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Monte Carlo simulation on backward steps of single kinesin molecule*

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Kinesin is a stepping molecular motor travelling along the microtubule. It moves primarily in the plus end direction of the microtubule and occasionally in the minus-end, backward, direction. Recently, the backward steps of kinesin under different loads and temperatures start to attract interests, and the relations among them are revealed. This paper aims to theoretically understand these relations observed in experiments. After introducing a backward pathway into the previous model of the ATPase cycle of kinesin movement, the dependence of the backward movement on the load and the temperature is explored through Monte Carlo simulation. Our results agree well with previous experiments.

Keywords: kinesin, backward steps, Monte Carlo, ATPase cycle
PACC: 8715, 8715D

1. Introduction

Kinesin is a stepping molecular motor which travels along the microtubule[1−3] with a regular 8 nm step size.[4−6] As an important molecular motor in cells, it plays an essential role in the course of organelle transport and division. Kinesin thus has attracted much attention, and several features of it have been revealed. In the ATPase cycle, this motor takes a hand-over-hand mechanism, according to which the two heads (motor domains) go forward coordinately by consuming the energy of ATP (adenosine triphosphate) hydrolysis. Generally, an ATPase cycle includes several states of kinesin defined by the states of both heads of the molecule.[7,8] Each head can reside in one of the three states (bound with ATP, ADP (adenosine diphosphate) or empty). An ATP molecule binding to the motor head in the empty state triggers the complicated conformation conversion and the ADP release.[9−11] Kinesin moves primarily in the forward direction (to the plus-end of the microtubule) and occasionally in the backward direction.[12−14] In the previous studies, the backward steps were often ignored for they are rare events as compared with the regular 8 nm steps in the forward direction. Recently, Carter and Cross observed the backward steps in a wide range of external loads.[15] Meanwhile, Taniguchi *et al* [16] confirmed that the backward steps probability is temperature and load dependent. Backward steps, like forward steps, are also triggered by ATP binding.[15] Furthermore, the backward step size is also 8 nm. When the load is near the stall force, the probability of forward stepping is equal to that of backward stepping. It is therefore a new challenge to understand how and why kinesin has backward steps in an ATPase cycle. Especially, how to understand the backward steps as a part of the kinesin movement? In the present paper, we propose a simple and intuitive model to describe the kinesin movements, especially the backward movement through Monte Carlo simulation.

2. Model

Considering the structure of the molecule, kinesin is simply modelled by two connected heads, trailing and leading heads. Each head can bind with ADP, ATP or just be empty. For convenience, they are denoted as D, T and O states, respectively. Generally, an ATP cycle comprises several states of kinesin, such as (D,O), (T,D) and (T,O) (Fig.1),[7,8] in which the first part in the bracket represents the state of the trailing

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head and the last part is the state of the leading head. In the present model, the backward movement and the T state of the head which were ignored in the previous studies are considered. Consequently, the kinetics of the system in an ATPase cycle is written as

$$
\begin{aligned}
&(\mathrm{D},\mathrm{D}) \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} (\mathrm{D},\mathrm{O}),\\
&(\mathrm{D},\mathrm{O}) \xrightarrow{k_{23}} (\mathrm{D},\mathrm{T}) \xrightarrow{k_{34}} (\mathrm{T},\mathrm{D}) \xrightarrow{k_{45}} (\mathrm{T},\mathrm{O}),\\
&(\mathrm{D},\mathrm{O}) \xrightarrow{k_{23}} (\mathrm{D},\mathrm{T}) \xrightarrow{k_{37}} (\mathrm{O},\mathrm{T}),\\
&(\mathrm{T},\mathrm{O}) \xrightarrow{k_{56}} (\mathrm{T},\mathrm{T}) \xrightarrow{k_{63}} (\mathrm{D},\mathrm{T}),\\
&(\mathrm{T},\mathrm{O}) \xrightarrow{k_{52}} (\mathrm{D},\mathrm{O}),\\
&(\mathrm{O},\mathrm{T}) \xrightarrow{k_{72}} (\mathrm{D},\mathrm{O}),
\end{aligned}
$$

in which k_{ij} are rate constants between i and j states, as shown in Fig.1. (T,O)→(T,T) suggests that an ATP molecule can bind to the head residing in the empty state.[17] The initial state of kinesin begins from the state (D,O) (Fig.1). More details of this model is described in our previous paper.[9] When the leading head in an empty state and the trailing head in an ADP state, both ADP and ATP molecules have opportunities to bind to the empty head. Once an ATP molecule binds to the leading head, the trailing head will move forward and bind to the next position with ADP release and ATP hydrolysis. But it is also possible that the trailing head will not move forward but stay at the original position, then ADP releases and ATP hydrolyzes, at the same time kinesin makes a backward step. Therefore, we introduce a backward routine in the ATPase cycle into our model. Thus, we assume that both the forward and the backward movements are coupled with ATP hydrolysis.[15,18,19] Based on this model (Fig.1), we employ Monte Carlo simulation to simulate the ATPase cycle. The transition rates have been given in the Ref.[9]. Especially, it is assumed that k_{37} is equal to k_{45}, for both of them describe the process of ADP release; k_{52} and k_{63}, the ATP hydrolysis rates ($k_{\mathrm{hydr}}(f)$ where f is the load), are both calculated from the phenomenologically assumed equation $k_{\mathrm{hydr}}(f) = k_{\mathrm{hydr}}(0)\left[1 - \left(\frac{f}{f_0}\right)^2\right]$,[20,21] where f_0, in the range of 9.1–10 pN, is the unbinding force.[7,22] However, the rate k_{72}, which describes the ATP hydrolysis in the backward routine, is taken as a constant because the minus-end load little influences the state (O,T). Therefore, the dwell times of the backward steps must

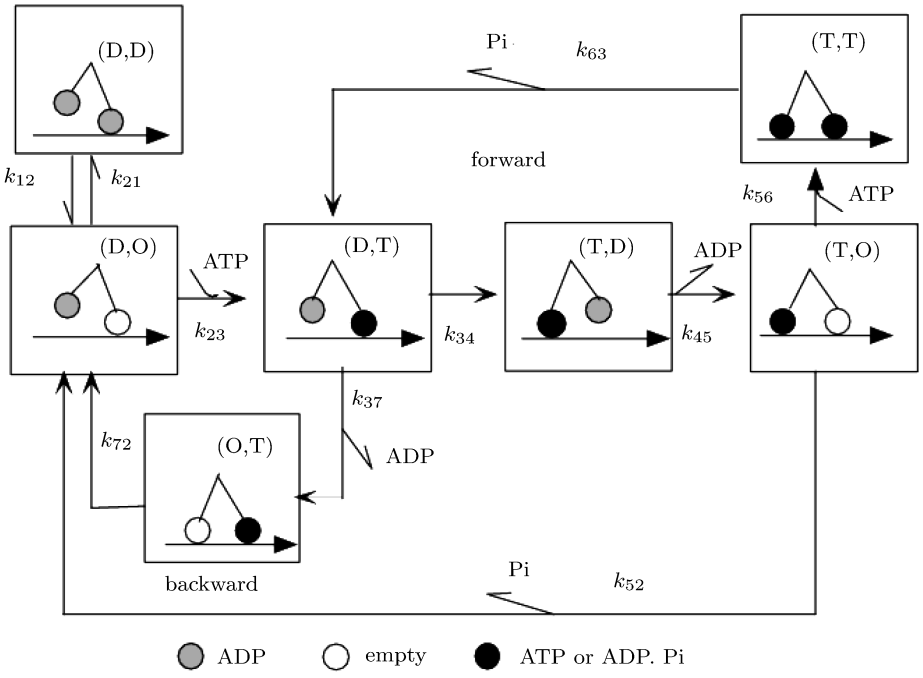


Fig.1. State transitions of the kinesin in an ATPase cycle. The black circles represent the ATP bound state (T), the grey circle represent the ADP bound state (D) and the hollow circles stand for the empty state (O). The direction of the arrow is the plus-end direction of the microtubule. k_b (k_{37}) and k_{45} are concerned with the binding of heads to the microtubule, k_f is k_{34}. (D,O)-(D,T)-(O,T)-(D,O) is the backward path.

be load-independent. In the present model, state (D,T) is very special because it determines the probabilities of forward and backward movements of kinesin. The transition rates as concerned with this state are k_{34} and k_{37} (denoted by k_f and k_b). We assume that the load dependence of k_f has the form[18,23] $k_f = k_f(0)\exp(-f\delta/k_{\rm B}T)$, where $\delta = 3.7 \times 10^{-9}$. Meanwhile, for the backward transition rate, because the force needs to overcome the detaching force of the front head, f_0, and at $f = f_0$, k_b takes the maximum value, we phenomenologically assume that it has a Gaussian form $k_b = k_b(0)\exp[-(f - f_0)^2/2\sigma^2]$, where $\sigma = 2.5 \times 10^{-12}$N and $k_b(0) \simeq 400\,{\rm s}^{-1}$. On the other hand, all the transition rates are influenced by the temperature. We thus introduce the Arrhenius law, which gives $k(T) = k_0\exp(-E/k_{\rm B}T)$, into the model, where E is the energy difference between (O,T) and (D,O) for the backward movement. It reads $k(T_2) = k(T_1)\exp[-E(1/k_{\rm B}T_2 - 1/k_{\rm B}T_1)]$, in which $E \simeq 5.3k_{\rm B}T$ (The energy produced by one ATP hydrolysis is about 12 $k_{\rm B}T$. Meanwhile, the unbinding force in ATP state is about 9 pN and the unbinding force in the ADP state is about 4 pN.[23] Therefore, the energy difference between (O,T) and (D,O) is $E = (4/9) \times 12k_{\rm B}T \simeq 5.3k_{\rm B}T$.)

3. Results and discussion

The simulated steps are divided into two groups: forward steps and backward steps. The probabilities of the forward steps and the backward steps, which alter with loads, are shown in the same figure. We obtain the results for the temperatures 7°C and 35°C respectively, in Figs.2(a) and 2(b). The results agree well with Yanagida *et al*'s experimental observations.[16,19]

When the load is smaller than 4 pN, the fraction of forward steps is almost equal to 1. The fraction of the forward steps gradually decreases when the load increases. Meanwhile, the fraction of backward steps distinctly increases during the foregoing course. From Fig.2 we can see that when the load is large (close to 9 pN), the probability of backward steps increases slowly. At about 9 pN, the fraction of the backward steps reaches a maximum. The same phenomena are observed more obviously in the range of high temperatures (35°C). The logarithm of the ratio of the forward steps to backward steps is a linear decreasing function of the load (Fig.3), which is in good agreement

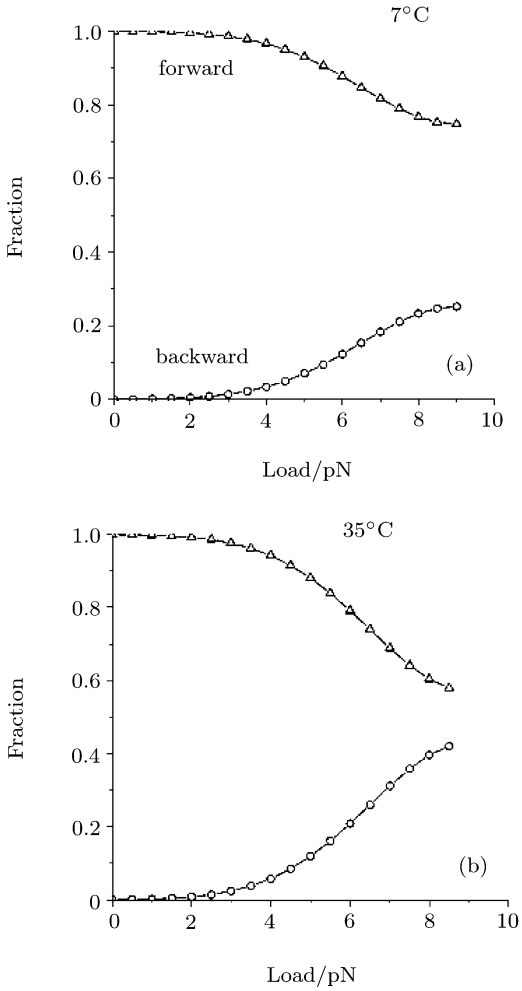


Fig.2. Load dependence of the proportion of forward steps to backward steps at different temperatures, (a) 7°C, (b) 35°C. The data are the mean values of fractions. The kinetic parameters used are taken from Ref.[9], f_0=9.0 pN, $k_0 = 3 \times 10^{-9}$.

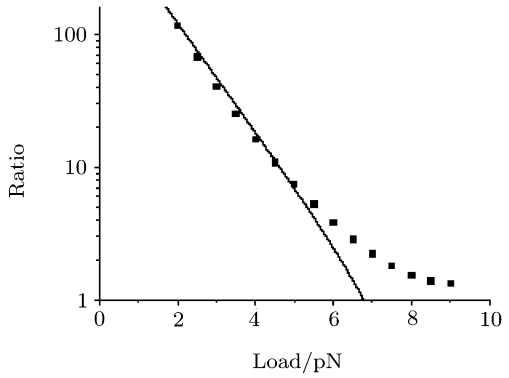


Fig.3. Fraction of the forward movement versus load. Data are fitted by a linear function. The fitted slope, k, is –0.94.

with the result of Carter *et al*[15] for the low load. Its fitted slope is –0.94. When the load is near to the stall force, the simulated results start to deviate from the line. This fitted straight line shows that the

fraction of the number of forward steps exponentially depends on the load. More interestingly, the fraction of the forward movement is only dependent on the load and independent on the concentration of ATP (data not shown). Experimentally, Carter *et al* [15] and Nishiyama *et al* [19] have already come to the same result. In addition, we obtain the distribution of the dwell times between the steps, which changes with the load and temperature. These curves are fitted by the function: $C\gamma^2 t\exp(-\gamma t)$, in which γ is a parameter and t is time (Fig.4).[21] The dwell time distribution illustrates that γ increases with increasing temperature but decreases with increasing load.

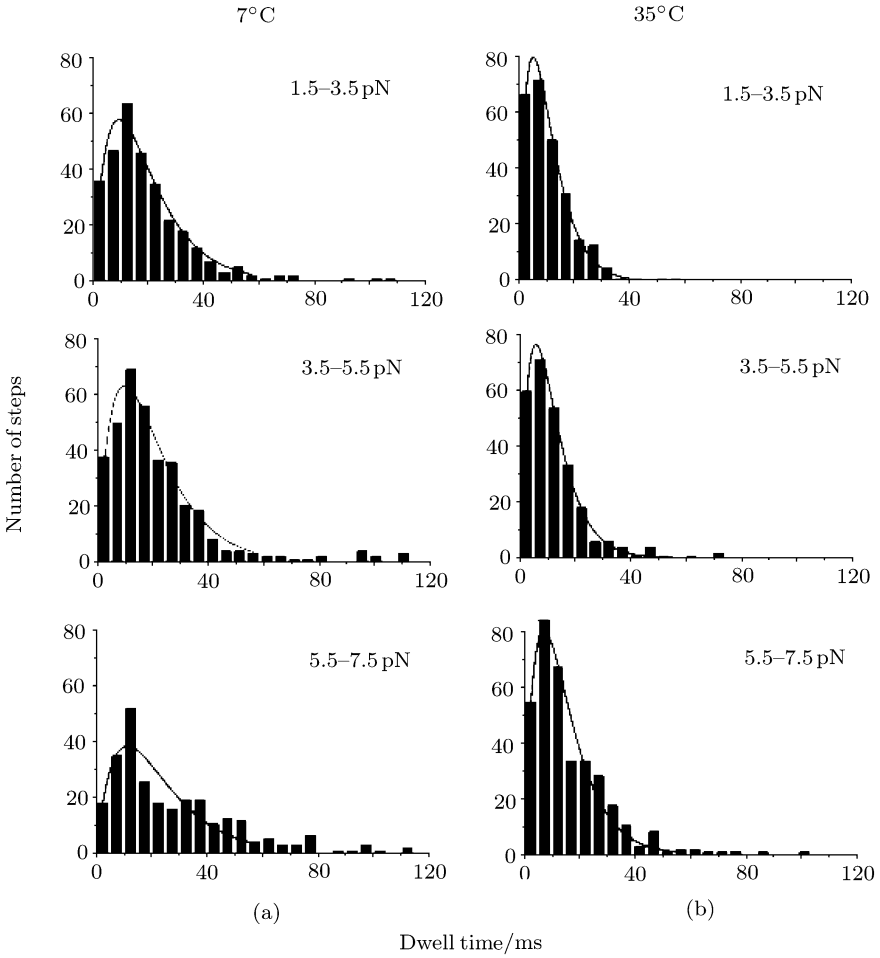


Fig.4. Dwell time distributions obtained at various loads and temperatures. Curves are fitted by the function: $C\gamma^2 t\exp(-\gamma t)$. (a) At 7°C, γ values are $0.105\,\mathrm{ms}^{-1}$, $0.098\,\mathrm{ms}^{-1}$ and $0.088\,\mathrm{ms}^{-1}$, respectively, from top to bottom; (b) at 35°C, γ values are $0.180\,\mathrm{ms}^{-1}$, $0.169\,\mathrm{ms}^{-1}$ and $0.136\,\mathrm{ms}^{-1}$, respectively, from top to bottom.

4. Conclusions

Kinesin is a stepping molecular motor, which plays a significant role in cells. Recently, the backward movements observed under different loads and temperatures have attracted many experimental studies. After improving the previous kinetic model, an ATP molecule can bind to the empty head of kinesin, and the trailing head in the D state can bind both forward and backward after the release of ADP. We have explored the dependence of backward movement on the load and temperature through Monte Carlo simulations. It reveals that the backward probability alters with the load and the temperature. The fraction of backward movement has a remarkable probability only when the load is greater than 4 pN, which explains why it is rarely observed in experiments and thus shows the consistence with the previous knowledge. Our simulation results agree with the previous experimental measurements quantitatively.

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