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Biophysical Chemistry 67 (1997) 117–125

Biophysical Chemistry

Length dependence of displacement fluctuations and velocity in microtubule sliding movement driven by sea urchin sperm outer arm β dynein in vitro

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Received 9 October 1996; revised 18 January 1997; accepted 10 February 1997

Abstract

We have studied the dependence on microtubule length of sliding velocity and positional fluctuation from recorded trajectories of microtubules sliding over sea urchin sperm outer arm β dynein in a motility assay in vitro. The positional fluctuation was quantified by calculating the mean-square displacement deviation from the average, the calculation of which yields an effective diffusion coefficient. We have found that (1) the sliding velocity depends hyperbolically on the microtubule length, and (2) the effective diffusion coefficients do not depend on the length for sufficiently long microtubules. The length dependence of the sliding velocity indicates that the duty ratio, defined as the force producing period over the total cycle time of β dynein interaction with microtubule, is very small. The length independence of the effective diffusion coefficient indicates that there is a correlation in the sliding movement fluctuation of microtubules.

Keywords: Sliding movement; Dynein; Microtubule; Cell motility; Protein motor

1. Introduction

A recently-developed in vitro motility assay has made it possible to study the sliding movements of individual cytoskeletal filaments generated by protein motors under well-defined conditions [1–4]. By analyzing the fluctuation of the sliding movement of individual filaments we can elucidate some aspects of the dynamics and kinetics of the mechanisms involved in the sliding movement [5]. In our previous work, we studied the positional fluctuation in microtubule sliding movement generated by kinesin in vitro under no external load [6]. There we quantified the positional fluctuation by calculating the mean-square displacement deviation from the average as a function of time, which yields an effective diffusion coefficient. One intriguing result we obtained in the study is that the effective diffusion coefficient of a sliding filament, which will be referred to as the motional diffusion coefficient, does not depend on the filament length when the concentration of kinesin

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0301-4622/97/$17.00
PII S0301-4622(97)00028-8

is large enough. This is in sharp contrast to the ordinary Brownian movement of filamentous particles, in which the diffusion coefficient, a measure of the positional fluctuation, is proportional to the inverse of the filament length. Thus, our previous results have provided evidence for the presence of correlation in the fluctuation of the microtubule sliding movement generated by kinesin in vitro [6].

In this study we have extended our fluctuation analysis to the microtubule sliding movement generated by sea urchin sperm outer arm β dynein in vitro. We have found that, as in the case with kinesin [6], the motional diffusion coefficient of microtubules sliding over β dynein does not depend on the filament length when the length is sufficiently long. Here we also studied the length dependence of the sliding velocity of microtubules. We have found that, unlike the sliding velocity of microtubules over kinesin, the velocity over β dynein depends on the microtubule length and hyperbolically increases with the increase in the length, as was previously found with *Paramecium* 22S dynein by Hamasaki et al. [7]. The significance of these findings will be discussed in the light of the filament length dependence.

2. Materials and methods

2.1. Proteins

Flagellar outer arm 21S dynein was prepared from sea urchin (*Pseudocentrotus depressus*) sperm. Complexes of dynein β heavy chain and intermediate chains were purified from the 21S dynein as described [8]. We used this preparation for motility assay and refer to it as β dynein in this paper. The β dynein is considered to be a single-headed motor. Tubulin was prepared from porcine brains by two cycles of temperature-dependent polymerization, followed by phosphocellulose chromatography [9]. Microtubules were polymerized in the presence of 9% dimethylsulfoxide and then stabilized with 40 μM taxol [3].

2.2. Motility assays in vitro

We performed the dynein motility assay using a microscope perfusion chamber at 25°C. 15 μl of a β dynein (600 $\mu g\,ml^{-1}$) solution was first infused into the chamber. After 3 min, unbound proteins were washed away by perfusing an assay buffer: 10 mM Tris-HCl (pH 7.5), 50 mM K-acetate, 4 mM $MgSO_4$, 1 mM EGTA and 1 mM DTT. Microtubules and 1mM ATP were then perfused into the chamber in the assay buffer.

We examined the microtubule movement over β dynein attached to a glass surface, by dark field microscopy using a Nikon microscope equipped with a 100 W mercury light source, a heat filter, an Olympus dark field condenser (1.2–1.33 N.A.) and a Nikon $\times 40$ objective lens. We took images of the filaments with an SIT camera (Ikegami CTC-9000) and recorded their movements onto a video cassette tape recorder (SONY EVO-9650).

2.3. Data collection

A video cassette tape recorder was connected to a video image digitizer board (I · O DATA, Japan) in an EPSON microcomputer (PC-486GR), on which we run custom software (written in C + +) to control the operation of the video tape recorder through an RS232C circuit [6]. By transferring the video images played back from the video tape recorder to the computer, we then displayed the images on the computer screen frame by frame. We next entered the x–y coordinate position of microtubule ends into the EPSON computer, using a mouse-driven video cursor on the screen. The digitization accuracies for the x and y coordinates were 145 and 142 $nm\,pixel^{-1}$, respectively. The sampling interval for the data collection was 1/30 s. We usually collected more than 100 digitized data points from a single trajectory of sliding movements in vitro.

2.4. Calculation of the sliding velocity

We calculated the sliding velocity by

$$V = \langle \Delta r(\Delta t)/\Delta t \rangle \quad (1)$$

where $\Delta r(\Delta t)$ is the displacement of a filament along its trajectory during a fixed time interval (Δt), and $\langle \ldots \rangle$, such as that on the right-hand side of Eq. (1), shows the average (see below).

The velocities are fitted to the following equation [7]

$$V = V_0 L/(K_L + L) \quad (2)$$

using the simplex method [10], where L is the filament length, and V_0 and K_L are the parameters (see Section 4, Discussion).

2.5. Calculation of the mean-square displacement fluctuation around the average

We calculated the mean-square fluctuation by

$$F_r^2 = \langle [\Delta r(\Delta t) - \langle \Delta r(\Delta t) \rangle]^2 \rangle \quad (3)$$

$$= 2 D_m \Delta t \quad (4)$$

Eq. (4) yields a coefficient, D_m, which is referred to in this study as the motional diffusion coefficient. For the average calculations in Eq. (1) and Eq. (3), we used an average calculation within a single trajectory of a filament [11], which is referred to in this study as single trajectory averaging [6,12].

2.6. Smoothing of the trajectories

Since the trajectories were not smooth enough to conduct single trajectory averaging, we smoothed the trajectories by low-pass digital filtering with a Hann window at 3 Hz (see [10] and [12] for more details). The smoothing does not significantly affect either the fluctuation or the steady characteristics inherent to the filament sliding movements generated by protein motors in vitro, as we have shown by a Monte Carlo study [12].

3. Results

A measure of the fluctuation inherent to the sliding motion is given by the motional diffusion coefficient [12]. As described in Section 2, Materials and Methods, the mean-square displacement deviation from the average as a function of time yields the motional diffusion coefficient. The main concern in this study has been to examine the dependence of the motional diffusion coefficients of microtubules on their filament length. To examine this dependence precisely, we needed to measure the motional diffusion coefficients for microtubules with various lengths. For the average calculation to obtain a motional diffusion coefficient, there are two methods: (1) an average calculation within a single trajectory of a filament [11], which is referred to as the single trajectory averaging, and (2) an average calculation over many filaments having lengths within a certain range. The single trajectory averaging has an advantage over the other [12], because the former, unlike the latter, yields a motional diffusion coefficient for each individual microtubule. In this work, we thus used the single trajectory averaging. The single trajectory averaging also yields a precise value for the sliding velocity of a single microtubule [12].

Fig. 1(A) shows a digitized trajectory of a microtubule sliding over β dynein. The unprocessed trajectory is noisy because of the errors introduced upon digitizing the microtubule position on the computer screen.

For the single trajectory averaging, noise needs to be removed from trajectories such as that shown in Fig. 1(A). Hence we smoothed trajectories by low-pass filtering as described in Section 2, Materials and Methods. By computer simulation, we have examined the cut-off frequency for smoothing noisy trajectories and found that a cut-off frequency of 3Hz is

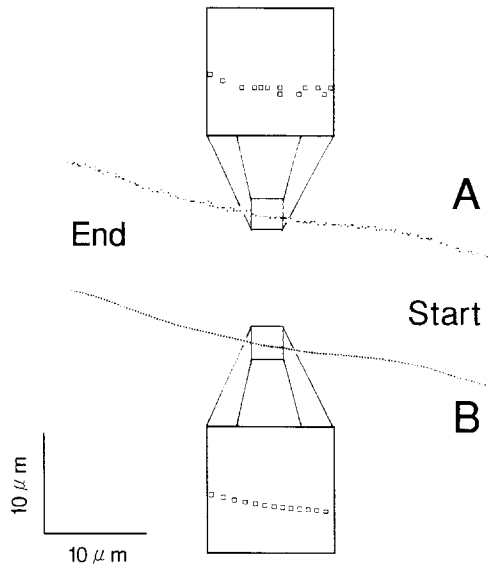

Fig. 1. An example of the trajectories of a microtubule sliding over β dynein (600 $\mu g\,ml^{-1}$) attached to a glass surface at 25°C. The microtubule length: 6.2 μm. (A) The original digitized trajectory before smoothing, consisting of 151 positional data points collected with a sampling interval of 1/30 s. (B) The trajectory after smoothing by low-pass filtering at a 3 Hz cut-off frequency as described in Section 2, consisting of the same number of positional data points as in A.

appropriate for the present analysis [12]. Fig. 1(B) shows the resulting smoothed trajectory obtained from the noisy trajectory shown in Fig. 1(A). Although the distances between the adjacent positional points along the smoothed trajectory appear to be almost homogeneous, smoothed trajectories retain the steady and fluctuation characteristics inherent to the sliding movement [12].

Fig. 2 shows an example of the mean displacement as a function of time for the single smoothed trajectory shown in Fig. 1(B). The slope of the plot yields the sliding velocity of the microtubule (see Eq. (1)).

By repeating similar calculations, we collected sliding velocity data for microtubules having various lengths. The results are summarized in Fig. 3, which shows original data and the binned averages of sliding velocity of microtubules as a function of filament length. Unlike the case of microtubules sliding over kinesin [6], the velocity of microtubules sliding over β dynein depends on the microtubule length. We found that the velocity dependency upon microtubule length is hyperbolic. Hamasaki et al. [7] reported similar length dependence of the velocity of microtubule sliding generated by *Paramecium* 22S dynein in vitro. Eq. (2) was fitted to the velocity data shown in Fig. 3 by the simplex method [10] which yielded values for the constants in Eq. (2), V_0 and K_L, as summarized in Table 1.

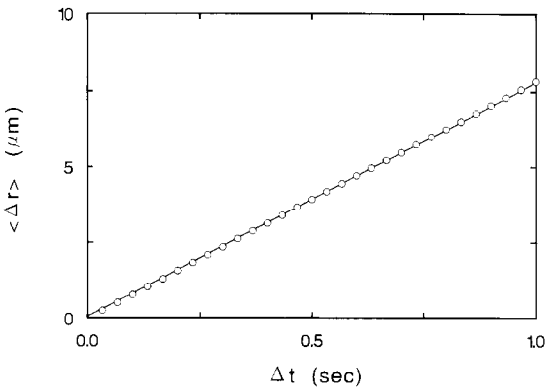

Fig. 2. The average displacement as a function of time interval. Calculated by single trajectory averaging within a single trajectory of a 6.2 μm microtubule (shown in Fig. 1(B)), as described in Section 2. We obtained the filled line using a linear regression method. The slope yields a value of 7.7 $\mu m s^{-1}$ for the sliding velocity.

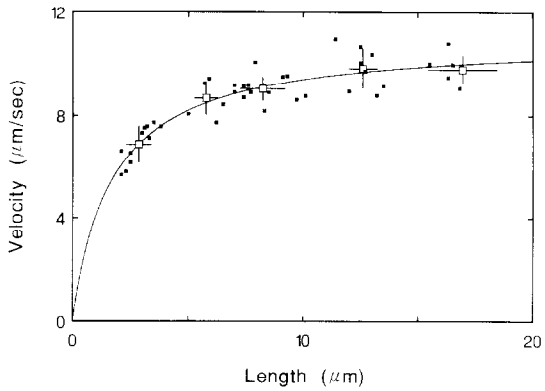

Fig. 3. The sliding velocities vs microtubule length. The curve shows a non-linear regression line of Eq. (2) fitted directly to all the 44 data points (■). For the regression, the simplex method [10] was used. The values of V_0 and K_L in Eq. (2) determined by the regression are listed in Table 1. Data for certain sets of microtubules were grouped and the averages are shown as follows. (□): 44 different microtubules in total; the data points from the left show the average taken over the sliding velocities for microtubules of ($N = 11$, $1 \sim 4$ μm), ($N = 6$, $4 \sim 7$ μm), ($N = 13$, $7 \sim 11$ μm), ($N = 8$, $11 \sim 15$ μm), and ($N = 6$, > 15 μm). Both the ordinate and abscissa values indicate the mean ± s.d. (for N shown above).

Fig. 4 shows an example of the mean-square displacement deviation from the average as a function of time interval, in the calculation of which we used single trajectory averaging. There is a lag in the graph at small time intervals. The lag is a result of the smoothing with low-pass filtering [12]. After the lag, there is a linear portion in the graph, as indicated by the line. The motional diffusion coefficient of a sliding single filament is given by half the linear slope (see Eq. (4)). The motional diffusion coefficient thus determined from this figure was $8.8 \times$

Table 1
Values of V_0 and K_L obtained by a curve fitting of Eq. (2) to data in Fig. 3, and estimated values of $N(K_L)$ and f in Eq. (6) for the sliding movement of microtubules over sea urchin sperm β dynein

	V_0 ($\mu m s^{-1}$)	K_L (μm)	$N(K_L)^c$	f^c
This work[a]	11	1.3	130	0.005
22S Dynein[b]	1.7	3.3	49	0.01

[a] The simplex method was used for fitting Eq. (2) to data in Fig. 3 (see Section 2).
[b] Hamasaki et al. [7].
[c] See text for $N(K_L)$ and f.

10^{-10} cm^2 s^{-1} for a microtubule of 6.2 μm. The deviation from the linearity at a long time interval is due to the increase in statistical errors [11,12].

Fig. 5 shows the dependence of the motional diffusion coefficients on the microtubule length. Here we used single trajectory averaging to obtain the motional diffusion coefficients. The motional diffusion coefficients of microtubules longer than 4 μm, unlike their sliding velocity, do not depend upon the microtubule length.

In our previous paper [6], we analyzed the length dependence of motional diffusion coefficients of microtubules sliding over kinesin. There we have shown that the motional diffusion coefficient of a microtubule sliding over kinesin can be expressed by the following equation (see the Appendix of [6])

$$D_m = kT/(L\zeta) + D_{ac} \quad (5)$$

where k is the Boltzmann constant, T is the absolute temperature, L is the microtubule length, ζ is the friction coefficient per unit length of the filament, and D_{ac} is a term due to the fluctuation in the active sliding force, a term that has been found to be length independent for kinesin. Eq. (5), which we proposed to explain the data for kinesin, is also consistent with the data for β dynein as shown by the broken line in

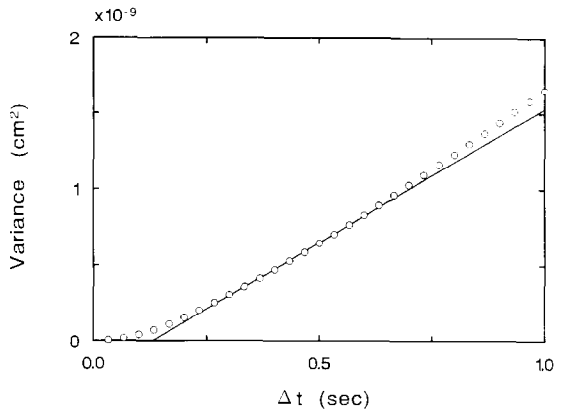

Fig. 4. The mean-square displacement deviation from the average (= variance) as a function of time interval. Calculated with positional data available within a single trajectory of a 6.2 μm microtubule (shown in Fig. 1(B)), as described in Section 2. We obtained the filled line using a linear regression method of Uyeda et al. [26]. Half of the slope yields a value of 8.8×10^{-10} cm^2 s^{-1} for the motional diffusion coefficient. The size of the linear range depends on the total number of data available from a single trajectory, and is larger when the number of data is greater.

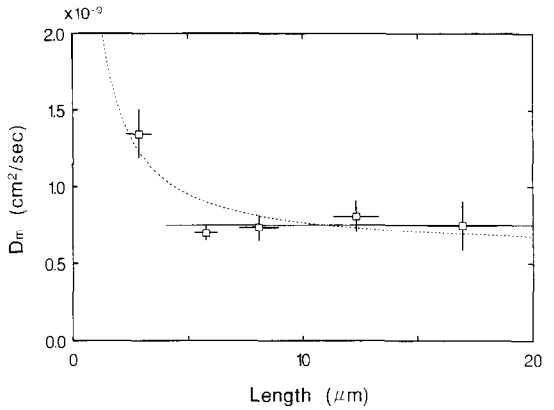

Fig. 5. The motional diffusion coefficients versus microtubule length. It is not appropriate to show all the individual data points because of the scatter. Hence, data for certain sets of microtubules were grouped as in Fig. 3. The ordinate values indicate the mean ± s.e.m. (for N shown as in Fig. 3), whereas the abscissa values indicate the mean ± s.d. (for N shown as in Fig. 3). The horizontal line shows an average of the open square data for the lengths > 4 μm. The dotted line shows a nonlinear regresion line of Eq. (5) fitted directly to all of the orignal 44 data points (not shown). See text for the values of ζ and D_{ac} determined by the regression.

Fig. 5. See Section 4 for more details of the broken line.

4. Discussion

By analyzing the trajectories of microtubules sliding over sea urchin sperm β dynein in vitro, we have examined the length dependence of the velocity and the displacement fluctuation in the sliding motion.

The velocity reflects steady characteristics of sliding movement. We found that the velocity of microtubules sliding over sea urchin sperm β dynein depends hyperbolically on the microtubule length. Similar hyperbolic dependence of the microtubule sliding velocity was also found by Hamasaki et al. [7] with *Paramecium* 22S dynein. Following the method of Hamasaki et al. [7], we fitted Eq. (2) to our velocity data in Fig. 3 to obtain V_0 and K_L values in the equation. Table 1 compares our values of V_0 and K_L obtained with sea urchin sperm β dynein, with those obtained by Hamasaki et al. [7] with Paramecium 22S dynein. Although the V_0 val-

ues are different between sea urchin sperm β dynein and *Paramecium* 22S dynein, the K_L values are relatively similar to each other.

The hyperbolic length dependence of the microtubule sliding velocity that is found with dyneins is in contrast to the length independence of the velocity of microtubules sliding over kinesin [6]. This difference between dynein and kinesin reflects their different duty ratios: the kinesin duty ratio is large, while the dynein duty ratio is small, as will be discussed below. The duty ratio is defined as the ratio of the force-producing (duty) phase over the total cycle time in the protein motor interaction with a filament [13].

Uyeda et al. [13] first studied the length dependence of actin sliding velocity in the motility assay with heavy meromyosin (HMM) in vitro. They used the equation

$$V = V_0\{1 - (1-f)^N\} \quad (6)$$

to estimate the duty ratio (f) for HMM, where V_0 is the velocity at which a motor moves during its duty phase, N is the average number of motor heads that interact with the filament, and V is the experimentally determined sliding velocity. By applying this equation to their data of actin sliding over HMM, Uyeda et al. [13] obtained $f = 0.05$ for HMM.

Since K_L in Eq. (2) is defined to be the microtubule length where $V = 0.5V_0$, Eq. (6) leads to

$$0.5 = (1-f)^{N(K_L)} \quad (7)$$

from which f can be calculated, as was calculated by Hamasaki et al. [7]. They thus obtained $f = 0.01$ for *Paramecium* 22S dynein.

To obtain a value of f for sea urchin sperm β dynein by using Eq. (7), we have to know the number of dynein heads that can interact with a 1 μm microtubule in our motility assay in vitro. We have estimated this number as follows:

1. We assume that all of the dynein molecules initially present in a solution (15 μl) which was infused into a perfusion chamber for motility assay were adsorbed on the glass surfaces within the chamber, and that the molecular weight of β dynein consisting of a β heavy chain (of molecular weight of 500000) and a few intermediate chains is 800000. The total surface area within the chamber is 3.6×10^8 μm^2 (= 18 mm $\times$ 10 mm $\times$ 2). Thus, the density of dynein adsorbed on the glass surfaces is 18.8×10^3 molecules μm^{-2}.
2. We assume that dynein molecules adsorbed on a surface right under a microtubule can interact with the microtubule. Since the surface area under a 1 μm microtubule is about 0.025 μm^2, we obtain 471 ($= 18.8 \times 10^3 \times 0.025$) for the number of dynein molecules present in this area.
3. We assume that 50–10% of the adsorbed dynein is active, because the adsorption of dynein on a glass surface may inactivate a fraction of dynein.

The above second and third assumptions lead to estimates of $250 \sim 50$ for the number of dynein molecules that can interact with a 1 μm microtubule. If we take 100 for this number, we obtain $f = 0.005$ for sea urchin sperm β dynein (Table 1) from Eq. (7). The duty ratio for sea urchin sperm β dynein is thus similar to that for *Paramecium* 22S dynein. These small values of the duty ratio for dyneins are in contrast to the large duty ratio for kinesin [14]. This is consistent with the report by Wang et al. [15] that the affinity of kinesin for microtubules is larger than that of cytoplasmic dynein for microtubules.

Our analysis also yielded values for the motional diffusion coefficient, which is a measure of the fluctuation of the sliding motion. This coefficient determined with sea urchin sperm β dynein has the following two major characteristics (Table 2). (i) The motional diffusion coefficient is smaller than the

Table 2
Diffusion coefficients of the microtubules undergoing either longitudinal random Brownian movement or sliding movement with fluctuation

	Movement	Diffusion coefficient ($cm^2 s^{-1}$)	References
Free diffusion[a]	random	8.9×10^{-9}	[25]
Over β dynein[a] (+ Vanadate)	random	1.3×10^{-10}	[16]
Over kinesin[b]	sliding	2.5×10^{-11}	[6]
Over β dynein[c]	sliding	7.5×10^{-10}	this work

[a] The longitudinal diffusion coefficient for a 5 μm microtubule.
[b] The average of the motional diffusion coefficients at a kinesin concentration of 100 $\mu g ml^{-1}$.
[c] The average of the motional diffusion coefficients at a dynein concentration of 600 $\mu g ml^{-1}$ for the microtubules longer than 4 μm.

longitudinal diffusion coefficient of a microtubule freely suspended in solution. For example, the motional diffusion coefficient of the microtubules sliding over β dynein is 7.5×10^{-10} cm^2 s^{-1}, about a tenth of the longitudinal diffusion coefficient for a microtubule of 5 μm ($= 8.9 \times 10^{-9}$ cm^2 s^{-1}). Note that the motional diffusion coefficient of microtubules sliding over kinesin [6] is much smaller than when sliding over β dynein. (ii) The motional diffusion coefficients are not proportional to the reciprocal of the microtubule length ($1/L$) when the filament is longer than 4 μm under the present experimental conditions (Fig. 5, horizontal line).

The first characteristic of the motional diffusion coefficient mentioned above indicates that the motional diffusion coefficient reflects the velocity fluctuation induced by the interaction of β dynein with microtubules, rather than by the random collision with solvent molecules [6].

Microtubules undergo one-dimensional, random Brownian movements when they are placed over β dynein in the presence of vanadate (a dynein ATPase inhibitor) plus ATP [16]. The diffusion coefficient of a microtubule of 5 μm under these conditions is 1.3×10^{-10} cm^2 s^{-1} (Table 2). This value is, as is the motional diffusion coefficient obtained by us here, much smaller than the diffusion coefficients of the microtubules freely suspended in solution (8.9×10^{-9} cm^2 s^{-1} for a 5 μm microtubule) (Table 2). Tawada and Sekimoto [17] previously proposed a "protein friction" model to explain the relatively small diffusion coefficient of the microtubules undergoing Brownian movement over dynein in vanadate plus ATP.

Eq. (5) described in Section 3, which we originally proposed to explain the data for kinesin [6], is also consistent with the data for β dynein shown in Fig. 5. Since the first term in the right-hand side of Eq. (5) is smaller for longer filaments while the second term in the right-hand side of Eq. (5) is length-independent, the first term vanishes and the second term remains for sufficiently long filaments. Hence, D_m will be practically length-independent when the filaments are long enough. To show this quantitatively, we fitted Eq. (5) directly to all the data (not shown) in Fig. 5 by the simplex method, a non-linear regression method. The resulting curve is shown by the broken line in the figure. The curve fits the data relatively well. The value of D_{ac} yielded by the curve fitting was 0.58×10^{-9} cm^2 s^{-1}. The average of data for filaments > 4 μm in Fig. 5, which is indicated by the horizontal line, is $(0.75 \pm 0.30) \times 10^{-9}$ (mean $\pm$ s.d., with $N = 33$) cm^2 s^{-1}. Thus, the estimated value of D_{ac}, the length-independent term in Eq. (5), is close to the average of the data for filaments > 4 μm.

In the above curve fitting, we obtained a value of 0.22 g s^{-1} cm^{-1} for ζ, which is about 10 times larger than the coefficient of the solvent friction for a microtubule (0.016 g s^{-1} cm^{-1}). This relatively large value of the friction coefficient which is obtained experimentally here is consistent with a value theoretically estimated for protein friction [18]. Such a large protein friction coefficient was proposed to explain the mechanism to limit the sliding velocity in the in vitro sliding movement [17].

In solution as well as in the above-mentioned protein environments [16], the diffusion coefficient of microtubules undergoing Brownian movements is approximately proportional to the inverse of the microtubule length. This is a direct consequence of the central limit theorem concerning the action of particles causing the random Brownian movements [19]. In contrast, as is shown in Fig. 5, the motional diffusion coefficient does not depend on the microtubule length when filaments are long enough. This length independence therefore indicates that the premises of the central limit theorem are violated in the directional sliding movements by dynein motors in vitro, as was pointed out in our previous work with kinesin [6]. In other words, there is a correlation in the sliding movement fluctuations.

The dynein used in the present study is monomeric, while the kinesin used in our previous study [6] is dimeric. Nonetheless, the motional diffusion coefficients of microtubules sliding over dynein and kinesin are both length-independent under certain conditions. Similar length-independence of the motional diffusion coefficients has also been found with actin filaments sliding over HMM in vitro (unpublished). Therefore, the length-independence of the motional diffusion coefficient, or the correlation in the sliding movement fluctuations, is a common property among cytoskeletal filament movements generated by (monomeric and dimeric) protein motors in vitro.

There are several possible explanations for the presence of correlation in the fluctuation in the microtubule sliding movement generated by dynein. Note that, unlike the single-headed 14S dynein from Tetrahymena cilia [3], sea urchin sperm β dynein does not induce rotation of microtubules during in vitro sliding movement [16]. Thus we do not consider the microtubule rotation for the cause of correlation in the fluctuation.

(i) The first possibility is to consider the effects on fluctuations of spatially non-uniform orientations of dynein motors attached on a glass surface. Under certain conditions, these non-uniform orientations can lead to the length-independence of the motional diffusion coefficient, as was shown theoretically [20].

(ii)The second possibility is to consider the effects of dynein density fluctuations in different regions on the surface. The density fluctuations may cause fluctuations in the sliding movement with length-independent motional diffusion coefficients.

The effects considered in the first and second possibilities could be also operative in the one-dimensional Brownian movements of microtubules occurring over dynein in the presence of vanadate (an ATPase inhibitor) and ATP as well, since the movements are considered to be generated by inactivated dynein heads through their weak interaction with microtubules [16]. However, the diffusion coefficients in the Brownian movements obey the $1/L$ law. This suggests that the first and second possibilities are unlikely.

The first and second possibilities can be checked experimentally by examining the positional fluctuations of the sliding movement of cytoskeletal filaments over protein motors with uniformly oriented heads and with little fluctuation of motor density. To do so, we with A. Yamada have been examining the length-dependence of positional fluctuations of actin filaments sliding along long native thick filaments of myosin from molluscan smooth muscle [21,22]. In the thick filaments, myosin heads are oriented and the head density fluctuations can be small. The results so far obtained, though preliminary yet, show length-independence of motional diffusion coefficients of actin filaments sliding along the native thick filaments. The following third possibility thus remains as a likely explanation for correlated fluctuations.

(iii) The third possibility is to assume cooperative action of dynein heads, as was discussed in our previous work with kinesin [6].

The length independence of the motional diffusion coefficient, if it is intrinsic to the sliding movement generated by protein motors, places a significant constraint on possible mechanisms of the sliding movement generation by protein motors in vitro. Holwill et al. [23] developed a model of ciliary motility and applied it to a computer simulation study of in vitro motility of microtubules over protein motors. They simulated the sliding displacement of microtubules having various lengths as a function of time. In their simulation results, the fluctuation of the microtubule displacement appears to be smaller for longer filaments, in contrast to our experimental results.

In the isometric force generation in vitro by myosin, the relative force fluctuation is proportional to $(\text{average force})^{-1/2}$ [24]. This means that the premises of the central limit theorem are not violated when there is no translational movement between the cytoskeletal filament and the protein motors, as was previously discussed [6].

To sum up, we came to the following two conclusions: (a) the fluctuation in the sliding movements of the microtubules is generated by the interaction of β dynein molecules with the microtubules; (b) when there is a translational sliding movement between a microtubule and β dynein molecules in vitro, the premises of the central limit theorem are violated in the sliding movement. These two conclusions with the foregoing discussions suggest that there is some coherence in the function of the β dynein molecules interacting with a sliding microtubule, although further studies are required to establish such coherence. (See Ref. [6] for some possible mechanisms of such coherent actions of protein motors.) The demonstration of the correlation in the sliding movement fluctuations with dynein and kinesin may thus lead to important insights about motor functions [6].

Acknowledgements

We thank Dr. Ken Sekimoto for discussion. We also thank Dr. John Murray for reading the manuscript. This work was in part supported by a grant from Asahi Glass Foundation to KT.

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