly. However, the variance analysis is comparatively robust: the ratio of the slope of the variance to the mean velocity is ≈ 1, equal to the step size, and is nearly independent of the noise level.

Simulations imply that for the purposes of fluctuation analysis, it is not critical to observe the displacement of a motor protein with an especially high signal-to-noise ratio. The reason for this is that the fluctuations for a nonstationary Markov process, such as motor stepping, grow without bound with time, whereas the measurement noise remains constant. The analysis is therefore analogous to determinations of diffusion coefficients from observations of Brownian motion with finite spatial resolution. In both cases, the microscopic fluctuations in the force that produce movement manifest themselves in macroscopic fluctuations in position.

More complicated reaction schemes than those involving a single rate-limiting step lead to nonexponential turnover interval distributions, $P_0(\tau)$. Although it is not feasible to compute explicitly the equivalent of the Poisson distribution (above) for the general case of $P_0(\tau)$, both the mean and variance for any such process can nevertheless be derived (see *Appendix*). In particular, consider the m-step sequential process

$$1 \xrightarrow{k_1} 2 \xrightarrow{k_2} \cdots \xrightarrow{k_{m-1}} m \xrightarrow{k_m} 1, \quad [4]$$

where each completed cycle of reactions is assumed to lead to one step. The average turnover time is $T_0 = 1/k_{cat} =$

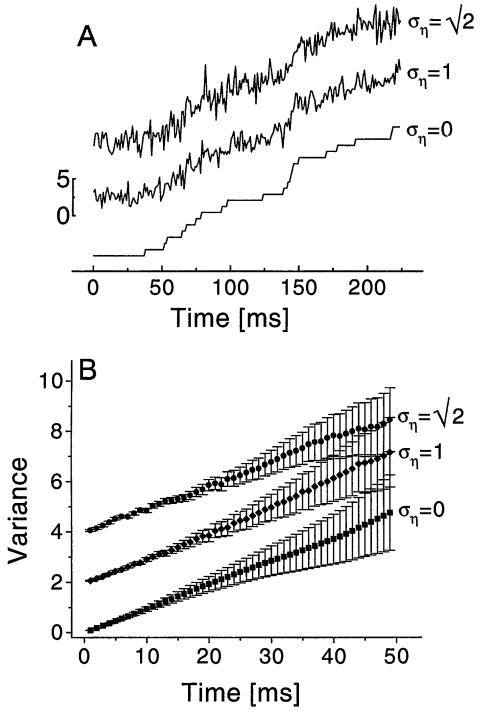

FIG. 1. Fluctuation analysis for the movement of simulated Poisson enzymes with Gaussian white noise, with mean step time τ_0 = 10 ms and step size $d = 1$. (*A*) Example of a single 20-step run subject to varying amounts of noise. (*B*) Displacement variance computed for three different noise levels, averaged over 35 separate runs. The y intercept gives $2\langle\eta^2\rangle$. Data are plotted as mean ± SEM.

$h\Sigma_{i=1}^{m} 1/k_i$ and the mean displacement is $\langle x(t)\rangle \approx (d/T_0)t$, with a variance

$$\langle (x(t) - \langle x(t)\rangle)^2\rangle \approx (d^2/T_0)t \sum_i T_i^2/T_0^2 + 2\langle \eta^2\rangle. \quad [5]$$

This expression is valid for $t \gg T_0$. For example, if $k_i = mk_{cat}$ for all i (i.e., when all the rate constants happen to be equal in Eq. **4**, hence $T_i = T_0/m$), the variance is reduced by a factor m^{-1} with respect to a Poisson enzyme. Thus, a two-step sequential process with comparable rates has precisely half the variance of a one-step process taking the same overall time. It can be shown that even in a cyclical scheme with reversible reactions, nonexponential step intervals arise only when two or more of the forward rates are rate limiting (the details of this derivation will be given elsewhere).

A convenient measure of step interval randomness, r, can be defined as

$$r = \frac{\langle (x(t) - \langle x(t)\rangle)^2\rangle - 2\langle \eta^2\rangle}{d\langle x\langle t\rangle\rangle}. \quad [6]$$

For the case of a Poisson enzyme, $r = 1$. For the scheme of Eq. **4**, $r = \Sigma T_i^2/T_0^2$, and therefore $r = 0.5$ for the two-step sequential process just considered. For the extreme case of completely regular movement, where the step interval distribution is described by a delta function (i.e., a molecular clock), $r = 0$. In principle, such a clock can be generated by an infinite sequence of reactions with finite overall turnover time, T_0, and any sufficiently large number of sequential steps with comparable rates will appear clock-like.

If, rather than leading to every step with certainty, each enzyme turnover produces a step with probability $p_s < 1$, one obtains

$$r = 1 - p_s(1 - \Sigma T_i^2/T_0^2) \quad [7]$$

for the scheme of Eq. **4** (see *Appendix*). Note that in the limit of small p_s, the enzyme spends most of its time cycling in a mechanically futile manner, and $r \approx 1$, independent of the particular distribution of stepping intervals.

EXPERIMENTAL RESULTS

We measured kinesin movement at a saturating (2 mM) ATP concentration and low mechanical load ($F < 1.5$ pN). Under these conditions, the thermal noise was too large and τ_0 was too short to measure step intervals directly (Fig. 2*A*). For quantitative analysis, we first characterized the Brownian noise of a bead in the absence of kinesin stepping, by tethering it to a MT via a kinesin molecule in the presence of the nonhydrolyzable ATP analog AMP-PNP, which produces a rigor-like linkage. The tethered bead was then driven through the detector zone of the interferometer at constant velocity (by moving the piezo stage carrying the experimental preparation) while the bead position was measured by interferometry. As expected for a tethered Brownian particle, the variance of the noise leveled off, and the correlation time was 1–2 ms (Fig. 2*B*). In contrast, the variance of bead movement driven by the ATP-dependent motion of a kinesin molecule increased linearly, with a slope of 2.79 nm^2/ms (computed over t = 3–50 ms). The mean kinesin velocity was 670 nm/s. Since d = 8 nm, Eq. **6** implies r = (2.79 nm^2/ms)/(670 nm/s × 8 nm) ≈ 0.52, significantly smaller than expected for a Poisson enzyme ($p < 0.004$).

Could this small value of r be due to an artifact? Most experimental sources of noise and error would be expected to increase the variance and hence r, not to decrease it. Possible sources of additional variance include (*i*) nonlinearities in the position detector and/or in the kinesin linkage compliance,

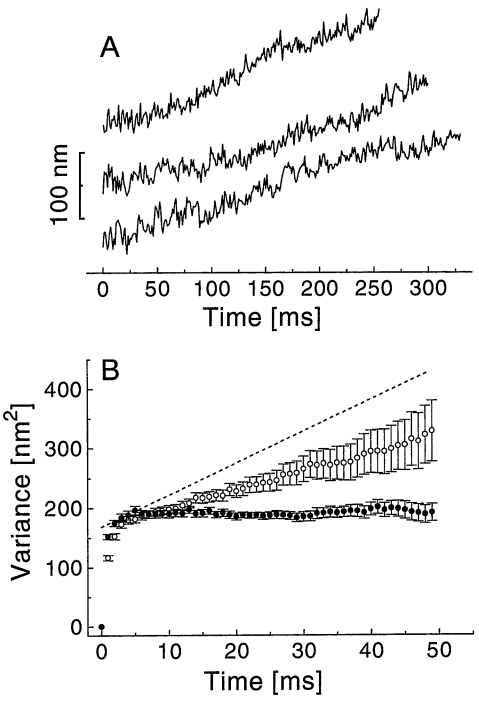

FIG. 2. Fluctuation analysis for kinesin movement at 2 mM ATP in the low-load regime. (*A*) Three examples of ≈20-step runs. (*B*) Displacement variance averaged over 35 runs for ATP-dependent kinesin movement (○); displacement variance averaged over 35 runs for a moving stage carrying beads tethered to MTs via kinesin motors immobilized with AMP-PNP (●); expected variance for a Poisson enzyme with mean speed v = 670 nm/s and step size d = 8 nm (- - - -).

both of which were assumed to be linear for this analysis, (*ii*) spatial heterogeneities in the MT lattice spacing (hence in the step size or step interval), (*iii*) heterogeneities in the kinesin population, leading to variable mean rates of advance, (*iv*) drift in the experimental conditions, and (*v*) rundown of the specimen. Since these factors, and other independent sources of noise, all make positive contributions to the variance, r = 0.52 probably represents an upper bound for the randomness of kinesin stepping. It is conceivable that two or more motors located on a single bead and coupled via elastic elements could generate motion characterized by a smaller effective step size and thereby a lower variance. However, given the exceedingly low kinesin concentrations used in these experiments and the fact that the probability for bead movement is well fit by a Poisson model, we consider this to be an unlikely explanation for the data (13). Equivalently, if single motors were to take smaller steps (<8 nm) under the conditions of these experiments, a lower variance could result.

DISCUSSION

Kinesin Kinetics. Analysis of motion driven by single kinesin molecules implies that the distribution of step intervals is nonexponential, because the variance is too low. The measured value r = 0.52 is consistent with a reaction scheme in which two or more sequential processes with comparable rates become limiting. Gilbert and Johnson (20) recently studied the transient kinetics of a recombinant, single-headed kinesin subfragment consisting of the presumptive motor domain and reported that ADP release is rate-limiting. Several explanations could reconcile their observations with ours. For example, it is possible that two (or more) kinetically distinct ADP-kinesin complexes with comparable lifetimes occur during the cycle. Alternatively, it may be that the

two-headed molecule used in our assays follows a different kinetic scheme from an isolated head, possibly because of coupling between the two motor domains. Given that kinesin molecules, like those of myosin, possess two heads, it is intriguing to speculate that the two kinetically distinct states suggested by these data reflect steps that are carried out in an obligatory sequence by each of the two motor domains, perhaps working "hand-over-hand." Certain classes of such models can lead to a reduced variance relative to that of a Poisson enzyme. However, not all two-headed mechanisms imply this property (21): some form of kinetic coupling between the heads must exist.

An interesting study suggested by these results will be to measure r as a function of ATP concentration. We predict that r should pass through a minimum when c is on the order of K_m, since the ATP binding step will be partially rate limiting. At limiting ATP, $c \ll K_m$, and displacement fluctuations reflect the ATP binding kinetics.

Relevance to Thermal Ratchet Models. Recently, a novel class of thermal ratchet models for molecular motors has been proposed (22–25). The motor protein is described as a particle moving in a periodic potential consisting of a series of asymmetric potential wells. The well depths fluctuate in time due to ATP hydrolysis, leading to a net particle flux in the direction toward the steeper part of the potentials. One allure of such models is that its only two essential elements are a periodic, locally asymmetric substrate and a time-varying binding energy, both of which seem plausible and are believed to be important for real biological motors. However, for the kinesin-MT system one such model predicts $p_s < 0.02$ (23). From Eq. 7, it follows that $r > 1 - p_s > 0.98$, independent of details of the biochemistry, which is close to unity and incompatible with our experimental estimate. A successful thermal ratchet model would have to take steps with much greater certitude in order to accommodate the low randomness of actual movement.

Estimate of the Stepping Probability, p_s. Can variance measurements be used to place a lower bound on p_s? The variation in stepping intervals arises from two distinct contributions (Eq. 7): the stochastic nature of the hydrolysis cycle itself (Eq. 5) and randomness in the efficiency of mechanochemical coupling, parametrized here by p_s. Assuming that the hydrolysis cycle were entirely clock-like (and thereby setting the stochastic contribution of all biochemistry to zero), then $\Sigma T_i^2/T_0^2 = 0$, and $p_s = (1 - r) = 0.48$. Any additional contributions from random biochemistry would therefore cause p_s to exceed this value. We conclude from the measured value of r that somewhere between one and two turnovers are required for a successful step, consistent with one:one (hydrolysis-to-step) coupling at low loads. Recent measurements of single-molecule force–velocity curves at both limiting and saturating ATP concentrations suggested that the load-dependent diminution in kinesin velocity might be due to a concomitant decrease in the stepping probability (13). The current findings suggest an independent way of checking this suggestion experimentally, since Eq. 7 predicts that the randomness, r, should approach unity as load is increased.

One:Many Coupling. A number of solution measurements of k_{cat} have been completed on native kinesin molecules, but typical values ($\approx 3\ s^{-1}$) (26) have not been consistent with the measured kinesin speed, $v \approx 670$ nm/s, a step size of $d = 8$ nm, and one:one coupling, since v/d should then equal k_{cat}. Perhaps the simplest explanation of the discrepancy (by a factor of ≈ 20) is that values obtained with the native molecule in solution are low due to the fact that the native molecule is in an inhibited state (21). Alternatively, it has been suggested that one ATP hydrolysis might generate a rapid series of n steps, where $n \gg 1$, so-called one:many coupling (27, 28). The smallness of the observed variance in kinesin motion argues strongly against this one:many coupling hypothesis. For example, let the cycle turnover time be τ_0, the duration of a step cluster be $\tau_c \ll \tau_0$, and hydrolysis intervals be exponentially distributed: this amounts to a Poisson enzyme with effective step size nd. Eqs. 2, 3, and 6 then imply $r \approx n \gg 1$, much larger than observed (note that stepping events no longer define a simple Markov process here; hence $r > 1$ is allowed).

Concluding Remarks. Fluctuation analysis is applicable whenever the output of a single enzyme can be measured. In its favor, the method is relatively forgiving with respect to signal-to-noise ratio and can be applied in situations where single enzyme turnovers cannot be resolved. Even when such events can be discerned, fluctuation analysis has some advantages. The construction of turnover interval distributions from noisy, finite-bandwidth data suffers intrinsically from the missing-event problem (29, 30), an issue that does not arise in fluctuation analysis. A drawback of the approach is that the interpretation of fluctuations is somewhat model-dependent. We anticipate that statistical analysis of variations in motor protein displacements will yield further insights into mechanochemistry.

APPENDIX

We seek to calculate the displacement variance for an enzyme with an arbitrary turnover interval distribution, $P_0(\tau)$. The probability that the enzyme has not completed its cycle a time τ after the completion of the preceding cycle is $\Phi_0(\tau) = \int_\tau^\infty P_0(\tau')d\tau'$. Let $P(N, t)$ be the probability of turning over exactly N times in time t. $P(N, t)$ can be expressed as the sum of the probabilities for the first turnover occurring in the interval $(\tau, \tau + \delta\tau)$ and the remaining $N - 1$ steps occurring in the remaining time $t - \tau$:

$$P(N, t) = \int_0^t d\tau P_0(\tau) P(N - 1, t - \tau), \qquad [8]$$

which has the Laplace transform

$$\tilde{P}(N, s) = \tilde{P}_0(s)\tilde{P}_0(N - 1, s). \qquad [9]$$

Noting that $P(0, t) = \Phi_0(t)$, we solve the recursion relation (Eq. 9) to obtain

$$\tilde{P}(N, s) = \tilde{P}_0^N(s)\tilde{\Phi}_0(s). \qquad [10]$$

The probability moment-generating function is defined as $G(\mu, t) = \Sigma_{i=0}^\infty \exp(\mu i)P(i, t)$, with the property

$$\langle N^k(t)\rangle = \left.\frac{\partial^k G}{\partial \mu^k}\right|_{\mu=0} \qquad [11]$$

In the Laplace transform domain, Eq. 8 can be summed to give

$$\tilde{G}(\mu, s) = \frac{1}{s}\frac{(1 - P_0(s))}{(1 - \exp(\mu)P_0(s))}, \qquad [12]$$

and therefore

$$\langle \tilde{N}(s)\rangle = \frac{1}{s}\left(\frac{P_0(s)}{1 - P_0(s)}\right) \qquad [13]$$

and

$$\langle \tilde{N}^2(s)\rangle = \frac{2}{s}\left(\frac{P_0(s)}{1 - P_0(s)}\right)^2 + \langle \tilde{N}(s)\rangle. \qquad [14]$$

For the multistep reaction scheme of Eq. **4**, $P_0(\tau)$ is the m-fold convolution of exponential distributions, $P_{T_i}(\tau) = T_i^{-1}\exp(-\tau/T_i)$, where $k_i^{-1} = T_i$, with Laplace transforms $\tilde{P}_{T_i}(s) = (T_i s + 1)^{-1}$. Therefore $\tilde{P}_0(s) = \Pi_{i=1}^{m}(T_i s + 1)^{-1}$. Computing $\langle\tilde{N}(s)\rangle$ and $\langle\tilde{N}^2(s)\rangle$ for small s (corresponding to large t) and transforming back into the time domain gives $\langle N(t)\rangle \approx t/T_0$, and for the variance $\langle(N(t) - \langle N(t)\rangle)^2\rangle \approx (t/T_0)\Sigma T_i^2/T_0^2$, which leads to Eqs. **5** and **7**.

Relaxing the requirement that each enzyme turnover must lead to a step, we now assume that each turnover leads to a step with some probability, p_s. For a given number of enzyme turnovers in time t, $N(t)$, the number of steps taken, $N_m(t)$, is binomially distributed with mean $p_s N(t)$ and variance $N(t)p_s(1 - p_s)$. Taking the fluctuations of $N(t)$ into account, we obtain $\langle N_m(t)\rangle = p_s\langle N(t)\rangle$ and $\langle N_m(t)^2\rangle = \langle N(t)^2\rangle p_s^2 + \langle N(t)\rangle p_s(1 - p_s)$. The variance of $N_m(t)$ is then

$$\langle(N_m(t) - \langle N_m(t)\rangle)^2\rangle = p_s t/T_0(1 - p_s(1 - \Sigma T_i^2/T_0^2)), \quad [15]$$

which leads directly to Eq. **7**.

Note Added in Proof. We have recently become aware of a proposal by Susan Gilbert, Kenneth Johnson, and colleagues, designed to reconcile their data on turnover rates of the kinesin biochemical cycle determined by pre-steady-state and steady-state kinetics. In their proposal, the two heads of the molecule are coordinated so as to dissociate sequentially from the microtubule, with each head release being a rate-limiting step (S. Gilbert and K. Johnson, personal communication). This form of two-step sequential reaction is sufficient to produce a diminished variance consistent with the physiology we report here.

We thank Howard Berg, Bert Halperin, George Oster, and Aravi Samuel for helpful discussions, Christoph Schmidt, Bruce Schnapp, and Russell Stewart for help with motility assays, and Egon Matijevic for the gift of uniform silica beads. This work was supported by the Rowland Institute for Science and a grant from the National Institute of General Medical Sciences to S.M.B.

1. Johnson, K. A. (1992) in *The Enzymes*, ed. Sigman, D. (Academic, New York), pp. 2–60.
2. Sakmann, B. & Neher, E. (1983) *Single-Channel Recording* (Plenum, New York).
3. Block, S. M. & Berg, H. C. (1984) *Nature (London)* **309,** 470–472.
4. Schafer, D. A., Gelles, J., Sheetz, M. P. & Landick, R. (1991) *Nature (London)* **352,** 444–448.
5. Kabata, H., Kurosawa, U., Arai, I., Washizu, M., Margarson, S. A., Glass, R. E. & Shimamoto, N. (1993) *Science* **262,** 1561–1563.
6. Finer, J. T., Simmons, R. M. & Spudich, J. A. (1994) *Nature (London)* **368,** 113–119.
7. Howard, J., Hudspeth, A. J. & Vale, R. D. (1989) *Nature (London)* **342,** 154–158.
8. Block, S. M., Goldstein, L. S. B. & Schnapp, B. J. (1990) *Nature (London)* **348,** 348–352.
9. Svoboda, K., Schmidt, C. F., Schnapp, B. J. & Block, S. M. (1993) *Nature (London)* **365,** 721–727.
10. Vale, R. D., Reese, T. S. & Sheetz, M. P. (1985) *Cell* **42,** 39–50.
11. Ray, S., Meyhofer, E., Milligan, R. A. & Howard, J. (1993) *J. Cell Biol.* **121,** 1083–1093.
12. Colquhoun, D. & Sigworth, F. J. (1983) in *Single-Channel Recording*, eds. Sakmann, B. & Neher, E. (Plenum, New York), pp. 191–263.
13. Svoboda, K. & Block, S. M. (1994) *Cell* **77,** 773–784.
14. Mitchison, T. & Kirschner, M. (1984) *Nature (London)* **312,** 232–237.
15. Svoboda, K. & Block, S. M. (1994) *Ann. Rev. Biophys. Biomol. Struct.* **23,** 247–285.
16. Qian, H., Sheetz, M. P. & Elson, E. L. (1991) *Biophys. J.* **60,** 910–921.
17. Song, Y. H. & Mandelkow, E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1671–1675.
18. Reif, F. (1965) *Fundamentals of Statistical and Thermal Physics* (McGraw–Hill, New York), p. 249.
19. Kramers, H. A. (1940) *Physica* **7,** 284–304.
20. Gilbert, S. P. & Johnson, K. A. (1994) *Biochemistry* **33,** 1951–1960.
21. Hackney, D. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6865–6869.
22. Magnasco, M. O. (1993) *Phys. Rev. Lett.* **71,** 1477–1481.
23. Astumian, R. D. & Bier, M. (1994) *Phys. Rev. Lett.* **72,** 1766–1769.
24. Prost, J., Chauwin, J. F., Peliti, L. & Ajdari, A. (1994) *Phys. Rev. Lett.* **72,** 2652–2655.
25. Peskin, C. S., Ermentraut, G. B. & Oster, G. F. (1994) in *Cell Mechanics and Cellular Engineering*, eds. Mow, V., Guilak, R., Tran-Son-Tay, R. & Hochmuth, R. (Springer, New York).
26. Hackney, D. D., Levitt, J. D. & Wagner, D. D. (1991) *Biochem. Biophys. Res. Commun.* **174,** 810–815.
27. Yanagida, T., Harada, Y. & Ishijima, A. (1993) *Trends Biochem. Sci.* **18,** 319–324.
28. Taylor, E. W. (1993) *Nature (London)* **361,** 115–116.
29. Draber, S. & Schultze, R. (1994) *Biophys. J.* **66,** 191–201.
30. Hawkes, A. G., Jalali, A. & Colquhoun, D. (1992) *Philos. Trans. R. Soc. London B* **337,** 383–404.