Mechanical and Chemical Properties of Cysteine-Modified Kinesin Molecules

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ABSTRACT: To probe the structural changes within kinesin molecules, we made the mutants of motor domains of two-headed kinesin (4−411 aa) in which either all the five cysteines or all except Cys45 were mutated. A residual cysteine (Cys45) of the kinesin mutant was labeled with an environment-sensitive fluorescent probe, acrylodan. ATPase activity, mechanical properties, and fluorescence intensity of the mutants were measured. Upon acrylodan-labeled kinesin binding to microtubules in the presence of 1 mM AMPPNP, the peak intensity was enhanced by 3.4-fold, indicating the structural change of the kinesin head by the binding. Substitution of cysteines decreased both the maximum microtubule-activated ATPase and the sliding velocity to the same extent. However, the maximum force and the step size were not affected; the force produced by a single molecule was 6−6.5 pN, and a step size due to the hydrolysis of one ATP molecule by kinesin molecules was about 10 nm for all kinesins. This step size was close to a unitary step size of 8 nm. Thus, the mechanical events of kinesin are tightly coupled with the chemical events.

Kinesin moves along microtubules to the plus end, utilizing the energy of ATP hydrolysis (*1, 2*). *Drosophila* kinesin contains two heads, each of which includes a catalytic domain (N-terminal 1−340 aa) and an α-helical stalk (350−975 aa) (*3*). The catalytic domain, which has ATP binding and microtubule binding sites, is highly conserved among kinesin family members (*4*). Recently, the three-dimensional atomic structure of the catalytic domain was obtained by X-ray crystallography (*5*). Microtubule binding sites on the catalytic domain were identified by alanine scanning mutagenesis (*6*).

The motility of single kinesin molecules has been investigated using optical trapping nanometry (*7*−*9*). Kinesin moves with a step of 8 nm in the fashion of a hand-over-hand mechanism (*7, 9, 10*). Analysis of the step dwell time suggested that each kinesin head moves with a step of 16 nm, or a kinesin molecule, center of mass of two heads, moves with a step of 8 nm (*9*). Statistical analysis of step intervals and ATPase activity was consistent with an 8 nm step per hydrolysis of one ATP molecule by kinesin (*9, 11, 12*). Thus, one mechanical step of 8 nm is coupled to one ATP hydrolysis cycle; that is, there appears to be a tight coupling between mechanical and chemical reactions. This contrasts with the long step size per one ATP hydrolysis cycle (longer than 20 nm) produced by myosin on actin filaments (*13*−*15*), which suggests a looser coupling of the mechano-chemical reaction, although some data showed a shorter step size of 4−11 nm (*16, 17*).

In an attempt to probe structural changes that occur within a two-headed kinesin molecule (4−411 aa) (*3*), we have been substituting existing cysteine residues with other amino acids and putting new cysteines at the desired positions to be labeled. In this study, we prepared kinesin mutants to determine if these mutants retained the characteristics of kinesin. We substituted all cysteines or all except Cys45 by the other amino acids and labeled the residual cysteine with the fluorescent dye aclyrodan. These mutants resulted in a proportionate decrease in the sliding velocity of single molecules and the ATPase rate. The step size, calculated as the ratio of the sliding velocity to the ATPase rate, as well as the force generated were the same for all kinesins studied. These results suggest that modification of cysteine does not affect the basic process of force generation and the step size per ATP molecule hydrolyzed.

MATERIALS AND METHODS

Construction of pGEX-DK411-BDTC. The coding sequences for the 411 N-terminal residues of *Drosophila* kinesin heavy chain (DKH4−411) and biotinylated peptide in *E. coli* for a segment coding a part of the 1.3S subunit of *Propionibacterium shermanii* transcarboxylase (BDTC) were obtained from the *Sac*II−*Eag*I fragment of pBS 1-2 (*18*) and the *Sal*I−*Sma*I fragment of pinpoint Xa-1 (Promega), respectively. These fragments were constructed in tandem into the *Xho*I site of pGEX-4T-1, which is the glutathione *S*-transferase (GST) expression vector (Pharmacia Biotech). This GST kinesin fusion plasmid was designated pGEX4T-

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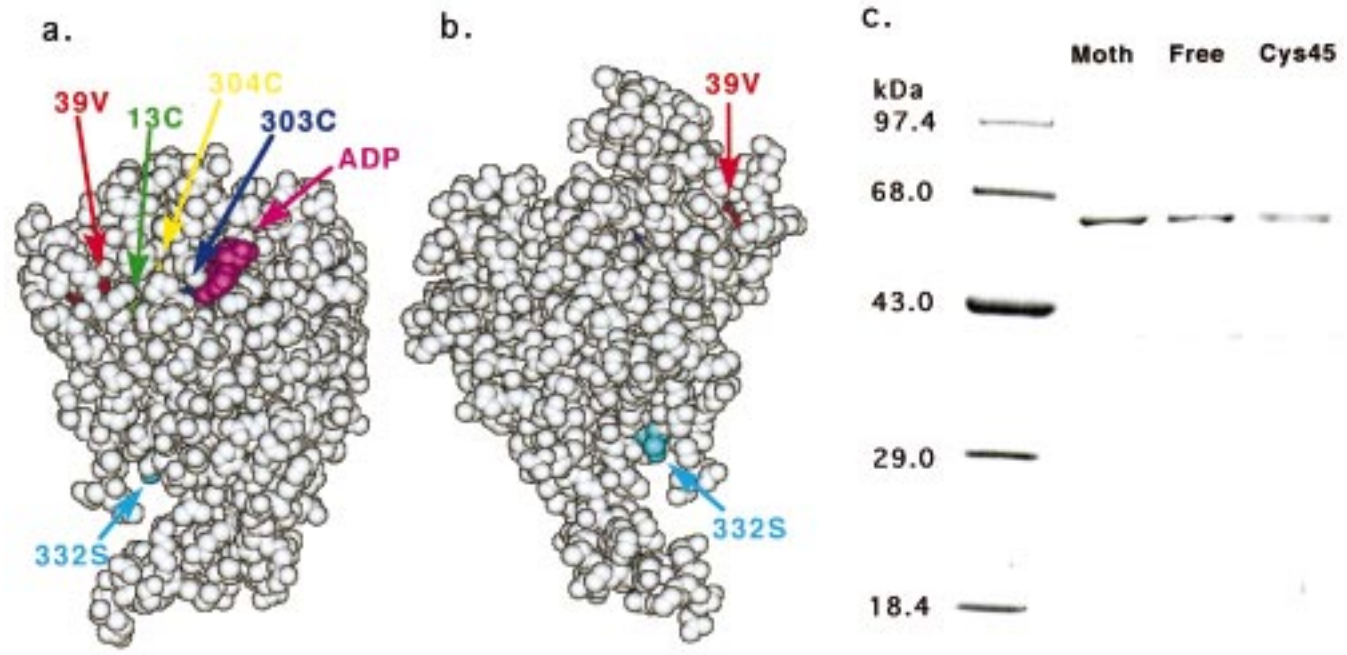

FIGURE 1: Positions of cysteines on the three-dimensional atomic structure and purity of kinesin mutants. (a and b) Space-filling models of the kinesin motor domain oriented on the ADP binding side (a) and its backside (b). Positions of cysteine residues 17C, 45C, 309C, 310C, and 338C of *Drosophila* kinesin correspond to these of 13C (green), 39V (red), 303C (blue), 304C (yellow), and 332S (cyan) of rat kinesin, respectively (*27*). Magenta balls indicate ADP. (c) SDS−PAGE of kinesin mutants. Left lane, molecular mass markers, with masses shown on the left. Moth, mother mutants (DKH411-BDTC) of kinesin heavy chain with BDTC; Free, cysteine-free DKH411-BDTC; Cys45, cysteine 45 containing DKH411-BDTC.

Table 1: Mutants of Kinesin

	no. of amino acids				
	17	45	309	310	338
mother	C	C	C	C	C
Cys-free	V	A	T	V	S
Cys45	V	C	T	V	S

DKH411-BDTC. The DKH411-BDTC protein, named mother kinesin, contains five cysteine residues at positions 17th, 45th, 309th, 310th, and 338th (Figure 1). A mutant in which all cysteines are substituted (C17V, C45A, C309T, C310V, and C338S), referred to as Cys-free kinesin, and a mutant in which all but Cys45 are substituted (C17V, C309T, C310V, and C338S), referred to as Cys45 kinesin (Table 1), were made by the LA PCR in vitro Mutagenesis Kit (TAKARA), using specific oligonucleotide primers. The correctness of the PCR-derived fragments was confirmed by double-stranded DNA sequencing using the high chemiluminescent DNA sequencing kit (Toyoba). This construct was designated pGEX4T- DKH411cf-BDTC.

Expression and Purification. E. coli 71-18 was transformed with pGEX4T-DKH411-BDTC and pGEX4T-DKH411cf-BDTC for expression of GST-DKH411-BDTC and GST-DKH411cf-BDTC, respectively. Cells were grown with shaking at 25 °C in 500 mL of LB media (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter) supplemented with ampicillin (0.05 mg/mL) until $OD_{595} = 1.0$. To express these fusion proteins, isopropyl β-D-thiogalactoside (IPTG) was added to the culture media at a final concentration of 1 mM and incubated for 10 h. The cells were chilled and collected by centrifugation at 3500*g* for 10 min and suspended with 8 mL of cold lysis buffer (16 mM Na_2HPO_4, 4 mM NaH_2PO_4, 150 mM NaCl, 1 mM EGTA, 1 mM p-APMSF, 1 mM DTT). The cells (2.5 g) were lysed by sonication for 30 s 3 times, and the lysate was centrifuged at 20000*g* for 20 min to remove insoluble materials. One milliliter of 50% glutathione−agarose suspension (G-4510, Sigma) in Pipes solution [50 mM Pipes, pH 6.8, 150 mM NaCl, 1 mM $MgCl_2$, and 25 μM AMPPNP (5′-adenylyl imidodiphosphate)] was added to the supernatant to adsorb the GST−kinesin. A slurry of the glutathione was poured into a chromatography column (polyprep column, Bio-Rad) and washed thoroughly with the Pipes solution. Cys45 kinesin (2 μM) was labeled for 60 min at 25 °C with 40 μM acrylodan (A-433, Molecular Probes) in Pipes solution (a labeled Cys45 kinesin), and the unreacted dye was washed out with the Pipes solution containing 1 mM DTT. Then thrombin (30 units after addition) and $CaCl_2$ (2 mM after addition) were added to kinesin solution in the resin. After incubation for 60 min at 25 °C, GST-free kinesin was eluted with the Pipes solution. Affinity purification of the mutants was done with taxol-stabilized bovine brain microtubules in solution containing 0.1 mM AMPPNP, 80 mM Pipes (pH 6.8), 1 mM EGTA, and 2 mM $MgCl_2$ (*20*). After incubation for 10 min at 25 °C, microtubule−kinesin complex was pelleted by centrifugation at 355000*g* for 10 min. Kinesin was released from microtubules in solution containing ATP [250 mM sodium acetate, 80 mM Pipes (pH 6.8), 1 mM EGTA, 2 mM $MgCl_2$, and 0.5 mM ATP]. Then 1−5 μM purified kinesin mutants (mother, Cys-free, Cys45, and labeled Cys45 kinesins) was collected from the supernatant after centrifugation at 355000*g* for 5 min.

Microtubule used in the present study was obtained by polymerization of bovine brain tubulin. Protein concentrations of kinesin and microtubule were determined with Coomassie Brilliant Blue G-250 staining (Tonein TP; Ootuka Co., Japan), using BSA as a standard. The molar concentrations of the kinesin mutants and microtubules were calculated

using their molecular masses of 120 kDa for two-headed kinesin and 110 kDa for tubulin dimer.

Fluorescence Emission Spectrum. The fluorescence spectrum of acrylodan labeled to cysteine 45 of Cys45 kinesin was recorded using a Spex Fluorolog-2 spectrofluorometer (Fluorolog, Edison, NJ): 150 μL of 20−100 nM Cys45 kinesin in solution containing 80 mM Pipes, 1 mM EGTA, and 2 mM $MgCl_2$ in the absence and presence of microtubule and AMPPNP (final concentrations of 2 μM and 1 mM, respectively) was excited at a wavelength of 365 nm at 25 °C.

ATPase Assay. Mg-ATPase and microtubule-activated ATPase assays of kinesins were carried out at 25 °C using a modified Malachite Green method (*19*). The ATPase activities (P_i released per second per head) were measured at 25 °C in a solution (80 mM Pipes, 1 mM EGTA, 2 mM $MgCl_2$, 0.2 mg/mL casein, 40 mM taxol) containing 5−10 nM kinesin, 10 μM−1 mM ATP, and 50 nM−4 μM tubulin dimer.

Optical Trapping Nanometry. Motility assay of kinesin-bound beads was carried out by laser trapping nanometry as described previously (*20*, *21*). Briefly, fluorescently labeled microtubules were bound on the surface of a coverslip. BDTC-kinesin was bound to streptavidin-coated beads. The beads were trapped and brought into contact with the microtubules. The displacement of beads was detected by a quadrant photodiode. Trap stiffness was estimated to be 0.045 pN/nm from the equipartition theorem (*7*, *21*). The assay was performed in a solution containing 80 mM Pipes (pH 6.8), 1 mM EGTA, 2 mM $MgCl_2$, 10 μM−1 mM ATP, 10 mM glucose, 9 μg/mL catalase, 50 μg/mL glucose oxidase, 0.5% 2-mercaptoethanol, and 10 μM taxol at 25 °C.

RESULTS

Purification and Labeling of Kinesins. The catalytic domain of *Drosophila* conventional kinesin contains five cysteines. All five cysteines and four cysteines except Cys45 were substituted to prepare Cys-free kinesin and Cys45 kinesin, respectively. The expression and purity of mother (DKH411-BDTC), Cys-free, and Cys45 kinesins were confirmed with SDS−PAGE (Figure 1a). The purity of kinesins was about 90%. The molecular mass of these kinesin heads was estimated to be 60 kDa from SDS−PAGE, in agreement with the value calculated from the amino acid sequence (46 kDa for DK411 + 14 kDa for BDTC). Biotinylation of BDTC was also detected with streptavidin−alkaline phosphatase and bromochloroindolyl phosphate/nitro blue tetrazolium as substrate (BCIP/NBT) (*22*). Cys-free and Cys45 kinesins were labeled with a fluorescent probe, acrylodan. Acrylodan labeling per kinesin head, which is determined from the absorbance of labeled kinesin at a wavelength of 365 nm, was <0.1 and 1.0, respectively. Cys-free kinesin was not labeled, indicating that the labeling with acrylodan is specific to cysteine residues. Thus, we confirmed that we had successfully constructed and labeled these proteins.

Fluorescence Properties of Labeled Kinesin. Acrylodan attached to Cys45 kinesin was used to probe its microenvironmental change, since this dye is known to be sensitive to the environment (*23*). The emission fluorescence spectrum exhibits a peak at 520 nm, indicating that the probe is relatively exposed to the solvent (Figure 2). As compared

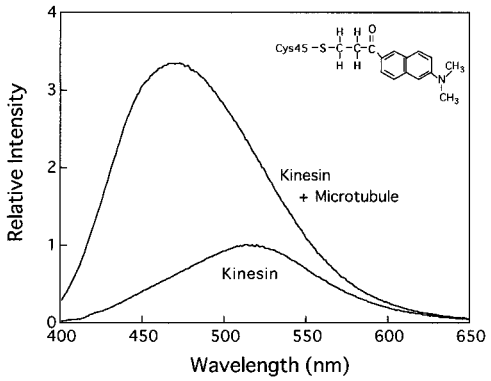

FIGURE 2: Fluorescence spectrum of acrylodan-labeled Cys45 in the presence and absence of microtubule. Excitation wavelength, 365 nm. Two-headed kinesin (0.1 μM) was mixed with microtubule (1 μM) in the presence of 1 mM MgAMPPNP.

to unreacted probe, the fluorescence intensity was enhanced by about 10 times and the peak position was shifted about 10 nm from 530 nm. Upon binding to microtubules in the presence of 1 mM AMPPNP, the peak further shifted from 520 to 470 nm with a 3.4-fold increase in the peak intensity (Figure 2). The binding of ATP dissociated kinesin from the microtubules and reversed the fluorescence change. Thus, we demonstrated that it is possible to monitor a local change in the protein with the probe that attached to the residual Cys45.

ATPase Assay. To study if these substitutions of cysteine residues and the labeling affect the properties of this protein, we measured the chemical and mechanical properties of these proteins. In the absence of microtubules, the ATPase rates of all kinesins examined were <0.1 s^{-1}. Microtubules activated the ATPase of kinesins. To determine the affinity of kinesin to microtubules and ATP and the maximum ATPase rate (*6*), the ATPase rate was measured as a function of the concentrations of both microtubules and ATP. ATPase rates of kinesin mutants per two heads increased with tubulin concentration (Figure 3a) and with ATP concentration in a hyperbolic manner (Figure 3b). The ATPase rates, V, were fit to Michaelis−Menten curves:

$$V = V_{max}[MT](K_{MT} + [MT])^{-1}[ATP](K_{ATP} + [ATP])^{-1} \quad (1)$$

where V_{max} is the ATPase rate at infinite concentrations of microtubules and ATP, [MT] and [ATP] are the concentrations of microtubules and ATP, and K_{MT} and K_{ATP} are the microtubule and ATP concentrations at which V is half of V_{max}, respectively. K_{MT} and K_{ATP} are measures of the affinities of kinesin to microtubules and ATP; the higher the value, the lower the affinity. The values of V_{max}, K_{MT}, and K_{ATP} obtained for each kinesin are summarized in Table 2. For mother kinesin, V_{max} was 68 s^{-1}, similar to another report (*24*). The substitution of four cysteines except Cys45 reduced V_{max} to 40 s^{-1}, 0.55 of mother kinesin. Further substitution of Cys45 did not change V_{max}. The labeling at Cys45 with acrylodan further decreased V_{max} to 26 s^{-1}, 0.32 of mother kinesin (Table 2). The affinities of kinesin for microtubules and ATP were reduced by mutation and labeling in a similar manner. Thus, the V_{max} and the affinity for microtubule and ATP were decreased by substitution of cysteines and labeling.

Motility of Recombinant Kinesins. The motility of single molecules of recombinant kinesins was measured using laser

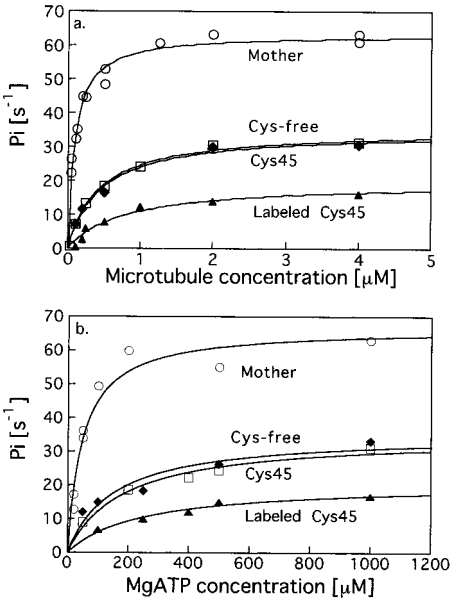

FIGURE 3: Microtubule-activated ATPase of kinesin mutants. (a) ATPase rate per two heads as a function of the concentration of tubulin dimers in microtubule at a fixed concentration of MgATP of 1 mM. (b) ATPase rate as a function of the concentration of MgATP at a fixed concentration of microtubule of 2 μM. Mother kinesin molecule (○); cysteine-free kinesin (◆); kinesin with cysteine 45 (□); and acrylodan-labeled Cys45 kinesin (▲). Best-fit curves shown by solid lines were calculated from the modified Michaelis−Menten equation (eq 1 in the text).

Table 2: Microtubule-Activated ATPase Activity per Two Heads of Kinesin Mutants

	V_{max} (s^{-1})	K_{MT} (μM)	K_{ATP} (μM)	$V_{1\ ATP}$ (s^{-1})[a]
mother	68	0.093	48	64
Cys-free	40	0.45	153	34
Cys45	38	0.44	185	32
labeled Cys45	26	0.80	247	20

[a] $V_{1\ ATP}$ is the ATPase rate at 1 mM MgATP and infinite concentration of microtubule present.

trapping and nanometry. BDTC fused at the C-terminus of recombinant kinesins was bound to streptavidin that was linked to beads. The displacement of bead was measured with a quadrant photodiode with nanometer precision. Figure 4 shows the bead-displacement and the force exerted by kinesins. The displacement of the bead increased in a stepwise fashion until it was saturated (Figure 4). A stall force, defined as a force when beads stayed for a period longer than 0.5 s, was similar for all kinesins. The stall force of all kinesin mutants was 6.0−6.5 pN (Figure 5a). In contrast to the force at saturation, the rise time was slower than that of mother kinesin, depending on mutation and labeling. The velocity at low force was obtained from the slopes of the displacement transients between a force of 0.5 and 1.5 pN by taking into account the attenuation factor of 1.3 due to the compliance of bead-to-glass (*9*, *20*). The velocity of mother kinesin was 670 nm s^{-1}. The velocities of Cys-free, Cys45, and labeled Cys 45 were reduced to 0.61, 0.49, and 0.27 of that of mother kinesin, respectively (Figure 5b). The decrease was similar to that for the ATPase decrease.

Step Size of Cysteine-Modified Kinesin. From the ATPase rate and the velocity, we could calculate a step size produced by hydrolysis of a single ATP molecule. Figure 6a shows

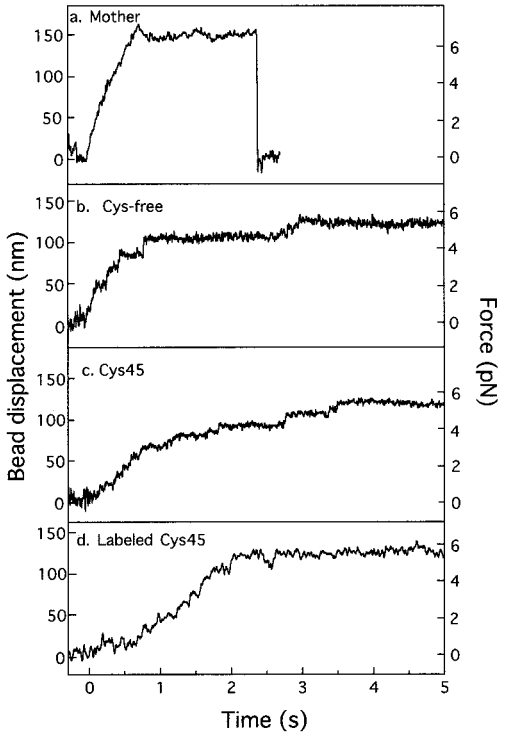

FIGURE 4: Force generation of single molecules of kinesin mutants. Mother kinesin (a), Cys-free (b), Cys45 (c), and acrylodan-labeled Cys45 (d). BDTC of kinesin mutants was bound on avidin-coated bead. The displacement of a bead was detected by a quadrant photodetector. The forces were obtained as a product of bead displacement and trapping stiffness of 0.045 pN/nm.

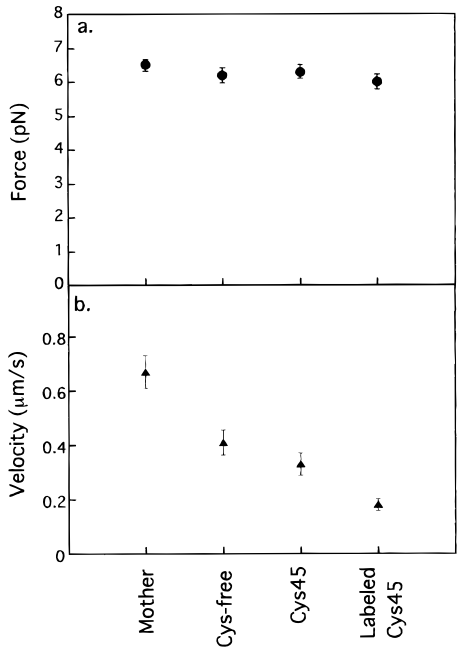

FIGURE 5: Force and velocity of single molecules of kinesin mutants. Force was measured at maximum and stalling for a period longer than 0.5 s. The velocity at 0.5−1.5 pN force was calculated from the bead displacement multiplied by the attenuation factor (1.3) of the compliance of bead−kinesin linkage. Bars, standard errors at sample numbers of 18−24 (a) and 26−30 (b).

the relation between the velocity at about 1 pN force and the ATPase rate under the same condition. The velocity of two-headed kinesins at 1 pN was reported almost the same as at no load (*9*, *20*). The sliding velocity was basically

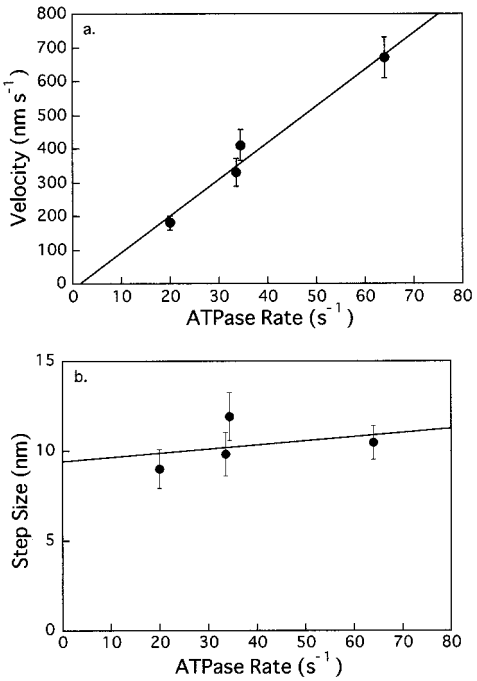

FIGURE 6: Step size of kinesin mutants. (a) Motile velocity was taken from data shown in Figure 5a. ATPase activity per two heads was calculated from the eq 1 at 1 mM MgATP and infinite concentration of microtubule. Bars, SD. (b) The step size per hydrolysis of one ATP molecule by the kinesin molecule or two heads was estimated by dividing the motile velocity by the ATPase activity. Bars, SD. Lines, linear regression lines.

proportional to the ATPase activity. The ATPase rate, $V_{1\,ATP}$ (the last column in Table 2), was calculated using eq1 with the K_{MT} and K_{ATP} values obtained above (Table 1), [ATP] = 1 mM at which the motility assay was carried out, and [MT] = infinite because the tubulin concentration around kinesin heads during movement in the assay is expected to be very high.

The linear relation (Figure 6a) indicates that the step size per ATP molecule hydrolyzed is independent of kinesin mutants. The step size per hydrolysis of a single ATP molecule by a two-headed kinesin was estimated as the ratio of the motile velocity to the ATPase activity (Figure 6b). The kinesin molecule moves with a step size of 9−12 nm (10.3 nm on average for four kinesins) independent of kinesin mutants. Thus, the modification and the labeling did not affect the step size per ATP molecule hydrolyzed of kinesin.

DISCUSSION

The catalytic domain of *Drosophila* conventional kinesin contains five cysteines. Three of them, the 17th, 309th, and 310th, were conserved among the conventional kinesin families (*4*). To exchange the cysteine residue, 309th and 310th, for another amino acid while preserving the activity of kinesin, we referred to the corresponding sequence (threonine and valine) of the other kinesins of XlEg5, SpoCut7, ScKip1, and LcKin (*4*). In the beginning of the experiment, we exchanged the 17th, 45th, and 338th cysteine for serine because of similarity in size and polarity. However, these exchanges induced proteolysis of kinesin during purification. This suggests that changes of cysteine to serine induced structural change. To prevent the proteolysis of kinesin, cysteines 17 and 45 were exchanged for valine and alanine. These are hardly proteolysed as shown in Figure 1.

The major result of this study is that substitution of the cysteine residue of the molecular motor kinesin does not affect its force and step size, and these cysteine-free mutants can be used to study the structure−function relationship of kinesin. The catalytic domain of *Drosophila* kinesin contains five cysteines. All five or four except Cys45 were substituted by other amino acids using site-directed mutagenesis. The step size produced by hydrolysis of a single ATP and the force produced by a single molecule are the same for these mutants. Also, these values were not affected when they were labeled with a fluorescent probe, acrylodan. The step size estimated from the ratio of the velocity to the ATPase was 9−12 nm for all kinesins. This value is close to the elementary mechanical step size reported for the native kinesin head (8 nm) (*7*, *9*). These results are also consistent with recent results (*9*, *11*, *12*, *20*) that an 8 nm step of kinesin molecules occurs during hydrolysis of one ATP molecule (tight coupling mechanism).

The stall force obtained was 6−6.5 pN for all kinesins. This value is similar to that of native bovine kinesin of 6.7 pN (*21*). The work done by our kinesin mutants, which moves by a step of 8 nm at 6 pN, is 8 nm × 6 pN = 48 × 10^{-21} J. If an 8 nm step is produced during one ATP molecule hydrolysis, the energy efficiency of kinesin is about 60%, compared with the energy liberated from the hydrolysis of ATP, 80 × 10^{-21} J (*25*). Therefore, the energy efficiency of kinesin mutants is almost the same as that of native kinesin.

Despite invariant numbers of force and step size, the ATPase activity and motile velocity depended on the mutation and labeling. Both the ATPase activity and the motile velocity of Cys-free and Cys45 kinesin were about half those of mother kinesin. Their affinities for microtubules and ATP were 4−9 times lower than those of mother kinesin (Table 2). A single substitution of Cys45 did not affect these numbers. Cys45 in *Drosophila* kinesin is not conserved among kinesin families. Thus, it may not be important whether the position is occupied by cysteine. However, the labeling at this position further decreased the ATPase rate and motile velocity.

The affinity for microtubule decreased by 9 times after the mutation and labeling (Table 2), which may affect the processability of single kinesin molecules. The traveling distance of mother and single labeled Cys45 kinesins was longer than 450 nm measured at a lower trap stiffness of 0.02 pN/nm, less than half of that in Figure 4, by taking into account the attenuation factor of 1.3 due to the compliance of bead-to-glass (data not shown). This indicates that the labeled Cys45 kinesin has a high processability. This is consistent with the result that human kinesin K560, for which the K_{MT} (1.1 μM) is higher than that (0.80 μM) of labeled Cys45 kinesin, is processive (*6*).

Thus, the kinetic rate of the ATPase cycle and the interaction with microtubules and nucleotides are affected by the mutation and the labeling. However, the mechanical events remain tightly coupled with the chemical processes, and the mechanical force is produced in the same way. This finding reinforces the nature of the tight coupling of kinesin.

Fluorescently labeled Cys45 kinesin was used to detect changes in the microenvironment around Cys45. The fluorescence emission spectrum of acrylodan was shifted to lower wavelength with an increase in the intensity upon the binding of kinesin to microtubule. Acrylodan at Cys45 is in a more

hydrophobic environment when kinesin interacts with microtubule. This change indicates that the structural changes around Cys45 are induced by the binding of microtubule at sites away from Cys45. The microtubule binding domains are around loop 12 and loop 7, opposite the site of Cys45 (6). Cooke and colleagues detected that the Cys670 on the Ncd molecule, which corresponds structurally to *Drosophila* kinesin 334 aa in the neck region, changes by the binding of microtubule (*26*). The kinesin structure may change around the neck region and Cys45 by the binding of microtubules.

The cysteine residues can be specifically labeled with many different probes, e.g., fluorescence dyes, organic compounds, proteins, and gold. By introducing cysteine residues to the desired position of Cys-free kinesin and labeling them with various probes, we will be able to monitor the local environmental changes that occur due to conformational changes, the binding to microtubules, or the binding of ligand, or the state changes due to bound nucleotide. Since kinesin without cysteine retains the basic mechanical properties, this approach could be promising.

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REFERENCES

1. Vale, R. D., Reese, T. S., and Sheetz, M. P. (1985a) *Cell 42*, 39−50.
2. Vale, R. D., Schnapp, B. J., Mitchison, T., Steuer, E., Reese, T. S., and Sheetz, M. P. (1985b) *Cell 43*, 623−632.
3. Goldsteins, L. S. B. (1991) *Trends Cell Biol. 1*, 93−98.
4. Bloom, G., and Endow, S (1994) *Motor Proteins* Vol. 1, Academic, London.
5. Kull, F. J., Sablin, E. P., Lau, R., Fletterick, R. J., and Vale, R. D. (1996) *Nature (London) 380*, 550−555.
6. Woehlke, G., Ruby, A. K., Hart, C. L., Ly, B., Hom-Booher, N., and Vale, R. D. (1997) *Cell 90*, 207−216.
7. Svoboda, K., Schmidt, C. F., Schnapp, B. J., and Block, S. M. (1993) *Nature (London) 365*, 721−727.
8. Coppin, C. M., Finer, J. T., Spudich, J. A., and Vale, R. D. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 1913−1917.
9. Kojima, H., Muto, E., Higuchi, H., and Yanagida, T. (1997) *Biophys. J. 73*, 2012−2022.
10. Howard, J., Hudspeth, A. J., and Vale, R. D. (1989) *Annu. Rev. Physiol. 58*, 703−729.
11. Schnitzer, M. J., and Block, S. M. (1997) *Nature (London) 388*, 386−390.
12. Hua, W., Yong, E. C., Fleming, M. L., and Gelles, J. (1997) *Nature (London) 388*, 390−393.
13. Yanagida, T., Arata, T., and Oosawa, F. (1985) *Nature (London) 316*, 366−369.
14. Higuchi, H., and Goldman, Y. E. (1991) *Nature (London) 352*, 352−354.
15. Kitamura, K., Tokunaga, M., Iwane, A. H., and Yanagida, T. (1999) *Nature (London) 397*, 129−134.
16. Finer, J. T., Simmons, R. M., and Spudich, J. A. (1994) *Nature 368*, 113−119.
17. Molloy, J. E., Burns, J. E., Kendrick-Jones, J., Tregear, R. T., and White, D. C. S. (1995) *Nature 378*, 209−212.
18. Yang, J. T., Laymon, R. A., and Goldstein, L. S. B. (1989) *Cell 56*, 879−889.
19. Kodama, T., Fukui, K., and Kometani, K. (1986) *J. Biochem. 99*, 1465−1472.
20. Inoue, Y., Toyoshima, Y. Y., Iwane, A. H., Morimoto, S., Higuchi, H., and Yanagida, T. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 7275−7280.
21. Higuchi, H., Muto, E., Inoue, Y., and Yanagida, T. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 4395−4400.
22. Iwane, A. H., Kitamura, K., Tokunaga, M., and Yanagida, T. (1997) *Biochem. Biophys. Res. Commun. 230*, 76−80.
23. Prendergast, F. G., Meyer, M., Carlson, G. L., Iida, S., and Potter, J. D. (1983) *J. Biol. Chem. 258*, 7541−7544.
24. Jiang, W., Stock, M. F., Li, X., and Hackney, D. D. (1997) *J. Biol. Chem. 272*, 7626−7632.
25. Alberty, R. A. (1968) *J. Biol. Chem. 243*, 1337−1343.
26. Naber, N., Cooke R., and Pate, E. (1997) *Biochemistry 36*, 9681−9689.
27. Sack, S., Mueller, J., Marx, A., Thormehlen, Mandelkow, E. M., Brady, S. T., and Mandelkow, E. (1997) *Biochemistry 36*, 16155−16165.

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