Thermal Drift Is Enough to Drive a Single Microtubule Along Its Axis Even in the Absence of Motor Proteins

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ABSTRACT One-dimensional diffusion of microtubules (MTs), a back-and-forth motion of MTs due to thermal diffusion, was reported in dynein motility assay. The interaction between MTs and dynein that allows such motion was implicated in its importance in the force generating cycle of dynein ATPase cycle. However, it was not known whether the phenomenon is special to motor proteins. Here we show two independent examples of one-dimensional diffusion of MTs in the absence of motor proteins. Dynamin, a MT-activated GTPase, causes a nucleotide dependent back-and-forth movement of single MT up to 1 μm along the longitudinal axes, although the MT never showed unidirectional consistent movement. Quantitative analysis of the motion and its nucleotide condition indicates that the motion is due to a thermal driven diffusion, restricted to one dimension, under the weak interaction between MT and dynamin. However, specific protein-protein interaction is not essential for the motion, because similar back-and-forth movement of MT was achieved on coverslips coated with only 0.8% methylcellulose. Both cases demonstrate that thermal diffusion could provide a considerable sliding of MTs only if MTs are restricted on the surface appropriately.

INTRODUCTION

Motor proteins translocate biological polymers such as actin filaments and microtubules (MTs) by ATP hydrolysis. In vitro motility assay has been a powerful technique to identify motor proteins as well as to elucidate the mechanism of the force generating enzymes (Howard et al., 1989; Ishijima et al., 1991; Uyeda et al., 1991; Vale et al., 1985). The movement of polymers by motor proteins is distinct from the Brownian movement by thermal drifts in a way that it is restricted to one dimension along the longitudinal axes of the polymer, unidirectional, and well correlated with the hydrolysis of ATP.

One-dimensional diffusion of MTs has been reported on dynein-coated coverslip after nucleotide hydrolysis is inhibited by vanadate (Vale et al., 1989). This phenomenon is a Brownian movement of MTs restricted to its longitudinal axes (aperiodic back-and-forth motion of MTs), which does not correspond to the dynein ATPase hydrolysis. It was implicated to show a state of a weak binding interaction between MTs and dynein, which may play an important role in the force-generating cycle of dynein ATPase (Vale et al., 1989; Vale and Oosawa, 1990). However, the nature of the weak binding interaction which would allow longitudinal thermal drifts of MTs remained unknown. Is it due to a very rapid association-dissociation reaction between polymer and its binding nucleotidase (Vale et al., 1989)? Is it a very special feature of dynein and MT?

Here we show two examples of one-dimensional diffusion of MTs in the absence of motor protein. One is in the presence of dynamin, a GTPase that interacts with MTs in a nucleotide-dependent manner (Maeda, et al., 1992). The second case is in the presence of methylcellulose without any nucleotides. Furthermore, the Methocell case showed clearly that specific protein-protein interaction such as motor protein-polymer interaction is not always necessary to such movement. From these observations, we consider the mechanism of one-dimensional diffusion and emphasize the effect of the coverslip surface on the phenomenon. We further discuss the role of thermal drifts in the motor protein mechanism.

MATERIALS AND METHODS

Purification of dynamin

Dynamin was purified from 5-w rat whole brain as described previously (Maeda et al., 1992). Briefly, flow-through fraction of brain cytosol (Fig. 1 *G, lane 1*) was incubated with taxol-polymerized MTs. MT pellets were extracted with 2.5 mM AMP-PNP and 5 mM GTP (*lane 2*) and then extracted with 10 mM GTP (*lane 3,* sup; *lane 4,* ppt). The second extracted sup was again subjected to DE52 to remove tubulin. The flow-through fraction (*lane 5*) was applied to 5%-20% sucrose density gradient. Each fraction was analyzed by SDS-PAGE and the purest dynamin fraction was used for motility assay.

Motility assay

Porcine brain tubulin, purified by phosphocellulose column, was polymerized into MTs in 1 mM GTP in PEM buffer (100 mM PIPES, 2 mM EGTA, 2 mM $MgCl_2$, (pH 6.8)) at 37°C for 2 min. Then taxol was added (final concentration, 6 μM) and diluted 10 times with the motility buffer. Ten microliters of purified dynamin in the motility buffer (10 mM Tris-Cl (pH 7.0), 30 mM sodium glutamate, 2 mM $MgSO_4$, 2 mM EGTA) was introduced on an 18 × 18 mm no. 0 coverslip at a concentration of 100 μg/ml and allowed to stand for 5 min, followed by adding nucleotides and 1 μl of 50 μg/ml taxol-polymerized MT. MTs were visualized on a Zeiss Axiophoto microscope with DIC using a 100-watt mercury light source. The image was projected onto a Hamamatsu c2400 Newvicon camera and contrast-enhanced with a Hamamatsu Argus 100 and recorded using a Sony 5800H U-matic video tape recorder. Selected frames were frozen and the

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position of the both ends of MT were measured on Argus 100. Methocell motility assay was performed as described above except the coverslip was incubated with Methocell solution instead of dynamin solution. Stock 2% Methocell solution in water was centrifuged at 15,000 rpm for 10 min. Supernatant was mixed with the same volume of the motility buffer without proteins or nucleotides and then incubated for 5 min on coverslips, followed by adding taxol-polymerized MT (final Methocell concentration, 0.8%).

Quantification of the movement of the microtubules

The images of the MTs recorded on videotape were analyzed with the Argus 10. The position of each of the ends of the MT ($x_1(t)$, $y_1(t)$), ($x_2(t)$, $y_2(t)$) was measured by the Argus 10 at 0.1-s interval (3 frames). The mean of the positions of both ends was used as the position of the MT ($x(t)$, $y(t)$) = (($x_1(t) + x_2(t)$)/2, ($y_1(t) + y_2(t)$)/2) The direction ($u(t)$, $v(t)$), and the length ($l(t)$) was calculated as

$$u(t) = x_2(t) - x_1(t), \quad v(t)=y_2(t) - y_1(t), \quad l(t) = \{u(t)^2+v(t)^2\}^{1/2} \tag{1}$$

From these variables, the differential displacement of the MT parallel to its long axis ($d_1(t)$) and perpendicular to it ($d_2(t)$) was calculated by the following formulae:

$$d_1(t) = \frac{u(t)\Delta x(t) + v(t)\Delta y(t)}{l(t)}, \quad d_2(t) = \frac{u(t)\Delta x(t) - v(t)\Delta y(t)}{l(t)} \tag{2}$$

where

$$\Delta x(t) = x(t + 0.1) - x(t), \quad \Delta y(t) = y(t + 0.1) - y(t)$$

The net displacement parallel to its long axis ($D_1(t)$) and perpendicular to it ($D_2(t)$) were calculated by summing up these variables:

$$D_1(t) = d_1(0) + d_1(0.1) + \cdots + d_1(t), \tag{3}$$

$$D_2(t) = d_2(0) + d_2(0.1) + \cdots + d_2(t),$$

We used $D_1(t)$ and $D_2(t)$ as the raw data of the position of the MTs.

Quantitative analysis of the movement

We have plotted $D_1(t)$ and $D_2(t)$ against t and the histogram of $d_1(t)$ and $d_2(t)$. In the experiment with dynamin or Methocell, no linear trends were found in D, and the distribution of d was symmetrical to the zero axis. In the control experiment using kinesin, $D_1(t)$ was almost linearly increased against t, and $d_1(t)$ fluctuated around its mean (significantly larger than zero). We have used the fluctuation of $d_1(t)$ around its mean for the estimation of the accuracy of our measurement, for Gelles et al. (1988) have shown that the unidirectional movement of MT by kinesin is so smooth that the fluctuation of its position is much smaller than that can be measured by our system.

The autocorrelogram and the power spectra of $d_1(t)$ were estimated by Burg's maximum entropy method (Burg, 1967) to clarify the nature of the fluctuation of $d_1(t)$ and to compare the fluctuation in the experiment of dynamin with that of Methocell.

To test the hypothesis that fluctuation of $d_1(t)$ is due to an independent random process, we have tested whether the mean square displacement increased linearly against time (Papoulis, 1984). From eight independent data, the mean of $\{D_1(t)\}^2$ was calculated for t = 0, 5, 10, 15, and 20 s and plotted against t. These MTs were 1.5 μm (SD = 0.5 μm) in length. The points were fitted to $\{D_1(t)\}^2 = at$ with least mean square error methods, and its fitting was tested by F-test (Snedecar and Cochran, 1980).

Calculation of diffusion coefficient

The observed diffusion coefficient along the long axis of MT (D_L) was estimated as $D_L = a/2 = t/2$(mean square displacement). The observed diffusion coefficient was 0.13×10^{-10} cm^2/s (parallel to the long axis, with dynamin), and 0.22×10^{-10} cm^2/s (parallel, with Methocell). The theoretical diffusion coefficient due to solvent molecule (D_s) of unconstrained MTs is calculated according to Vale et al. (1989). Briefly, diffusion coefficient D is described as:

$$D = kT/\zeta \tag{4}$$

where k is Boltzmann constant, T is absolute temperature, and ζ is the drag coefficient. For a long cylinder of length l and radius r moving parallel to its long axis at height h above a surface, the drag coefficient is calculated as

$$\zeta = 2\pi\eta l/\ln(2h/r) \tag{5}$$

while the drag coefficient for motion perpendicular to the long axis of MT is

$$\zeta = 4\pi\eta l/\ln(2h/r) \tag{6}$$

Thus a MT of length 2 μm and radius 12 nm at an estimated height 20 nm (single molecular structure of dynamin is 10–20 nm; Maeda et al., 1992), in a medium of viscosity (η) 1 cP (without Methocell) and viscosity 100 cP (with 0.8% Methocell; Uyeda et al., 1990), at 293 K has a diffusion coefficient 40×10^{-10} cm^2/s (parallel, without Methocell), 20×10^{-10} cm^2/s (perpendicular, without Methocell), 0.4×10^{-10} cm^2/s (parallel, with Methocell), and 0.2×10^{-10} cm^2/s (perpendicular, with Methocell). Apparently, the effect of the protein-protein interaction or the effect of the Methocell polymer is neglected in these theoretical values.

Microtubule binding assay

Dynamin was incubated with taxol-polymerized MTs in the motility buffer without nucleotides and centrifuged at 100,000 $\times$ g for 20 min. The pellet was resuspended with the motility buffer containing nucleotides and incubated for 20 min at 37°C. Then the samples were again centrifuged at 100,000 $\times$ g for 20 min at room temperature. The resulting supernatants and pellets were subjected to 7.5% SDS-PAGE.

RESULTS

We found back-and-forth movements of MTs on dynamin-coated coverslips (Fig. 1 *A*). It was controversial that dynamin, a MT-activated GTPase, is a motor protein, because MT motility was not reported except for the initial report (Sphetner and Vallee, 1989) and contamination with kinesin was suspected. Our preparation of dynamin is essentially free of cytoplasmic dynein and kinesin (Fig. 1 *G*). Purified dynamin consistently exhibit high MT activated GTPase activity (K_m = 25 μM, V_{max} = 5.5 min^{-1}; Maeda et al., 1992). However, no unidirectional MT movement was observed at any nucleotide condition, as we reported previously (Maeda et al., 1992). Instead, we found that short MTs (<3 m) undergo continual aperiodic backward and forward displacements along its longitudinal axes. The back-and-forth linear movement of MTs required the presence of dynamin on the glass surface as well as nucleotides (10 mM ATP, 5 mM GTP, and 2 mM AMP-PNP at 2 mM Mg^{2+}) in the solution (Table 1). Electron microscopy of the samples for motility assay revealed that virtually all short MTs <3 μm are single MTs (data not shown).

Quantification of the displacements of parallel and perpendicular to the longitudinal axes of MTs undergoing back-and-forth motion confirms that the movement was essentially

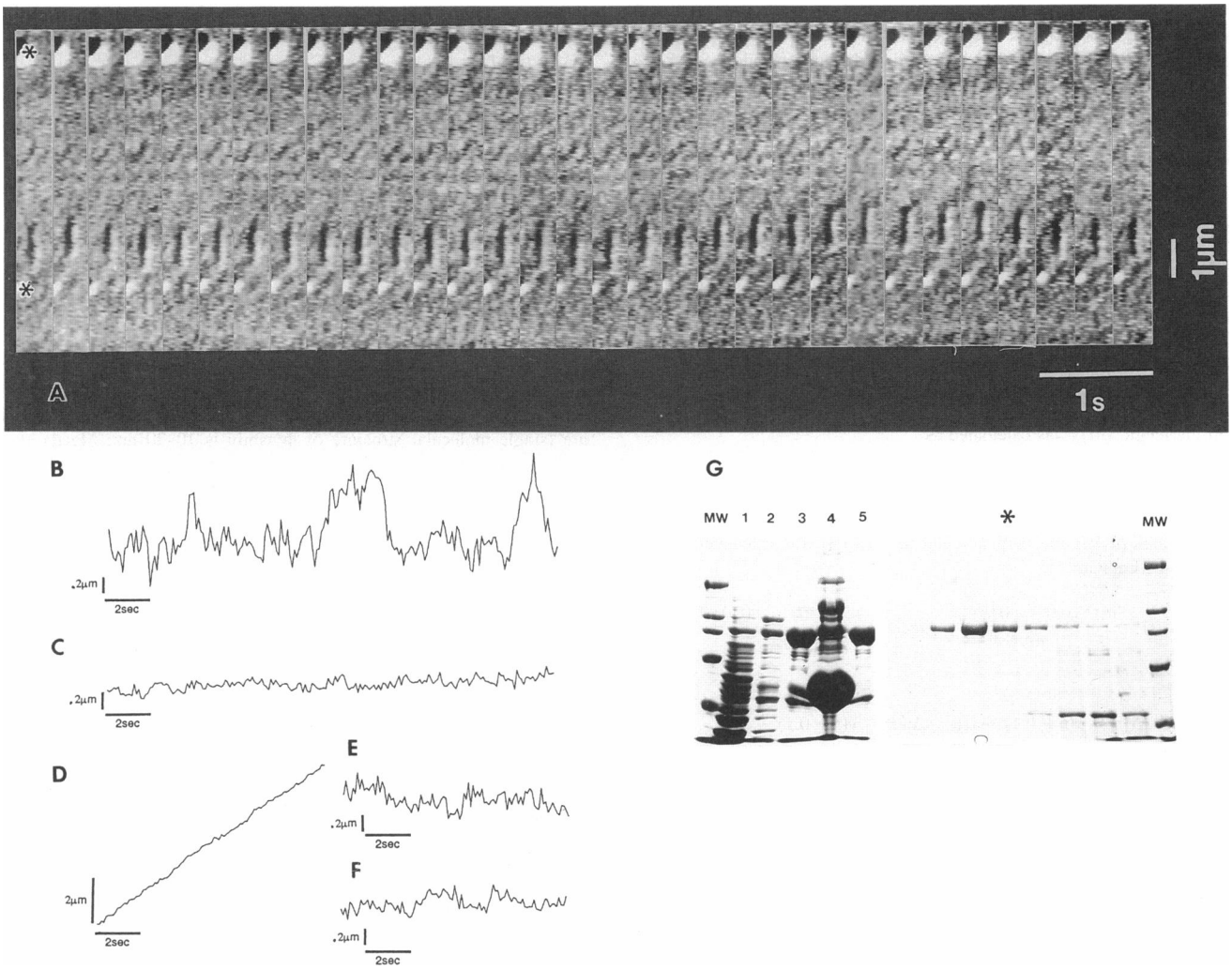

FIGURE 1 Back-and-forth motion of MT on dynamin-coated coverslip in the presence of 5 mM GTP, 10 mM ATP, and 2 mM AMP-PNP. Sequential micrographs of a typical back-and-forth movement (*A*). The intervals between successive images was 0.33 s. The position of the two stationary markers on the glass surface are indicated by *asterisks* in the first panel. Quantification of the back-and-forth motion of the MT in directions parallel (*B*) and perpendicular (*C*) to the MT longitudinal axes are shown for each 0.1-s time interval. (*D–F*) Quantification of the unidirectional movement of MT on kinesin-coated coverslips in directions parallel (*D*) and perpendicular (*E*) to the MT longitudinal axis. (*F*) Residual error ($(D) - \bar{v} \cdot t\bar{v}$:mean velocity of MTs), showing the level of error in measurement. (*G*) Dynamin purification. DE52 flow-through fraction of brain cytosol (lane *1*) was incubated with taxol-polymerized MTs. MT pellets were extracted with 2.5 mM AMP-PNP and 5 mM GTP (lane *2*) and then extracted with 10 mM GTP (lane *3*, sup; lane *4*, ppt). The second extracted sup were again subjected to DE52 to remove tubulin. The flow-through fraction (lane *5*) was applied to 5%-20% sucrose density gradient. Each fraction was analyzed by SDS-PAGE and the purest dynamin fraction was used for motility assay.

confined to the longitudinal axes of the MTs (Fig. 1, *B* and *C*). Their displacements perpendicular to their axes were within the range of the error of our measurement estimated from the control movement of MTs driven by kinesin used as the control (Fig. 1, *D–F*). This motion was quite different from the random movement of MTs detached from coverslips, a vibrational movement with no apparent preferred direction of the movement. But this back-and-forth motion of MTs on dynamin-coated coverslips was similar to Brownian movement in that it did not contribute to the net translocation of MT in either direction even after observation for several minutes, and that it looked like stochastic vibration (Fig. 1). Its amplitude was in the order of 1 μm, which is much larger than the single molecular size of dynamin ~10 nm (Maeda et al., 1992), indicating that MTs were not simply tethered to one or more dynamin.

We tested whether this motion of MTs was thermal drift of MTs weakly interacting with dynamin in a nucleotide-dependent manner, as proposed by Vale et al. (1989), in the dynein case.

First, the quantitative analysis of the movement showed that the motion was essentially a stochastic process (Fig. 4). Analysis of the typical movement along the longitudinal axes at intervals of 0.1 s up to 30 s showed that the differential displacement $d_1(t)$ fluctuated around 0, with a Lorenzian power spectrum (Fig. 4 *A*). Thus $d_1(t)$ obeys the Langevin

TABLE 1 Nucleotide and dynamin-dependent attachment and back-and-forth movement of MT

Sample	Nucleotides	Attachment	Movement
Dynamin	No addition	Yes	No
Dynamin	5–15 mM GTP	Yes	No
Dynamin	5 mM ATP	Yes	No
Dynamin	10 mM ATP	Weak	Back-and-forth*
Dynamin	15 mM ATP	No	
Dynamin	5 mM GTP+ 10 mM ATP	Weak	Vibration
Dynamin	5 mM GTP+ 10 mM ATP+ 2 mM AMP-PNP	Yes	Back-and-forth‡
Dynamin	5 mM GDP+ 10 mM ATP+ 2 mM AMP-PNP	Yes	Back-and-forth§
Kinesin	5 mM ATP	Yes	Unidirectional
Kinesin	5 mM GTP+ 10 mM ATP + 2 mM AMP-PNP	No	
Buffer	5 mM GTP+ 10 mM ATP + 2 mM AMP-PNP	No	

* Very few.
‡ More than 90% of MT less than 3 μm.
§ Approximately half of the MT less than 3 μm.

equation of Brownian movement:

$$d\,d_1(t)\,/\,dt = -\,\gamma d_1(t) + f(t) \quad (\gamma>0), \qquad (7)$$

where $f(t)$ is a white Gaussian noise term, indicating that the movement is driven by independent random process such as thermal drifts (Fig. 4 *A*). Later, we show that $-\gamma d_1(t)$ is mainly due to the protein friction between MTs and dynamin (see Eq. 9). Furthermore, mean square displacement of eight MTs increased linearly with the length of the time interval, also showing that the motion is driven by a random independent process (Pappoulis, 1984) (Fig. 4 *B*).

Second, the optimal condition for back-and-forth motility corresponds to the condition for MT-dynamin binding rather than that for dynamin's nucleotidase activity (Table 1, Fig. 2). Very high concentration of nucleotides is necessary for the motility and for the release of dynamin from MTs compared with the nucleotidase activity. GTP alone could not achieve the movements. Substitution of GTP by GDP in the nucleotide mixture caused the back-and-forth motion, while dynamin is a MT-activated GTPase rather than ATPase (Sphetner and Vallee, 1992; Maeda et al., 1992). In contrast, dynamin dissociates from MTs most efficiently with 15 mM ATP, then ATP + GTP, ATP + GTP + AMP-PNP, and dynamin barely dissociates with 15 mM GTP only (Fig. 2), indicating the extent of the interaction between MTs and dynamin in each nucleotide condition. This corresponds well to the behavior of MTs in the motility assay: detachment, then vibration, back-and-forth motion, and tight attachment, respectively (Table 1). Furthermore, Mg^{2+} dependence of dynamin-MT binding also correlated with the MT behavior in the motility assay (Fig. 2, Table 2). MTs dissociated from coverslips and MTs in low concentration of Mg^{2+} and attached tightly to coverslips in high concentration of Mg^{2+}. Appropriate Mg^{2+} concentration was necessary to achieve the back-and-forth motion.

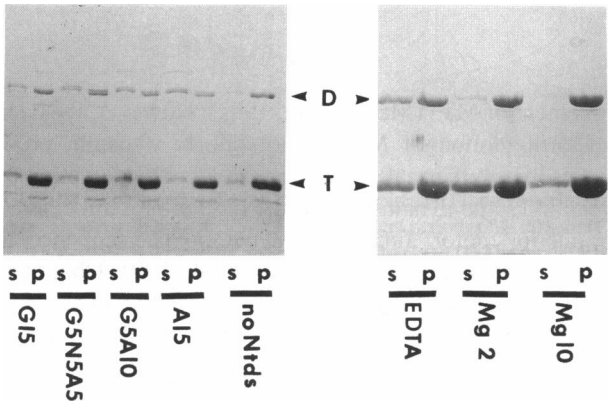

FIGURE 2 MT binding of dynamin in various nucleotide conditions were analyzed with SDS-PAGE. Each lane shows sup(s) and ppt(p) after extraction of dynamin-MT complex with 15 mM GTP (G15), 5 mM GTP + 5 mM AMP-PNP + 5 mM ATP (G5N5A5), 5 mM GTP + 10 mM ATP (G5A10), and 15 mM ATP (A15), at 2 mM Mg^{2+}. Gel on *right* shows magnesium dependence of dynamin binding to MTs after extraction with nucleotide mixture (5 mM GTP, 2 mM AMP-PNP, and 10 mM ATP) in the presence of 5 mM EDTA, 2 mM Mg^{2+}, and 10 mM Mg^{2+}, respectively. D, dynamin; T, tubulin.

Third, we could reproduce quite similar movement of MTs in 0.8% methylcellulose (Methocell) solution in the motility buffer in the absence of such proteins nor nucleotides (Fig. 3, *A–C*). MTs attached well and most of the short MTs made one-dimensional back-and-forth movement on Methocell-coated cover glass. Once they detach, they vibrate in all directions (Fig. 3 *D*), showing that the motion is specific to the MTs attached to coverslips. This is in contrast with the repetitive movement of actin filament in Methocell solution (Uyeda et al., 1990).

Because no motor proteins exist in the Methocell case, the motion should be due to the thermal drifts. Quantification and its analysis showed that the back-and-forth motion by

TABLE 2 Magnesium dependence of MT attachment and movement

Mg^{2+}	Attachment	Back-and-forth movement*
20 mM	yes	0/100
10 mM	yes	0/100
2 mM	yes	43/59
10 μM	no	
5 mM EDTA	no	

* Number of MT with back-and-forth movement/total number of MT less than 3 μm counted.

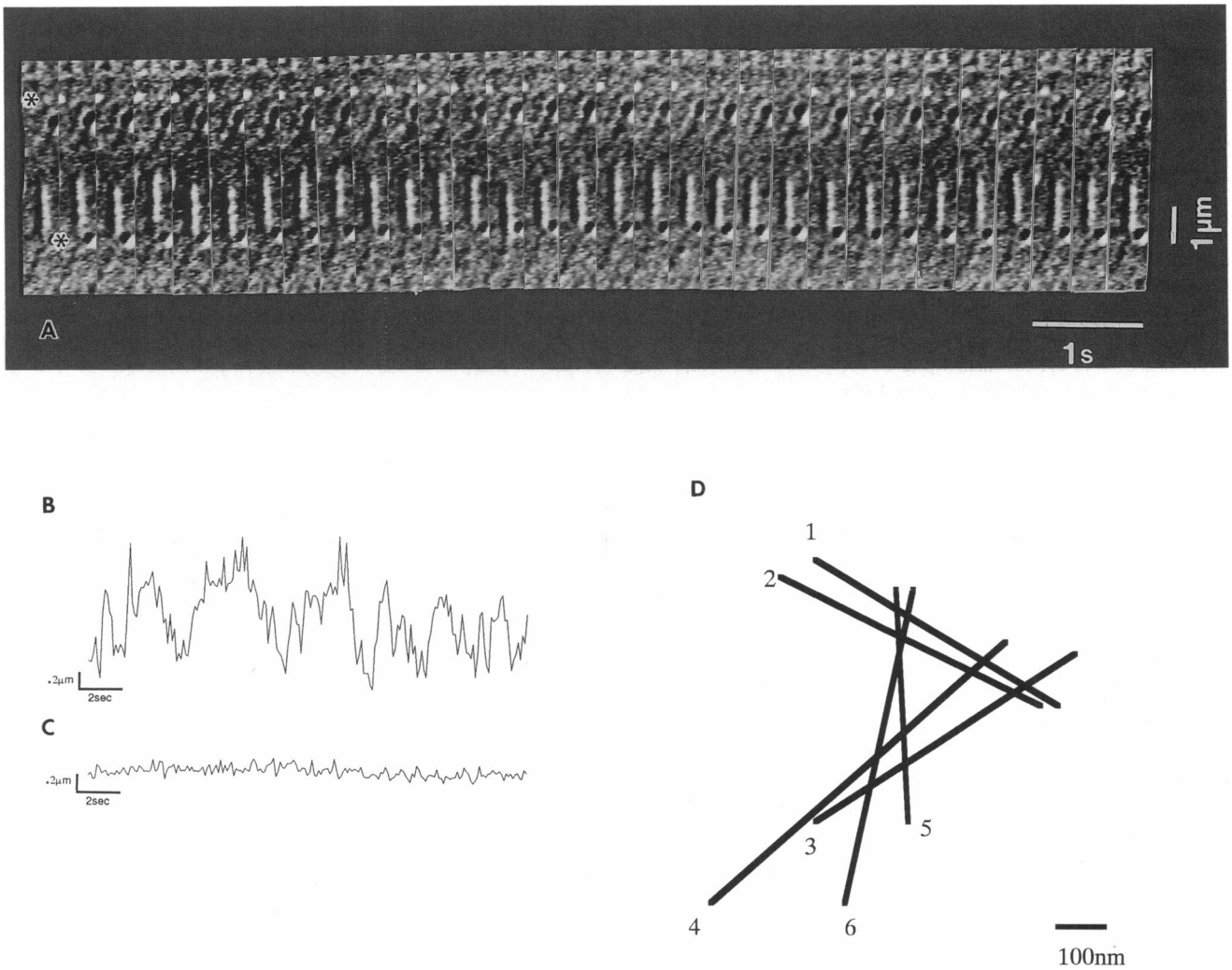

FIGURE 3 MTs show the back-and-forth movement in 0.8% Methocell. (*A*) Sequential micrographs of a MT undergoing back-and-forth movement at 0.33-s interval. (*B*) Quantification of the back-and-forth motion of a MT as described in Fig. 1, *B* and *C*. (*D*) Tracing of video images of MTs detached from the surface of coverslip at 0.2-s intervals in a Methocell solution. A MT that had performed one-dimensional diffusion suddenly detached from the coverslip (1), rotated (2–5), and again touched the coverslip (6).

Methocell was quite similar to the motion by dynamin (Figs. 3 and 4), suggesting that the driving force of MTs is the same in both cases.

DISCUSSION

Back-and-forth motion of MTs is not due to "motor" function of dynamin

We showed that dynamin causes the back-and-forth motion of MTs in a nucleotide-dependent manner. Although dynamin was first reported to move MTs in vitro (Shpetner and Vallee, 1989), subsequent studies from the same laboratory and other laboratories could not show MT motility again (Sphetner and Vallee, 1992; Scaife and Margolis, 1990; Maeda et al., 1992). In the present study, using highly purified dynamin essentially free of other contaminating motor proteins, we succeeded in showing MT movement on dynamin-coated coverslips quite reproducibly, although purified dynamin did not cause a consistent unidirectional movement of MTs (Maeda et al., 1992). However, the back-and-forth motion of MTs is not due to dynamin power strokes, but to thermal driven forces in weak interaction between MTs and dynamin. Thus the motion presented here is not due to "motor" function of dynamin. The sequence homology with other proteins (Nakata et al., 1991; Obar et al., 1990; Chen et al, 1991, Rothman et al., 1990; van der Bliek and Meyerowitz, 1991) suggests a role for dynamin as a molecular switch for membrane traffic (Noda et al., 1993).

It is not known why a nucleotide mixture is required for one-dimensional diffusion of MTs on dynamin-coated coverslips. One possibility is that MT, which moves back and forth, associates with dynamin molecules on a number of different molecules, and that the appropriate proportion of number of attached GTP-dynamin, ATP-dynamin, and AMP-PNP-dynamin is necessary for the one-dimensional diffusion. Another possibility is that the addition of ATP and AMP-PNP may effect the interaction between adjacent dynamin molecules within oligomers (Maeda et al., 1992)

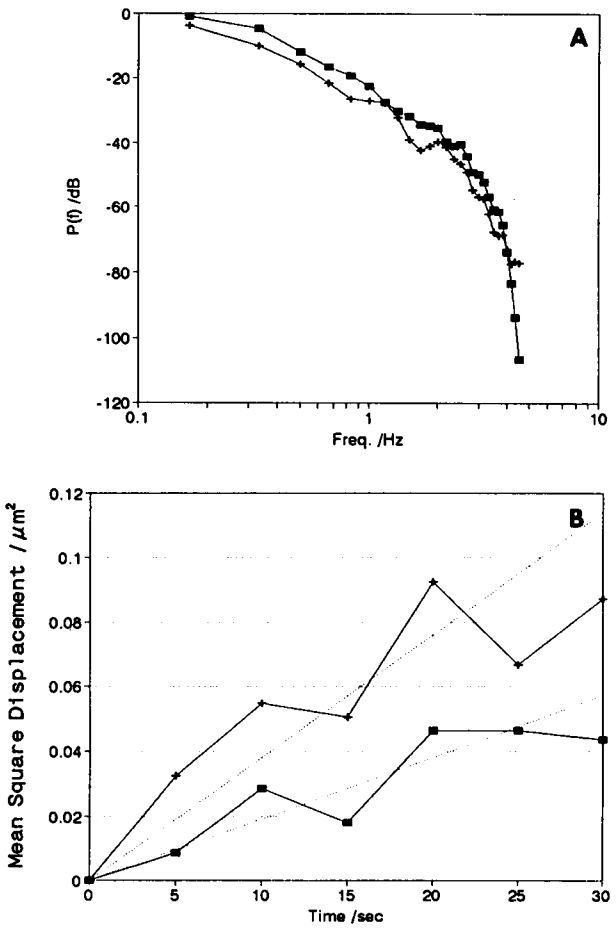

FIGURE 4 (*A*) Power spectra of typical back-and-forth motion of a MT by dynamin and Methocell. (*B*) Mean square displacements of the back-and-forth motion of each of the eight MTs (1.5 μm in length) by dynamin and Methocell.

and thus secondarily effect the association of these dynamin molecules and MT. In fact, the combination of the nucleotides appears to affect the binding between MTs and dynamin (Fig. 2).

Different mechanisms of MT constraints cause a similar one-dimensional diffusion of MTs

One-dimensional diffusion indicates that the diffusion coefficient perpendicular to the long axis of MTs (D_P) is much smaller than that along the long axis (D_L). However, the observed D_L was also smaller than the theoretical value for unconstrained diffusion. In the case of dynein, the observed D_L was 2×10^{-10} cm^2/s (dynein β chain) and 0.9×10^{-10} cm^2/s (dynein, intact), whereas the theoretical value was 67×10^{-10} cm^2/s (Vale et al., 1989). In the case of dynamin, D_L was 0.18×10^{-10} cm^2/s, whereas the theoretical value was 40×10^{-10} cm^2/s. The discrepancy between the observed and theoretical values could be attributed to the mechanical constraint between MTs and the proteins (dynein or dynamin).

If we allow the drag coefficients along the longitudinal axis of MTs due to the solvent molecules and the dynein or dynamin be ζ_s and ζ_d, respectively, then the Langevin equation describing the Brownian motion of the MT is:

$$m(dd_1/dt) = -(\zeta_s + \zeta_d)d_1 + f(t), \quad \langle f(t)\rangle = 0, \qquad \langle f(t)f(s)\rangle = xf\delta(t - s), \tag{8}$$

where d_1 is the velocity of the MT, and m is the mass of MT (Tawada and Sekimoto, 1991). Using Einstein's equation (4), the apparent diffusion coefficient D_L is divided into

$$D_L^{-1} = D_s^{-1} + D_d^{-1} \tag{9}$$

where $D_s = kT/\zeta_s$, $D_d = kT/\zeta_d$.

Because the theoretical value (D_s) is much smaller than the observed D_L, we see that the observed D_L can be approximately D_d, which is due to the protein-MT interactions. The difference between the observed D_L between dynein β chain, intact dynein, and dynamin could be attributed to the difference of the friction coefficient between MTs and these molecules. In the case of Methocell, the observed D_L fitted well with the calculated D_s parallel to the long axis of MT. However, the reduced mobility of MT perpendicular to its long axis could not be explained by the calculation (Eq. 6) in Materials and Methods. This might be explained by considering the rheological properties of polymer solvent (Methocell), such as reptation model (de Gennes, 1991). However, it should be emphasized that MTs move back and forth only on the surface of the coverslip (Fig. 3 *D*), clearly different from the case of actin filament (Uyeda et al., 1990). It may be possible that electrostatic charges between MTs and coverslips might play a role in this system. The effect of surface on the reptile movement in polymer solution would be an interesting question, and the present MT motion would present a simple example for analyzing the issue.

Although the mechanism for the restriction of MT movement might be different between Methocell and dynamin cases, the observed motion was quite similar in both cases. Thus this phenomenon appears to occur under the appropriate restriction of the MT movement on the surface of coverslip, whether it is a specific protein-protein interaction or nonspecific restriction by a polymer lattice. The restriction of the movement may be produced through rapid association/dissociation of motor protein and MTs (Tawada and Sekimoto, 1991), but the Methocell case showed that the phenomenon itself is not special to the motor protein.

Motor protein and diffusion

One-dimensional diffusion of MTs has been proposed to play an important role in directional movement by motor proteins (Vale and Oosawa, 1990). Our examples showed that motor protein is not necessary for one-dimensional diffusion of MTs. On the other hand, the present study provided an experimental evidence that thermal drifts have certainly enough power to translocate MTs along longitudinal axes without considering any effect caused by motor protein. Thus concerning the relationship between one-dimensional diffusion and motor protein mechanism, two possibilities are raised. One possibility is that one-dimensional diffusion is an in

vitro artifact and plays no role in motor mechanism. However, another possibility is that biological motors use the thermal drifts as a source of their driving force. In such a case, ATP hydrolysis by motor proteins might be used as Maxwell's demon (Vale and Oosawa, 1991). In fact, recent data shows the myosin step size is considerably larger than the estimated size of conformational change of myosin head (Ishijima et al., 1991). Models have been proposed that motor mechanism is a ratcheted Brownian movement (Vale and Oosawa, 1990; Cordova et al., 1992; Huxley, 1957; Alberts and Miake-Lye, 1992). In these models, $f(t)$ in Eq. 8 should be produced by the thermally generated structural fluctuations of motor head f_d. In our dynamin example, it is difficult to differentiate the contribution of f_d from that of f_s (due to the atomic collision of the solvent molecules with the MTs). The case of Methocell suggests the major contribution of f_s to the motion. Even if it is the case, it should be noted that MTs show thermal driven motility in a system which is commonly used in the motility assay of motor proteins in vitro, and the force or motility we see in motor protein assay is a combination of the thermal and "motor" power, whether or not thermal power facilitates or disturbs the motor protein-dependent motility.

Modified Brownian movement may be involved in various cellular events such as enzyme access to substrate (Adam and Delbruck, 1968), DNA binding protein (Winter et al., 1981), chromosome moving (Koshland et al., 1988), and filopodia formation (Peskin et al., 1993). Further study of these restricted diffusion processes in microscopic systems is necessary, and the Methocell system may be a useful simple model to evaluate the role of diffusion in biological polymer behavior (de Gennes, 1979). Our data further showed an importance of glass surface in the diffusional motion of MTs, which we should consider seriously in both in vitro and in vivo motility assay.

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