Understanding mechanochemical coupling in kinesins using first-passage-time processes

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(Received 29 June 2004; published 8 March 2005)

Kinesins are processive motor proteins that move along microtubules in a stepwise manner, and their motion is powered by the hydrolysis of ATP. Recent experiments have investigated the coupling between the individual steps of single kinesin molecules and ATP hydrolysis, taking explicitly into account forward steps, backward steps, and detachments. A theoretical study of mechanochemical coupling in kinesins, which extends the approach used successfully to describe the dynamics of motor proteins, is presented. The possibility of irreversible detachments of kinesins from the microtubules is explicitly taken into account. Using the method of first-passage times, experimental data on the mechanochemical coupling in kinesins are fully described using the simplest two-state model. It is shown that the dwell times for the kinesin to move one step forward or backward, or to dissociate irreversibly, are the same, although the probabilities of these events are different. It is concluded that the current theoretical view—that only the forward motion of the motor protein molecule is coupled to ATP hydrolysis—is consistent with all available experimental observations for kinesins.

DOI: 10.1103/PhysRevE.71.031902 PACS number(s): 87.16.Nn, 05.40.−a, 82.37.Rs

I. INTRODUCTION

There are several classes of enzymes, called molecular motor proteins, that are critical for many biological processes, but especially they are important for cellular transport and motility, cell division, and transfer of genetic information [1–3]. The motor proteins, such as kinesins, myosins, and DNA and RNA polymerases, move in a stepwise motion along rigid molecular tracks (microtubules, actin filaments, and DNA molecules). The motion of motor proteins is fueled by the hydrolysis of ATP or related compounds. However, the exact mechanism of the coupling between the chemical energy of hydrolysis and the mechanical motion of motor proteins is still unknown, and it remains one of the most important problems in biology.

Kinesins provide the most convenient system to investigate the mechanochemical coupling in motor proteins since biophysical, chemical, and mechanical properties of these molecules are now well studied at the single-molecule level [4–13]. Conventional kinesins are dimeric two-headed molecules, which hydrolyze ATP and move stochastically in 8.2-nm steps along the microtubules. These motor proteins can make hundreds of steps before dissociating from the microtubules, and they can be processive even against the opposing load as high as 7–8 pN [8–10]. Kinesins move preferentially in the forward direction (plus end of microtubules); however, at high loads the frequency of backward steps (in the direction of minus end of the microtubule) is increasing [11].

In order to understand how the motor proteins function, it is important to investigate how the chemical energy of ATP hydrolysis is transformed into the mechanical motion of proteins. To approach this fundamental problem, first, several critical questions should be answered: (1) How many ATP molecules consumed for each kinesin's step? (2) Are ATP molecules hydrolyzed for any step, forward or backward? (3) Is there a futile hydrolysis in kinesin motion—i.e., ATP consumption without actual moving of the motor protein?

In recent experiments [11], the mechanism of mechanochemical coupling in motor proteins has been studied by correlating the forward and backward movements of single kinesin molecules to the hydrolysis of ATP. Using an optical trapping nanometry system, the time trajectories of single kinesin molecules have been measured for different external forces and for different ATP concentrations. It was found that the dwell times before the forward and backward steps are the same at all external forces and at all ATP concentrations. A biased Brownian motion model with asymmetric potentials was developed to explain the bidirectional motions of kinesins. Based on this model, it was concluded that the hydrolysis of single ATP molecules is coupled to either forward or backward steps of kinesins.

Although the theoretical picture presented in Ref. [11]—that both forward and backward steps of kinesins are created by the same mechanochemical transduction mechanism—seems to be able to describe several features of the kinesin motility, there are serious fundamental problems with this view. It contradicts the current biochemical view of this process and earlier studies [6,14,15] that show a tight mechanochemical coupling; i.e., one ATP molecule is hydrolyzed per each forward 8-nm step. According to the theoretical model of Nishiyama *et al.* [11], one ATP molecule is hydrolyzed when the motor protein moves one step forward or backward. Note, however, that earlier experimental investigations [6,14,15] mainly neglected the backward steps in their statistical analysis. In addition, the asymmetric potential used in the biased Brownian motion model breaks the periodic symmetry of the system, and it violates the principle of microscopic reversibility since the backward processes are not taken into account. Furthermore, this model fails to account for irreversible detachments of kinesin molecules from the microtubules, which are observed in experiments.

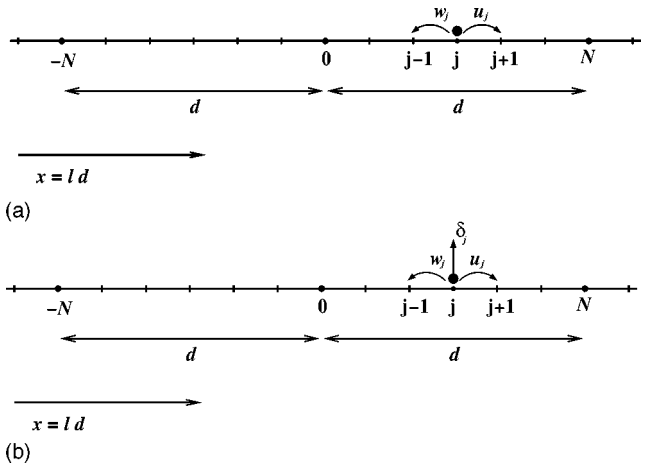

FIG. 1. (a) General schematic view of periodic multistate stochastic models. A motor protein particle in state j can make a forward transition at rate u_j, or it can undertake a backward transition at the rate w_j. The states $j=\ldots,-N,0,N,\ldots$ correspond to the strongly bound states. (b) General scheme of periodic multistate stochastic models with irreversible detachments. The particle in state j can dissociate with a rate δ_j.

Clearly, a better quantitative theoretical description, which does not violate the basic physical and chemical principles, is needed in order to satisfactorily understand the mechanochemical coupling in kinesins. The aim of this article is to discuss in detail such a theoretical approach.

We present a theoretical analysis of mechanochemical coupling and dynamics of kinesin molecules which utilizes the first-passage-time processes [16] in periodic discrete-state stochastic models. This is an extension of a recently developed approach [17–23], which has been used successfully to analyze in detail the dynamics of single conventional kinesin molecules [24] and myosin-V [25]. We argue that the experimental observations by Nishiyama *et al.* [11] can be described by the simplest $(N=2)$-state model with irreversible detachments, in which ATP hydrolysis is tightly coupled only to the forward steps of motor proteins. Also note that the dynamics of motor proteins has been studied theoretically using other methods and approaches [3,26–28].

II. THEORETICAL APPROACH

A. Chemical kinetic models

Our approach is based on using multistate discrete stochastic, or chemical kinetic, models. The main assumption of the simplest periodic sequential chemical kinetic model, which is shown in Fig. 1(a), is that a motor protein molecule is viewed as a particle that moves along a periodic linear track from one binding site to the next one through the sequence of N biochemical conformations. The particle in state j can jump forward to state $j+1$ with the rate u_j, or it can slide one step backward to the site $j-1$ with the rate w_j. After moving N sites forward the motor protein comes to the same biochemical state but shifted by a step size distance d. For kinesins this distance is 8.2 nm, and it is equal to the size of a tubulin subunit in microtubules [3]. The states $j=lN$ $(l=0,\pm1,\pm2,\ldots)$ represent the biochemical conformations where the motor protein molecule is tightly bound to the track—i.e., to the microtubule in case of kinesins—and without the ATP fuel molecule. ATP binding corresponds to the transitions from states $j=lN$ to $j=1+lN$, while other forward transitions describe the ATP hydrolysis and subsequent release of hydrolysis products. It is important to note that, although the motor protein moves preferentially in one direction, the reverse transitions cannot be ignored in any reasonable model of motor protein motility, and the backward steps are frequently observed experimentally at certain conditions (high loads) [6,11].

In the periodic sequential multistate stochastic model the dynamics of the motor protein can be viewed as the motion of the particle on a periodic one-dimensional lattice (with a period N). This observation allows one to derive an explicit analytical expressions for the mean velocity $V(\{u_j,w_j\})$,

$$V=\lim_{t\to\infty}\frac{d\langle x(t)\rangle}{dt}, \tag{1}$$

in terms of transition rates $\{u_j,w_j\}$ for any value of N [19,20]. Here, $x(t)$ measures the position of the single molecule on the linear track. Specifically, the mean velocity is given by [21]

$$V=d\frac{1-\prod_{j=0}^{N-1}(w_j/u_j)}{R_N}=d(u_{eff}-w_{eff}), \tag{2}$$

where the effective forward and backward rates are defined as

$$u_{eff}=1/R_N,\ w_{eff}=\frac{\prod_{j=0}^{N-1}(w_j/u_j)}{R_N}, \tag{3}$$

with

$$R_N=\sum_{j=0}^{N-1}r_j,\ r_j=\frac{1}{u_j}\left(1+\sum_{k=1}^{N-1}\prod_{i=j+1}^{j+k}w_i/u_i\right). \tag{4}$$

Note also the periodicity of transition rates—i.e., $u_{j\pm N}=u_j$ and $w_{j\pm N}=w_j$.

Similar arguments can also be applied to obtain closed-form exact analytic formulas for the dispersion $D(\{u_j,w_j\})$ (or the effective diffusion constant) of the motion, which is defined as follows:

$$D=\frac{1}{2}\lim_{t\to\infty}\frac{d}{dt}[\langle x^2(t)\rangle-\langle x(t)\rangle^2]. \tag{5}$$

The simultaneous knowledge of both the velocity V and the dispersion D determines the bounds on rate-limiting biochemical transitions and thus provides a valuable information about the mechanism of motor proteins motility [8,21,24].

One of the advantages of using chemical kinetic models to describe the processivity of motor proteins is the ability to easily incorporate the effect of external force F on their dy-

namics [19,20,24]. This can be done with the introduction of load-distribution factors θ_j^+ and θ_j^- (for $j=0,1,\ldots,N-1$), which modify the transition rates in the following way:

$$u_j \Rightarrow u_j(F) = u_j^0 \exp(-\theta_j^+ Fd/k_BT),$$

$$w_j \Rightarrow w_j(F) = w_j^0 \exp(\theta_j^- Fd/k_BT). \tag{6}$$

This is a consequence of the fact that the external load F modifies the activation barriers for forward and backward transitions, and the load-distribution factors reflect how they changed. It is also reasonable to assume that

$$\sum_{j=0}^{N-1} (\theta_j^+ + \theta_j^-) = 1, \tag{7}$$

since the motor protein, making a step d against an external force F and going through N intermediate steps, produces a work equal to Fd. A force at which the motor protein stops moving is called a stall force.

1. First-passage time processes

In many single-molecule experiments on motor proteins the fractions of forward and backward steps and dwell times between the consecutive events are measured [11,12,29]. In terms of chemical kinetic models discussed above, these experimental quantities can be associated with the so-called splitting probabilities and conditional mean first-passage times, correspondingly. First-passage processes for sequential multistate stochastic models are well studied [16,30], and thus the available results can be easily adopted for the description of motor proteins dynamics.

Consider a motor protein particle in state j, as shown in Fig. 1(a). Recall that the sites $-N$, 0, and N correspond to the binding sites for motor proteins. Now let us define $\pi_{j,N}(\{u_j,w_j\})$ as the probability that the particle starting from state j will reach the site N, before backtracking to the previous binding site $-N$. Similarly, we can define $\pi_{j,-N}(\{u_j,w_j\})$ as the probability for the particle to advance to state $-N$ for the first time before reaching the forward binding site N. These quantities are called the splitting probabilities [16]. We are mainly interested in the case of $j=0$, since the probabilities $\pi_{0,\pm N}$ give us the forward and backward fractions of stepping for the motor protein particle. The explicit expressions for splitting probabilities are known [16], and for the periodic N-state stochastic models we obtain a simple relation

$$\pi_{0,N} = 1 - \pi_{0,-N} = \frac{1}{1 + \prod_{j=0}^{N-1} (w_j/u_j)}. \tag{8}$$

In a similar fashion, we can define the conditional mean first-passage times $\tau_{j,\pm N}(\{u_j,w_j\})$, which represent the average time the particle, that starts at site j, spends before advancing forward or backward to sites $\pm N$, correspondingly. Then it is easy to conclude that the dwell times for the forward steps of motor proteins correspond to $\tau_{0,N}$, while the dwell times for the backward steps are given by $\tau_{0,-N}$. The explicit expressions for the dwell times within the periodic N-state chemical kinetic model can be derived from more general equations that are not restricted by periodicity conditions (see Pury and Caceres [30]), yielding

$$\tau_{0,N} = \frac{\pi_{0,N}}{u_{eff}}, \quad \tau_{0,-N} = \frac{\pi_{0,-N}}{w_{eff}}, \tag{9}$$

where the effective transition rates u_{eff} and w_{eff} are defined in Eqs. (3) and (4). Then applying the Eqs. (3) and taking into account the relations for the forward and backward fractions [see Eq. (8)], we conclude that

$$\tau_{0,N} = \tau_{0,-N}. \tag{10}$$

This is a specific but very important result derived from the general calculations of mean first-passage times for a single particle on a lattice [30] applied for periodic systems that describe the motion of motor proteins along the molecular tracks. It indicates that the dwell times for the forward and backward steps are *always* equal to each other for *any* set of transition rates, although the probabilities of these steps may differ significantly. It is also important to note that periodic conditions in the system are crucial for this conclusion.

B. Effect of detachments

Motor proteins do not always stay bounded to the linear track; they can dissociate and diffuse away. For kinesins moving along the microtubules the effectively irreversible detachments have been observed experimentally [9,11]. Theoretically, the effect of detachments on the drift velocity, dispersion, and stall force has been investigated [21] using an extension of the simplest sequential multistate stochastic model. However, to the best of our knowledge, the problem of how the motor protein dissociations change the mean first-passage-time processes—namely, the fractions and mean dwell times of forward and backward steps—has not been studied at all. Below we outline how this effect can be solved by mapping it onto another sequential multistate stochastic model but *without* detachments, for which the results are already known.

Consider a motor protein particle in state j as shown in Fig. 1(b). It can move forward (backward) with the rate u_j (w_j), or it can dissociate irreversibly with the rate δ_j. We again define $\pi_{j,N}$ and $\pi_{j,-N}$ as splitting probabilities of reaching for the first time the forward (at N) or the backward (at $-N$) binding site. In addition, we introduce a new function $\pi_{j,\delta}$ as a probability for the motor protein, which starts at the site j, to detach before reaching the forward or the backward binding states. These probabilities are related through the normalization condition

$$\pi_{j,N} + \pi_{j,-N} + \pi_{j,\delta} = 1. \tag{11}$$

Now we may recall that the particle at the site j has to jump to the site $j+1$ or $j-1$, or it will detach. These jumps have the probabilities $u_j/(u_j+w_j+\delta_j)$, $w_j/(u_j+w_j+\delta_j)$, and $\delta_j/(u_j+w_j+\delta_j)$, correspondingly. Then the expression for the forward splitting probability is given by [16]

$$\pi_{j,N} = \frac{u_j}{(u_j + w_j + \delta_j)} \pi_{j+1,N} + \frac{w_j}{(u_j + w_j + \delta_j)} \pi_{j-1,N}, \quad (12)$$

for any $-N < j < N$ and with the obvious choice of boundary conditions

$$\pi_{N,N} = 1, \quad \pi_{N,-N} = 0. \quad (13)$$

Similar equations can be derived for the backward splitting probabilities $\pi_{j,-N}$.

Equation (12) can be easily rewritten as a difference equation—i.e.,

$$u_j \pi_{j+1,N} + w_j \pi_{j-1,N} - (u_j + w_j + \delta_j) \pi_{j,N} = 0. \quad (14)$$

Assume that the solution of this equation can be presented as

$$\pi_{j,N} = \phi_j \pi^*_{j,N}, \quad (15)$$

where the function $\pi^*_{j,N}$ is the splitting forward probability for a new system without detachments and the auxiliary function ϕ_j is yet to be determined. Substituting Eq. (15) into the Eq. (14) we obtain

$$u_j \phi_{j+1} \pi^*_{j+1,N} + w_j \phi_{j-1} \pi^*_{j-1,N} - (u_j + w_j + \delta_j) \phi_j \pi^*_{j,N} = 0. \quad (16)$$

If we define new rates for the stepping process without detachments as

$$u^*_j = u_j \phi_{j+1}, \quad w^*_j = w_j \phi_{j-1}, \quad (17)$$

and also require that

$$u^*_j + w^*_j = u_j \phi_{j+1} + w_j \phi_{j-1} = (u_j + w_j + \delta_j) \phi_j, \quad (18)$$

then Eq. (16) is easily transformed into

$$u^*_j \pi^*_{j+1,N} + w^*_j \pi^*_{j-1,N} - (u^*_j + w^*_j) \pi^*_{j,N} = 0, \quad (19)$$

with the boundary conditions $\pi^*_{N,N} = 1$ and $\pi^*_{-N,N} = 0$. These boundary conditions also mean that $\phi_{-N} = \phi_N = 1$. Examining Eq. (19), one can observe that this is the expression to determine the forward splitting probability of the sequential multistate stochastic process with rates $\{u^*_j, w^*_j\}$ but without detachments, for which solutions are available [16]. It leads to an explicit equation for the forward splitting probability. Similar arguments can be developed for the backward splitting probabilities.

Our analysis relies on the ability to compute the functions ϕ_j, which can be accomplished by utilizing the Eq. (18). However, it is more convenient to look at ϕ_j as elements of the left eigenvector of a $(2N+1) \times (2N+1)$ matrix **M**, for which the nonzero elements are given by

$$M_{ij} = \begin{cases} -(u_j + w_j + \delta_j), & \text{for } i = j, \\ w_j, & \text{for } i = j - 1, \\ u_j, & \text{for } i = j + 1, \end{cases} \quad (20)$$

with $-N < i, j < N$.

The effect of detachments for conditional mean first-passage times can be investigated in a similar way. Here we again define $\tau_{j,N}$ ($\tau_{j,-N}$) as the mean time to reach the forward (backward) binding state N ($-N$) for the first time. In addition, we define $\tau_{j,\delta}$ as a mean first-passage time for the motor protein particle to dissociate from the molecular track before reaching the forward or backward binding sites $\pm N$, provided that it started from the state j. The mean first-passage times can be found by solving the backward master equation (see [30])

$$u_j \tau_{j+1,N} + w_j \tau_{j-1,N} - (u_j + w_j + \delta_{j,N}) \tau_{j,N} = -1, \quad (21)$$

with the boundary conditions $\tau_{\pm N,N} = 0$. Again, looking for a solution in the form $\tau_{j,N} = \phi_j \tau^*_{j,N}$ and using Eqs. (17) and (18), we obtain the expression

$$u^*_j \tau^*_{j+1,N} + w^*_j \tau^*_{j-1,N} - (u^*_j + w^*_j) \tau^*_{j,N} = -1, \quad (22)$$

which determines the forward mean first-passage time for the system without detachments. Because exact solutions for this case are available [16,30], expressions for the mean first-passage times for the system with detachments can beeasily obtained.

The general equations for splitting probabilities and mean first-passage times are quite complex, and we present in the next subsection the expressions only for the simplest cases $N=1$ and $N=2$. However, it can be shown generally, using the explicit mathematical formalism presented in Ref. [30], that for any N the calculations of the mean dwell times to move forward, backward, or to dissociate lead to the important relation

$$\tau_{0,N} = \tau_{0,-N} = \tau_{0,\delta}. \quad (23)$$

This is one the main result of our theoretical analysis. It can be understood in the following way. The motion of the motor protein along the molecular track, which takes place through the sequence of biochemical transitions, can be viewed as a Markov process. The average lifetime of the state when the motor protein is bounded to the track does not depend on the direction of where the particle will go after this state—forward, backward, or to detach irreversibly. Only the probabilities to move forward, backward, or to dissociate irreversibly are different. This is because there is no memory in the Markov processes [16]. Equation (23) expresses this statement in a mathematic form.

C. Results for $N=1$ and $N=2$ models

To illustrate our method, let us consider two simple cases: $N=1$ and $N=2$ periodic sequential stochastic models with detachments. When the period of the system is $N=1$, the auxiliary function ϕ_0 can be easily calculated,

$$\phi_0 = \frac{u + w}{u + w + \delta}, \quad (24)$$

and also recall that $\phi_{-1} = \phi_1 = 1$. This leads to the simple relations for the splitting probabilities,

$$\pi_{0,1} = u/(u + w + \delta), \quad \pi_{0,-1} = w/(u + w + \delta), \quad \pi_{0,\delta} = \delta/(u + w + \delta), \quad (25)$$

and for the mean first-passage times,

$$\tau_{0,1} = \tau_{0,-1} = \tau_{0,\delta} = 1/(u + w + \delta). \quad (26)$$

For the $N=2$ case, the calculations become more tedious. The results for the functions ϕ_{-1}, ϕ_0, and ϕ_1 are given by

$$\phi_{-1} = \frac{u_0 u_1^2 - u_0 w_1^2 + w_1(u_0 + w_0 + \delta_0)(u_1 + w_1 + \delta_1)}{[(u_0 + w_0 + \delta_0)(u_1 + w_1 + \delta_1) - (u_0 w_1 + u_1 w_0)](u_1 + w_1 + \delta_1)}, \tag{27}$$

$$\phi_0 = \frac{u_0 u_1 + w_0 w_1}{[(u_0 + w_0 + \delta_0)(u_1 + w_1 + \delta_1) - (u_0 w_1 + u_1 w_0)]}, \tag{28}$$

$$\phi_1 = \frac{w_0 w_1^2 - w_0 u_1^2 + u_1(u_0 + w_0 + \delta_0)(u_1 + w_1 + \delta_1)}{[(u_0 + w_0 + \delta_0)(u_1 + w_1 + \delta_1) - (u_0 w_1 + u_1 w_0)](u_1 + w_1 + \delta_1)}. \tag{29}$$

Then, after lengthy but straightforward calculations, it can be shown that the splitting probabilities are

$$\pi_{0,2} = \frac{u_0 u_1}{[u_0 u_1 + w_0 w_1 + \delta_0 \delta_1 + \delta_0(u_1 + w_1) + \delta_1(u_0 + w_0)]}, \tag{30}$$

$$\pi_{0,-2} = \frac{w_0 w_1}{[u_0 u_1 + w_0 w_1 + \delta_0 \delta_1 + \delta_0(u_1 + w_1) + \delta_1(u_0 + w_0)]}, \tag{31}$$

and $\pi_{0,\delta} = 1 - \pi_{0,2} - \pi_{0,-2}$. Similar calculations for the mean first-passage times yield

$$\tau_{0,2} = \tau_{0,-2} = \tau_{0,\delta} = \frac{u_0 + u_1 + w_0 + w_1 + \delta_1}{u_0 u_1 + w_0 w_1 + \delta_0 \delta_1 + \delta_0(u_1 + w_1) + \delta_1(u_0 + w_0)}. \tag{32}$$

Thus these examples again illustrate our main theoretical findings. For the motor proteins moving along periodic molecular tracks the mean first-passage times to go forward, backward, or to detach irreversibly are the same, while the probabilities of these events are always different. Note that in the case $\delta_0 = \delta_1 = 0$, Eqs. (26) and (32) give the correct expressions for the mean first-passage times without detachments [16,30]. Thus, the effect of dissociations might be estimated quite easily.

III. ANALYSIS OF KINESIN DATA

Structural, biochemical, and kinetic data on kinesins suggest that the protein molecule goes through at least four intermediate states [1,2]. However, a recent study of kinesin dynamics using ($N=2$)-state chemical kinetic model, which takes into account the irreversible detachments, provides a very reasonable description of some aspects of mechanochemical coupling in this system [24]. Thus, in order to analyze the experimental data of Nishiyama *et al.* [11] we adopt the simplest model which includes only two states. The states $j = \ldots, -2, 0, 2, \ldots$ would correspond to the kinesin with both molecular heads tightly bound to the microtubule and without an ATP molecule. The states $j = \ldots, -1, 1, \ldots$ label all other kinesin conformations after ATP binding and subsequent hydrolysis and release of its products.

It now follows that the forward ATP-binding transition depends linearly on ATP concentration, $u_0^0 = k_0^0[\mathrm{ATP}]$, where the superscript 0 indicates the case of zero load: see also Eq. (6). At the same time the next forward rate u_1 and the backward rate w_1 do not depend on the ATP concentration, while they may change under the effect of external forces.

The final backward rate w_0 might, in principle, depend on concentrations of ADP and inorganic phosphate, which both are the products of ATP hydrolysis. However, most current experiments on kinesins utilize an ATP regeneration system [5,8,9,11], in which there is no independent control of [ADP] and [P_i]. As a result, we adopt a phenomenological description of this backward transition—namely,

$$w_0^0 = k_0'[\mathrm{ATP}]/(1 + [\mathrm{ATP}]/c_0)^{1/2}, \tag{33}$$

where the parameter c_0 effectively describes the ATP regeneration process. This approach has been used successfully to describe the mechanochemical transitions in kinesin and myosin-V [24,25]. Note, however, that the specific description of the ATP regeneration process has a minimal effect in the fitting of experimental results.

The fitting of the model was done by minimization of a trial function defined as a sum of deviations between the calculated data and the experimentally observed values for both mean dwell times and fractions of different steps. This trial function also reflects the error bars in the data for mean dwell times. A combined scheme was used for the two parts (for dwell times and for the fractions) of the trial function with different weight factors.

After systematically exploring the multidimensional space of parameters and using Eqs. (30)–(33) the fractions of forward and backward steps and mean dwell times between the consecutive steps of kinesins can be well described by the rate constants

$$k_0^0 \simeq 5.1\ \mu\mathrm{M}^{-1}\,\mathrm{s}^{-1}, \quad k_0' \simeq 2.8\ \mu\mathrm{M}^{-1}\,\mathrm{s}^{-1},$$
$$c_0 \simeq 1.7\ \mu\mathrm{M}, \quad w_1^0 \simeq 5.5 \times 10^{-4}\ \mathrm{s}^{-1},$$
$$u_1^0 \simeq 121\ \mathrm{s}^{-1}, \quad \delta_0^0 \simeq 1.1\ \mathrm{s}^{-1}, \quad \delta_1^0 \simeq 1.6 \times 10^{-3}\ \mathrm{s}^{-1} \tag{34}$$

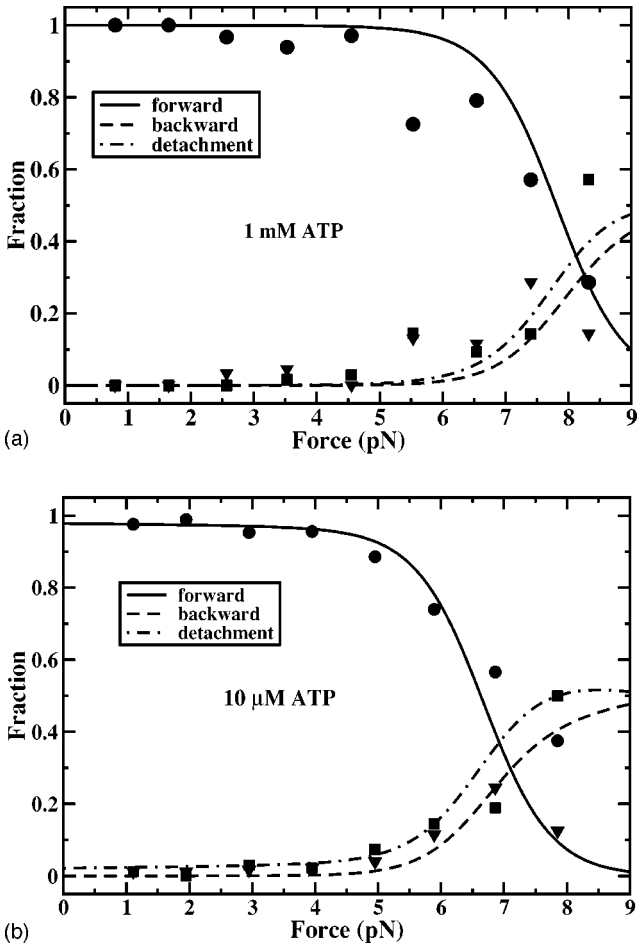

FIG. 2. Probabilities, or fractions, of forward steps (circles), backward steps (triangles) and detachments (squares), as a function of the external force at (a) [ATP]=1 mM, (b) [ATP]=10 μM.

and load-distribution parameters

$$\theta_0^+ \simeq 0.0, \quad \theta_0^- \simeq 0.391, \quad \theta_1^+ \simeq 0.086,$$

$$\theta_1^- \simeq 0.523, \quad \theta_0^\delta \simeq 0.047, \quad \theta_1^\delta \simeq 0.466. \qquad (35)$$

The results of the fitting of experimental observations are given in Figs. 2 and 3. Note that the values for the parameters reported here are in a good agreement with the other independent investigation of kinesin motility [24], where the multistate periodic stochastic models have been used to analyze the single-molecule experimental measurements of velocities, stall forces, and dispersions [8].

IV. DISCUSSION

Our theoretical analysis provides explicit expressions for the fractions of forward and backward steps and dissociations, and for the mean dwell times between consecutive steps of motor proteins. This allows us to investigate the problem of mechanochemical coupling between the motion of kinesins and ATP hydrolysis. Our main conclusion is that the mean dwell times to move forward, backward, or irreversibly detach are equal to each other independently of ATP

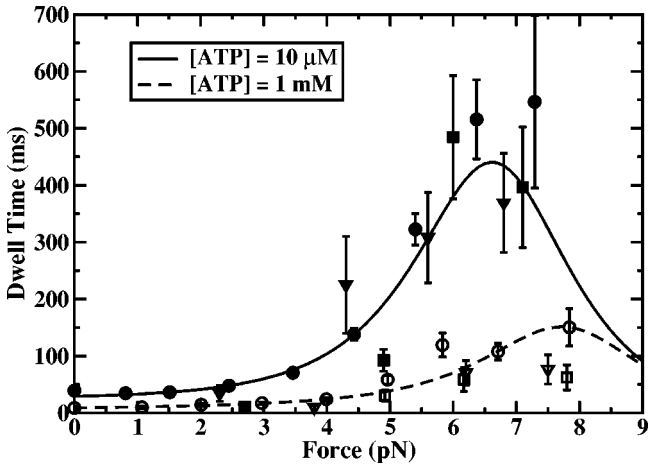

FIG. 3. Dwell times between the adjacent movements of the kinesin molecule as a function of external force. The solid symbols correspond to experimental measurements at [ATP]=10 μM, while open symbols describe the experiments at [ATP]=1 mM. The circles mark the experimental measurements for dwell times before the forward steps, the triangles correspond to experimental dwell times before the backward steps, and squares describe the dwell times before detachments.

concentration or external force. It means that the picture of tight coupling between ATP hydrolysis and forward steps of kinesins does not contradict the experimental findings of Nishiyama *et al.* [11]. Moreover, the proposed bidirectional biased-model [11], which assumes that a hydrolysis of a single ATP molecule is coupled to either forward or backward movement, is basically incorrect since it violates the principle of microscopic reversibility and breaks the symmetry of the system if the biochemical states of the motor protein belong to a single kinetic pathway.

Our theoretical results could also be understood in the following way. The mean dwell times between movements measured in single-molecule experiments actually correspond to the mean lifetimes of states when the motor protein binds strongly to the linear track. Then these lifetimes should be independent of what direction the motor protein will go in the next step, although the probability of these steps might be rather different.

The analysis of mean dwell times at different external forces, as shown in Fig. 3, suggests that there is a maximum at high loads. This maximum is close but not exactly at the stall force. When [ATP]=10 μM the maximum can be found at $F \simeq 6.6$ pN, while the stall force is approximately equal to 6.8 pN. At high ATP (1 mM) the position of maximum is shifted to 7.7 pN, with the calculated stall force $F_S \simeq 9.2$ pN. This can be understood in the following way. The external load decreases the forward transition rates, while accelerating the backward transitions. These two tendencies have an opposite effect on mean dwell times, and it leads to the observation of maximum at some specific value of external force.

Because our method provides exact expressions for dynamic characteristics, we are able to study the effects of ATP concentration and external forces on these parameters, and we can make a qualitative predictions that can be checked experimentally.

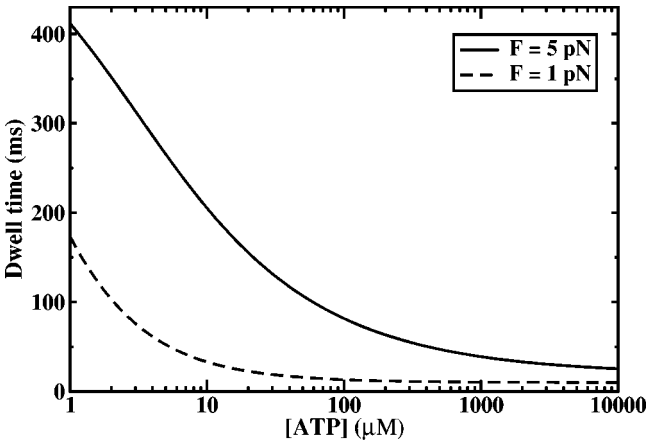

FIG. 4. Predictions for the dwell times as a function of [ATP] at low ($F=1$ pN) and high external load ($F=5$ pN).

First, we investigate how mean dwell times depend on [ATP] at different external loads. As shown in Fig. 4, the larger the external force, the larger is the mean dwell time. However, at constant force, the mean dwell time decreases with an increase in the concentration of ATP. This is in agreement with intuitive expectations since at large [ATP] the binding process is faster. At the same time the external force slows down the binding and other forward processes less than it accelerates the backward transitions. These observations are also consistent with a theoretical investigation of the processivity of motor proteins using a thermal ratchet approach [28].

The dependence of the fractions of different movements on ATP concentration at different external loads is presented in Fig. 5. The increase in [ATP] increases the probability of the forward steps, while making the fractions of backward steps and detachments negligible. Finally, the predictions for the force and velocity based on the fitted parameters are given in Fig. 6. These predictions are generally agree with the values of drift velocities and stall forces obtained in other single-molecule experiments on kinesins [8]. However, the shapes for force-velocity curves are different for ATP saturating conditions.

The results of the fitting of experimental data suggest that there are substeps at approximately $d(\theta_0^+ + \theta_1^-) \simeq 4.3$ nm when the kinesin makes 8.2-nm steps from one binding site to another. However, they are not found in experiments on the dwell times of kinesin molecules [11]. In addition, the experimental observations of Nishiyama *et al.* [10] indicate that there are no substeps at distances larger than 1 nm that would correspond to intermediate states with a lifetime of more than 1 ms. The apparent contradiction between the theoretical predictions and experimental data can be explained in the following way [31]. The kinesin molecules move along a complex three-dimensional potential energy surface, and the simplified one-dimensional energy landscape, as assumed in the discrete-state stochastic models, might not produce a correct description of intermediate states. The basic stochastic models can be extended to include more realistic three-dimensional energy potentials. However, most of the features of kinesin motility can still be well described by the one-dimensional chemical kinetic models utilized in this work.

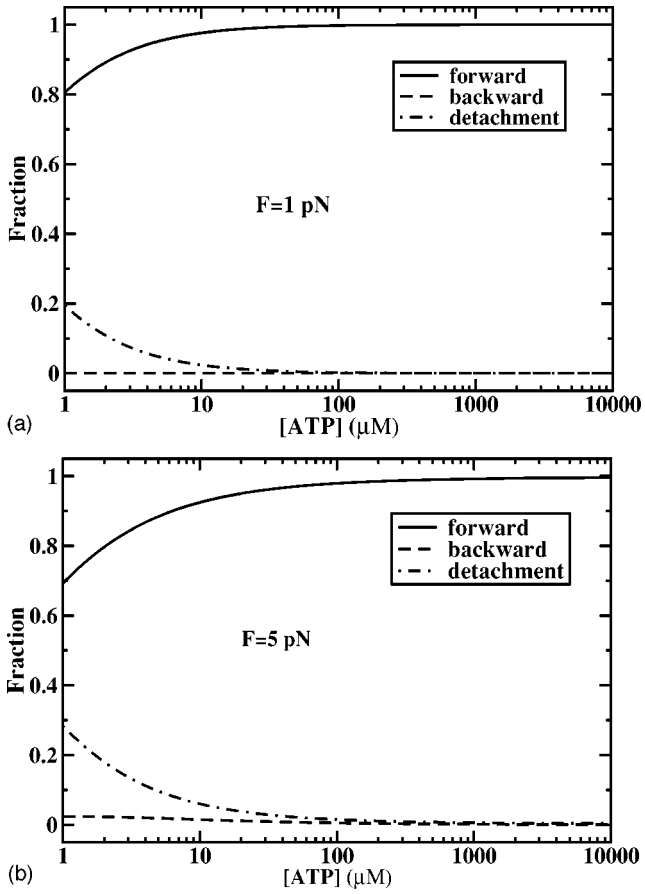

FIG. 5. Predictions for the variation of the fractions of forward steps, backward steps, and detachments at (a) $F=1$ pN and (b) $F=5$ pN.

V. CONCLUSIONS

In summary, we have presented a theoretical study of mechanochemical coupling in kinesins. The analysis of mul-

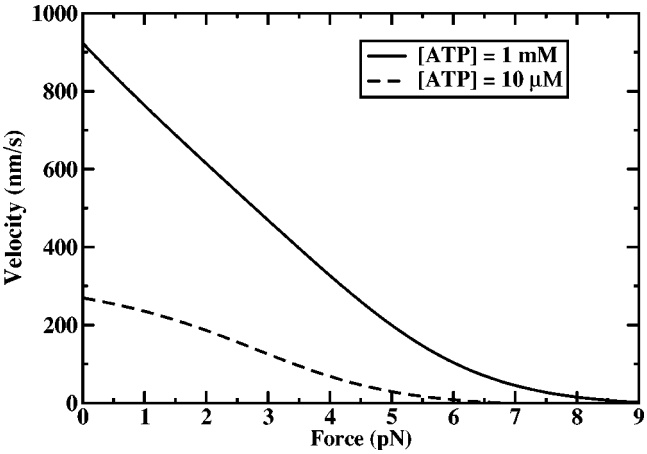

FIG. 6. Predictions for the force-velocity curves at different [ATP].

tistate stochastic models of motility using the method of first-passage times allowed us to obtain explicit formulas for fractions of steps in different directions and for the mean dwell times between the steps, including irreversible detachments. The experimental data on kinesins can be well described by this approach. Our analysis is consistent with the current theoretical view of a tight coupling between catalytic cycles and mechanical steps for kinesins; i.e., one ATP molecule is hydrolyzed per each forward step, and the rare backward steps correspond to ATP production. Although our theoretical approach seems to provide a reasonable and convenient framework for investigating the mechanochemical coupling in different motor proteins, further experiments are needed in order to validate our theoretical picture.

ACKNOWLEDGMENTS

We acknowledge support from the Camille and Henry Dreyfus New faculty Awards Program (Grant No. NF-00-056), the Welch Foundation (Grant No. C-1559), the Alfred P. Sloan foundation (Grant No. BR-4418), and the U.S. National Science Foundation through Grant No. CHE-0237105. We also thank M. E. Fisher and Hong Qian for critical discussions, suggestions, and encouragements.

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