

The Second Microtubule-binding Site of Monomeric Kid Enhances the Microtubule Affinity*[§]

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Chromokinesin Kid (kinesin-like DNA-binding protein) localizes on spindles and chromosomes and has important roles in generating polar ejection force on microtubules in the metaphase. To understand these functions of Kid at the molecular level, we investigated molecular properties of Kid, its oligomeric state, interaction with microtubules, and physiological activity *in vitro*. Kid expressed in mammalian cells, as well as Kid expressed in *Escherichia coli*, was found to be monomeric. However, Kid cross-linked microtubules in an ATP-sensitive manner, suggesting that Kid has a second microtubule-binding site in addition to its motor domain. This was ascertained by binding of Kid fragments lacking the motor domain to microtubules. The interaction of the second microtubule-binding site was weak in a nucleotide-insensitive manner. K_{mMT} of the ATPase activity of Kid was lower than that of the fragments lacking the second microtubule-binding site. Moreover, the velocity of Kid movement *in vitro* was not affected by the second microtubule-binding site, which is consistent with the weak binding of this site to microtubules. The second microtubule-binding site would be important to enhance the affinity to microtubules for the monomeric motor, Kid. Because the amino acid sequence of this region is highly conserved among species, it seems to have essential roles for the functions of Kid *in vivo*.

Mitosis is an essential phenomenon of living cells, in which the genetic materials are segregated accurately into the daughter cells. This process, mainly comprised of the spindle formation and the chromosome segregation, is regulated spatially and temporally by the activity of motor proteins in a coordinated manner. Both cytoplasmic dynein and the members of the kinesin superfamily play critical roles in centrosome separation and assembly of bipolar spindles (bimC, cut7, Eg5, Klp61F), spindle pole formation (CHO1, cytoplasmic dynein, MKLP1, Ncd, XCYK2), organizing and maintaining spindle structure (bimC, Cin8, Kar3, Kip1 and Xklp1), positioning

chromosomes at the spindle equator (CENP-E, cytoplasmic dynein, Kin I, Nod and Xklp1), and chromosome segregation (MCAK and XKCM1) (1–4).

Kid (kinesin-like DNA-binding protein), one of the chromokinesins, was identified as a member of the chromosome-associated kinesin family (5). The N-terminal half of Kid contains the kinesin-like motor domain, and there is a helix-hairpin-helix DNA binding domain at its C terminus. It has been reported that the subcellular localization of Kid changes dramatically during cell division (5). Kid is distributed exclusively in the nucleus in the interphase. Following the breakdown of the nuclear membrane at the end of prophase, Kid localizes on the spindles and along the length of the chromosomes during prometaphase and metaphase. In the anaphase, Kid is enriched to the spindle pole-proximal side on the chromosomes. In the progression to telophase and cytokinesis, Kid localizes around the chromosomes and then in the nucleus. Xkid, a *Xenopus* ortholog of Kid, has been shown to be essential for proper chromosome alignment on the metaphase plate and plays important roles generating a polar ejection force (6, 7). Kid is also necessary for chromosome orientation and oscillation of chromosome arms (8). It was recently shown that Xkid has a role in the meiotic cell cycle (9). Based on these reports about mitosis, Kid is thought to have important roles in chromosome alignment. However, the functions of Kid at the anaphase and telophase remain unknown in cell division.

To understand the molecular mechanism of known Kid function and to find clues to elucidate unknown functions of Kid, it is important to characterize the Kid molecule in itself. Compared with conventional kinesin, C-terminal kinesins, and bimC kinesins, our knowledge of chromokinesins is limited; therefore, analysis of the Kid molecule is critical for understanding spindle dynamics during mitosis and meiosis. Furthermore, study of the Kid molecule will provide important information about the structural organization and the mechanistic significance of structural diversity among kinesin superfamily members.

Although Kid has been shown to be a plus-end-directed motor (10), there have been few reports about the molecular properties of Kid as a motor protein. Here, we report the molecular structure of Kid and its interaction with microtubules (MTs)¹ using expressed proteins of Kid fragments.

EXPERIMENTAL PROCEDURES

Construction, Purification, Electrophoresis, and Western Blotting—The diagrams of all constructs used here are shown in Fig. 1A. To construct plasmids for expression of Kid fragments in *Escherichia coli*,

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[§] The on-line version of this article (available at <http://www.jbc.org>) contains two movies.

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¹ The abbreviations used are: MT, microtubule; aa, amino acid; Pipes, 1,4-piperazinediethanesulfonic acid; KS, kinesin stalk; Kidfull, full-length Kid; GST, glutathione *S*-transferase; GSΔC, gelsolinΔC.

the cDNAs encoding 1–515 aa (Kid515), 1–441 aa (Kid441), and 1–388 aa (Kid388) were amplified by PCR from pBS-C1–6 (5), and each fragment was introduced at *NdeI*-*Bam*HI sites of pKN172 (kind gift from A. Weeds, MRC-Laboratory of Molecular Biology, Cambridge, UK) for expression. For a fusion protein with a part of *Drosophila* kinesin stalk (402–560 aa, KS) and human gelsolin (1–732 aa, gelsolin Δ C), the corresponding DNA fragment was amplified from pBS1–2 (11) and human gelsolin in pKN172, respectively, and the PCR fragments were ligated at the 3'-end of the cDNA of Kid515 and/or Kid441 in the pKN172 vector. For the GST fusion constructs, the cDNA encoding 389–515 aa (Kid-(389–515)) and 389–441 aa (Kid-(389–441)) were amplified from pBS, and each fragment was inserted at a *Bam*HI site into pGEX-2T (Amersham Biosciences).

To construct plasmids for the expression of full-length Kid (Kidfull) in mammalian cells, the cDNA encoding full-length Kid was amplified and cloned at the *Eco*RI-*Bam*HI sites of a pME18S (12) or pMX-puro vector (13) for retrovirus production. Kidfull-KS was made following the method described above. Retroviruses were produced using PlatE packaging cell as described (13, 14).

All constructs, except for fusion proteins with GST, were tagged with His₆ at the 3'-end, and the DNA sequence of all constructs was confirmed using a Thermo Sequenase fluorescent-labeled primer-cycle sequencing kit (Amersham Biosciences).

For the preparation of His-tagged proteins expressed in *E. coli* (BL21Star(DE3), Invitrogen) following high-speed centrifugation, proteins were purified using nickel-nitrilotriacetic acid-agarose (Qiagen) and a P11 column (Whatman). For the purification of GST fusion proteins expressed in *E. coli* (BL21Star(DE3)), a high-speed supernatant was adsorbed to glutathione-agarose (Sigma) and eluted. Proteins purified from *E. coli* were analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue (Fig. 1B). Lower bands in Fig. 1B, lanes 5 and 7, were found to be degradation products by Western blotting, and it was confirmed that these degradation products did not affect the characterization of Kid. For the expression of Kidfull in mammalian cells, 293T cells transfected with pME-Kid-His or NIH3T3 cells infected with retrovirus were synchronized in the M phase by 100 ng/ml nocodazole treatment for 14–18 h. Kid proteins after high-speed centrifugation were purified using TALON metal affinity resin (Clontech). Proteins expressed in mammalian cells were Western-blotted (Fig. 1C).

Protein concentration was determined by the method of Read and Northcote (15) using bovine serum albumin as a standard. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as reported (16). Western blotting was carried out by the standard method with a monoclonal antibody against Kid (kind gift from Dr. T. Urano) (17) and goat anti-mouse IgG (KPL), using the kit BCIP/NBT membrane phosphatase substrate system 3-C (Kirkegaard & Perry Laboratories).

Analysis of Molecular Weight—The $s_{20,w}$ values of Kid proteins were determined by 10–25% sucrose density-gradient centrifugation (40,000 rpm, 24 h, 2 °C, P50S2, Hitachi) in an assay buffer (20 mM Pipes-KOH, 100 mM K-acetate, 4 mM MgSO₄, 1 mM EGTA, 1 mM dithiothreitol) or with additional 400 mM K-acetate (500 mM K-acetate assay buffer), including 100 μ M ATP. Aldolase (7.3 S), bovine serum albumin (4.5 S), ovalbumin (3.7 S), and carbonic anhydrase (3.2 S) were used as markers. Stoke's radius was measured by gel filtration chromatography (superose 6, Amersham Biosciences) with ~60 μ g of Kid in the 500 mM K-acetate assay buffer using a high-performance liquid chromatography system (TOSOH) at 4 °C. Thyroglobulin (8.9 nm), ferritin (6.1 nm), aldolase (4.8 nm), bovine serum albumin (3.6 nm), and carbonic anhydrase (3.1 nm) were used as markers. All these markers were purchased from Sigma. The fractions were concentrated by trichloroacetic acid precipitation and analyzed by SDS-PAGE and Western blotting. The peak position was determined by quantification of the protein bands using Photoshop (Adobe) followed by fitting with a Gaussian curve. Molecular weight was determined by $s_{20,w}$ and Stoke's radius as reported (18). The error of the analysis of the molecular weight is estimated to be within 10%.

MT Purification, Cross-linking Assay, and Co-sedimentation Assay—Tubulin was purified from porcine brains as reported (19). Tubulin labeled by rhodamine (Molecular Probes) (20) was polymerized at 37 °C for 30 min and stabilized with paclitaxel. Kid (75 nM) was mixed with MT (150 nM) in the presence or absence of 10 mM Mg-ATP in the assay buffer, incubated for 2 min at 25 °C, and then observed using an epi-fluorescence microscope (BX60, 100 \times /1.4 objective, Olympus). For the co-sedimentation assay, GST-Kid-(389–515) (2–45 μ M) or GST-Kid-(389–441) (4 μ M) mixed with MT (2 μ M) in the assay buffer in the absence or presence of 5 mM Mg-ATP, 1 mM Mg-AMP-PNP, and 5 mM Mg-ADP, respectively, was centrifuged (110,000 \times g, 25 °C, for 5 min).

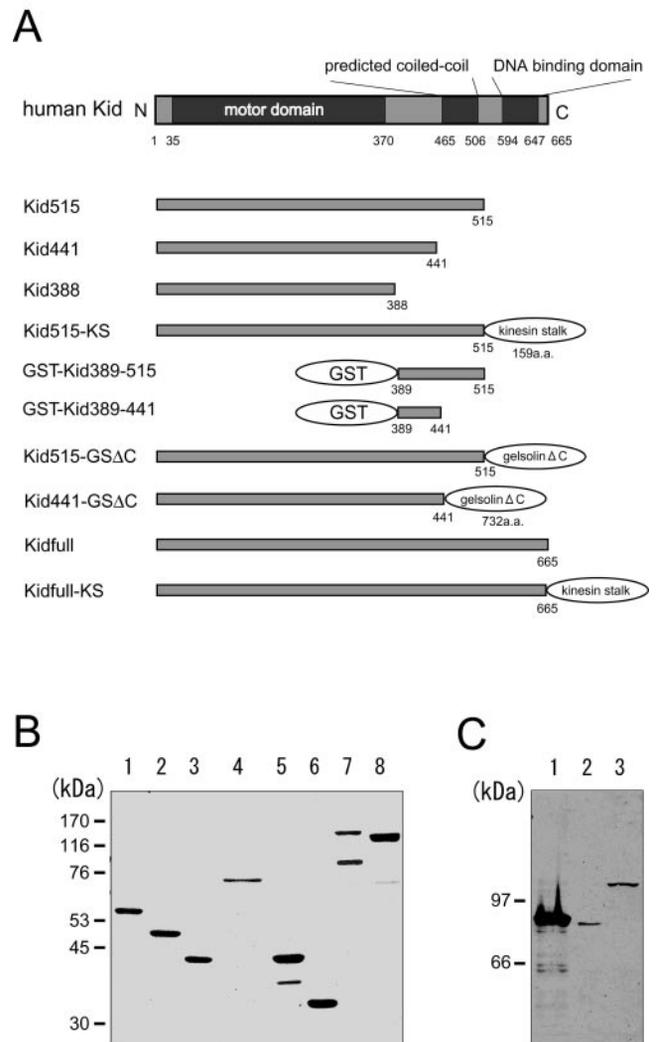


FIG. 1. Constructs and proteins. A, the domain structure of human Kid and the constructs used in this study. The numbers of the amino acid are shown below. B, proteins expressed in and purified from *E. coli* in SDS-PAGE 10% gel-stained with Coomassie Brilliant Blue. Lanes 1–8 correspond to Kid515, Kid441, Kid388, Kid515-KS, GST-Kid-(389–515), GST-Kid-(389–441), Kid515-GS Δ C, and Kid441-GS Δ C, respectively. Numbers on the left indicate the position of the molecular weight markers. C, Western blotting from 7.5% gel shows the proteins expressed in and purified from mammalian cells. Lane 1, Kidfull overexpressed in 293T cells. Lanes 2 and 3, the Kidfull and Kidfull-KS, respectively, which were expressed moderately (see “Results”) in NIH3T3 cells. Numbers on the left indicate the molecular weight markers.

The supernatant and the pellet were analyzed by SDS-PAGE.

ATPase and Motility Assays—The ATPase assay was performed using Enzchek (Molecular Probes) at 25 °C. The MT-stimulated activities of Kid fragments (10 nM) were measured in the presence or absence of MT (0–1000 nM) in the assay buffer. The motility assay using the Kid fragments fused with gelsolin Δ C was performed basically following the previous method (21). Because gelsolin Δ C severs and caps an actin filament in a Ca²⁺-insensitive manner (22, 23), Ca²⁺ ion was not added. The final concentration of Kid-gelsolin Δ C was 2–6 nM. Movements in the presence of 1 mM ATP and 0.1% polyoxyethylene sorbitan monolaurate (Nacalai Tesque) in the assay buffer were observed at 25 \pm 1 °C using an epi-fluorescence microscope (BX60, 100 \times /1.4 objective, Olympus) with a \times 5 relay lens and a camera (ICD-6100, Ikegami). Images were recorded using a videocassette recorder (WV-DR7, Sony) after averaging of four frames with an image processor (Arugus-10, Hamamatsu Photonics). The positions of Kid were analyzed by calculation of the center of gravity. Velocities were determined for Kid that moved continuously and uninterrupted for more than one micrometer by the linear fitting of traces measured every 1 s.

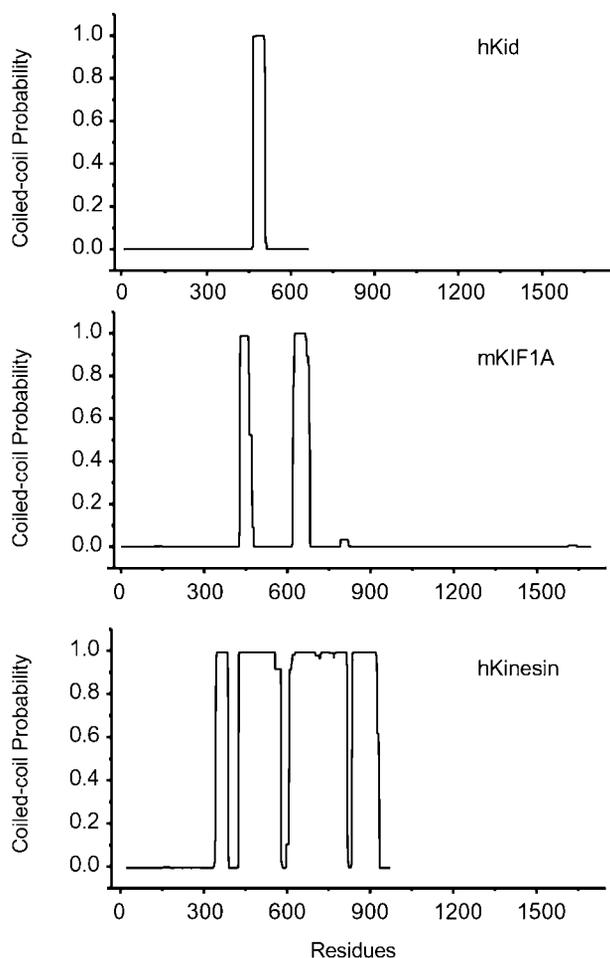


FIG. 2. **Predicted coiled-coil region.** Theoretical probabilities of coiled-coil formations of three kinesin-like proteins: Kid from *Homo sapiens* (accession number, AB017430), KIF1A from *Mus musculus* (accession number, D29951) and conventional kinesin from *Homo sapiens* (accession number, X65873) are shown. Probabilities were calculated using the program COLLS (25).

RESULTS

Oligomeric State of Kid—Information on the oligomeric state of Kid is very important to interpret Kid interaction with MTs. Kid has a theoretical predicted coiled-coil region at 465–506 aa (Fig. 2) (24, 25). However, it has not been ascertained experimentally whether Kid is a monomer or a dimer. The oligomeric state of Kid fragments, as well as that of Kidfull, was then examined with or without the predicted coiled-coil region (Table I). First, the $s_{20,w}$ values of Kid515, Kid441, and Kid388 prepared from *E. coli* (Fig. 1, A and B) were measured by sucrose density-gradient centrifugation in the assay buffer (100 mM K-acetate). These $s_{20,w}$ values (3.6–3.7 S) indicate that they are the same oligomeric form and have comparable $s_{20,w}$ values to those of the monomeric kinesin fragments (3.3–3.4 S) (26–28). Kid515 fused with the kinesin coiled-coil region (402–560 aa of *Drosophila* conventional kinesin) that dimerizes (Kid515-KS) showed a value of 5.3 S, which is similar to the $s_{20,w}$ values of dimeric kinesins as reported (5.1–5.2 S) (26–28). Therefore, Kid515, Kid441, and Kid388 fragments are thought to be monomeric. The $s_{20,w}$ of full-length Kid expressed in *E. coli* could not be measured because it was unstable at our experimental conditions. To know the oligomeric form of full-length Kid and to confirm that the oligomeric state is not affected by expression in *E. coli*, the Kidfull prepared from mammalian cells was examined (Fig. 1, A and C). To avoid the artifact by overexpression, the Kidfull was expressed in moderate amounts, by which

the cell cycle is not inhibited (17). The $s_{20,w}$ value of this Kidfull was 4.1 S, and Kidfull-KS in a dimeric form showed 5.9 S. Therefore, full-length Kid also seemed to be monomeric. Next, gel filtration chromatography was performed to measure the Stoke's radii of Kidfull, Kid515, and Kid515-KS in a high-salt condition (500 mM K-acetate) in which Kid can avoid adsorption to the resins. The Kidfull was overexpressed in mammalian cells, because the amount of Kid expressed in moderate amounts was too small to detect by gel filtration chromatography. We confirmed that the $s_{20,w}$ values of moderately expressed and overexpressed Kidfull were not different and that the difference between the 100 and 500 mM K-acetate conditions did not significantly change the $s_{20,w}$ values for Kidfull and its fragments measured here (Table I). The Stoke's radii of Kid515 (4.5 nm) and Kid515-KS in dimer (6.8 nm) were significantly different. Comparison of the molecular weights determined experimentally and calculated from the weight of the comprising amino acids indicates that both full-length Kid prepared from mammalian cells and Kid515 purified from *E. coli* have a monomeric form.

The Second MT-binding Site of Kid—When Kid fragment Kid515 was mixed with MTs, aggregation of MTs was observed, suggesting formation of MT bundles or MT networks (Fig. 3A, b). This MT aggregation was not observed in the presence of ATP (Fig. 3A, c). On the other hand, Kid441 and Kid388 did not aggregate MTs even in the absence of ATP (Fig. 3A, d and e). Because MT aggregation is caused by at least two distinct MT-binding sites in the mixed molecule, Kid515 is suggested to have an additional MT-binding site in 442–515 aa besides its motor domain. To confirm that there is the second MT-binding site in 442–515 aa in Kid, GST-Kid-(389–515) was constructed, and co-sedimentation assay with MTs was carried out using GST-Kid-(389–441) as a control (Figs. 1, A and B, and 3B). GST-Kid-(389–515), but not GST-Kid-(389–441) was co-sedimented with MTs in a nucleotide-independent manner. These results suggest that, in addition to the motor domain, Kid has a second MT-binding site. The dissociation of MT aggregation by Kid515 in the presence of ATP is thought to be because of the function of the motor domain. The dissociation constant of the second MT-binding site ($K_d = 12 \mu\text{M}$) (Fig. 3C) indicates that the interaction of the second site with MTs is weak. The amino acid sequence of this region is very conservative among the three species of human, mouse, and frog (Fig. 3D). (The sequence in mouse is obtained from the data base and is 81.3% identical throughout to human Kid.) Therefore, the second MT-binding site is likely to have essential roles for the functions of Kid (see "Discussion").

The Effect of the Second MT-binding Site on ATPase Activity—To elucidate the roles of the second MT-binding site of Kid, an ATPase assay was performed using Kid515, Kid441, and Kid388. The ATPase activities of these fragments activated by MTs followed Michaelis-Menten type kinetics (Fig. 4A). The k_{cat} of all the fragments was almost the same (12 s^{-1}); however, the $K_{m\text{MT}}$ of Kid515 (5.6 nM) was 5–6 times lower than that of Kid441 (35 nM) and Kid388 (29 nM). The similarity of k_{cat} shows that the second MT-binding site has no significant effect on the rate-limiting step of the ATPase cycle. The significant difference of $K_{m\text{MT}}$ is correlated to the existence of the second MT-binding site, indicating that the second MT-binding site enhances the affinity for MTs during ATPase cycles. Because Kid515 did not cross-link MTs in the presence of ATP (also ascertained in the same condition for ATPase assays), the affinity of Kid for MT seems to be enhanced by binding of the second MT-binding site to the same MT with which the motor domain interacts during the ATPase cycles.

The Effect of the Second MT-binding Site on Motile Activity—The motile property of Kid and the effect(s) of the second

TABLE I
Oligomeric states of Kid and its fragments

Fragment	Expression	$s_{20,w}$ (S)		R_s^a (nm)	MW		Oligomeric state
		100 mM K-acetate	500 mM K-acetate		Experimental	Theoretical	
Kid515	<i>E. coli</i>	3.7	3.4	4.5	63	59	Monomer
Kid441	<i>E. coli</i>	3.6					
Kid388	<i>E. coli</i>	3.6					
Kid515-KS	<i>E. coli</i>	5.3	5.0	6.8	139	72	Dimer
Kidfull	Mouse	4.1					
Kidfull-KS	Mouse	5.9					
Kidfull	Human	4.0	4.0	5.1	84	77	Monomer

^a Stoke's radius.

MT-binding site of Kid on motility were investigated. If the second MT-binding site were for fixation of Kid on an MT, Kid does not move along an MT. A type of motility assay was selected that would allow the second MT-binding site to interact with MT without sticking to a substrate, such as a bead or glass surface. MTs were fixed on the glass, and the movement of Kid along an MT was observed by a labeled actin filament that was capped by gelsolin Δ C fused to Kid (Fig. 1, A and B) (21). The deletion of 33 aa at the C terminus of gelsolin can sever and cap the actin filament in a Ca²⁺-insensitive manner (22, 23). In the case of this gelsolin Δ C in the absence of Ca²⁺ ion, it is easier to regulate the gelsolin activity of severing and capping the actin filament and MTs are stable in the absence of Ca²⁺ ion. It was checked that the fusion to gelsolin did not change $K_{m,MT}$ of either Kid515-GS Δ C or Kid441-GS Δ C of the ATPase activities ($K_{m,MT} \pm$ S.E., $k_{cat} \pm$ S.E.; 4.7 ± 1.0 nM, 9.0 ± 0.2 s⁻¹ of Kid515-GS Δ C, 24 ± 5 nM, 8.1 ± 0.3 s⁻¹ of Kid441-GS Δ C), confirming that the second MT-binding site can interact with MTs irrespective of gelsolin fusion. The movements of Kid515-GS Δ C and Kid441-GS Δ C along an MT visualized by an actin filament were observed as shown in supplemental Movies 1 and 2). Both Kid fragments moved smoothly and stably for several micrometers, showing that the second microtubule-binding site does not inhibit the motility of Kid. Some of these movements may be induced by several Kid molecules because it has been reported that the plural gelsolin molecules bound to the same actin filament (29). Therefore, it is unclear from our assay whether Kid is a processive motor or not. The average velocities of Kid515-GS Δ C and Kid441-GS Δ C were almost the same (150 nm/sec), indicating that the second MT-binding site of Kid does not reduce the velocity (Fig. 4B).

DISCUSSION

Monomeric Form of Kid—As a result of this study, the full-length Kid expressed in mammalian cells and all Kid fragments prepared from *E. coli* were found to be monomeric in form in the condition (100 mM K-acetate, Kid < 100 nM). Therefore, it was concluded that the Kid molecule has a monomeric form in the physiological condition. Compared with the dimeric conventional kinesin, Kid has a shorter region of predicted coiled-coil formation, as does KIF1A (Fig. 2), which is monomeric in form at low concentrations (30). This is consistent with the results obtained here.

On the other hand, it was recently reported that one kinesin-like motor protein, Unc104, is monomeric in form at low concentrations and can move along an MT similar to a conventional dimeric kinesin only when it dimerizes with its coiled-coil at high concentrations (>1 μ M) (31). It was also shown that Unc104 may dimerize while attaching to its cargo, vesicle membrane. Moreover, it has also been reported that Kar3, a kinesin-like protein, makes a hetero complex with Cik1, a nonmotor protein, by predicted coiled-coil region (32). It would be interesting to learn whether Kid can function in monomeric form *in*

vivo or dimerize by binding cargos (chromosomes) or make complexes with other proteins.

The Role of the Second MT-binding Site of Kid—The finding that Kid has a second MT-binding site in the 442–515 aa is consistent with a report showing direct interaction of the Kid C-terminal half (404–665 aa) with tubulin (33). In the preserved region of the second MT-binding site (Fig. 3D), there are some clusters of basic residues. MT-associated proteins such as MAP2 and tau bind and regulate MT assembly, and they have clusters of basic residues and an adjacent proline-rich region in the MT binding domain (34, 35). There are also some clusters of prolines and basic residues in two Ncd tail domains that bind MTs in an ATP-insensitive manner (36, 37). In addition, it has been shown that the binding affinity of tau for MTs becomes low by removing an adjacent proline-rich region (38). Kid has 10 basic conservative residues but not an adjacent proline-rich region in the second MT-binding site (Fig. 3D), which is consistent with weak binding activity. It was reported that regulation of the activity of a second MT-binding site at the C-terminal tail of CENP-E by phosphorylation is required for proper localization and the function (39). Recently, the localization of Kid was also found to be regulated by phosphorylation of Thr-463 (17). It is interesting that the conserved Thr-463 is near the cluster of basic residues in the second MT-binding region. Thus, the MT-binding activity of this region may be controlled by the phosphorylation of Thr-463 similar to the regulation mechanism of CENP-E.

The activity of the second MT-binding domain at the CENP-E tail is ATP-insensitive and is assumed to have a role in cross-linking antiparallel MTs in the spindle (39). It has been shown that Ncd acts in meiosis I spindle assembly of *Drosophila* by cross-linking microtubules (40). These domains of both Ncd (described above) and CENP-E are assumed to have a role in bundling MTs by fixation of themselves on MTs. However, based on the results of this study, the second MT-binding site of Kid seems to have other roles than bundling MTs, because the affinity for MTs is enhanced but the velocity of Kid is not reduced by its weak binding. Even when Kid is moving or generating force, Kid would be able to keep binding on an MT with high affinity by the second MT-binding site. Generally, the monomeric form has a lower affinity than an oligomeric form. The affinity of monomeric Kid for MTs is enhanced by the second MT-binding site, which may contribute to proper localization on the spindles without detaching and to efficient force generation as a polar ejection force to keep pushing chromosomes toward the metaphase plate.

The value of $k_{cat}/K_{m,MT}$ measured in ATPase assay, called chemical processivity, is known to be an indicator of mechanical processivity (41) when ATP hydrolysis is tightly coupled to the movement of a motor molecule; high value would suggest high “processivity.” The $k_{cat}/K_{m,MT}$ of Kid515 that have the second MT-binding site was $2100 \mu\text{M}^{-1} \text{s}^{-1}$, which is comparable with other processive motors, *e.g.* conventional kinesin

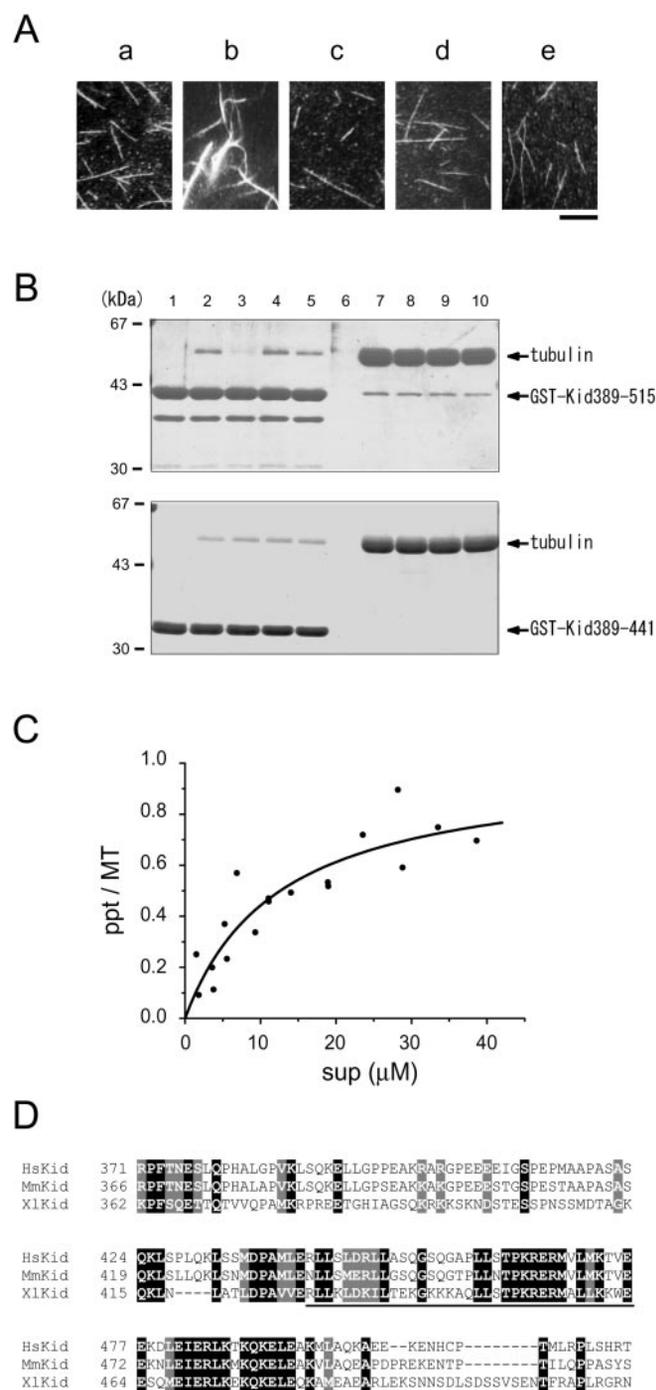


FIG. 3. The second MT-binding site of Kid. *A*, images observed by epi-fluorescent microscopy when Kid fragments were mixed with MTs. *a*, MT; *b*, Kid515 + MT; *c*, Kid515 + MT + ATP; *d*, Kid441 + MT; *e*, Kid388 + MT. Scale bar, 20 μm . *B*, the SDS-PAGE shows a co-sedimentation assay of GST-Kid-(389–515) (upper panel) and GST-Kid-(389–441) (lower panel) with MTs. Lanes 1–5, supernatant; lanes 6–10, the pellet. Lanes 1 and 6, GST-Kid protein without MT. Lanes 2 and 7, GST-Kid protein + MTs. Lanes 3 and 8, GST-Kid protein + MT + ATP. Lanes 4 and 9, GST-Kid protein + MT + AMP-PNP. Lanes 5 and 10, GST-Kid protein + MT + ADP. Numbers on the left indicate the position of the molecular weight markers. *C*, co-sedimentation of GST-Kid-(389–515) with MTs. Data are fitted using the simple quadratic equation showing $K_d = 12 \pm 4 \mu\text{M}$. *D*, the amino acid sequences 371–519 of human Kid were aligned with orthologs using the CLUSTAL W program (44). Identical amino acids are shaded in black, and conservative substitutions are shaded in gray. Hs, *Homo sapiens* (accession number, AB017430); Mm, *Mus musculus* (accession number, NW_000332; gene name, AL033313); Xl, *Xenopus laevis* (accession number, AF267850). Underlines show the region of the second MT-binding site determined in the study.

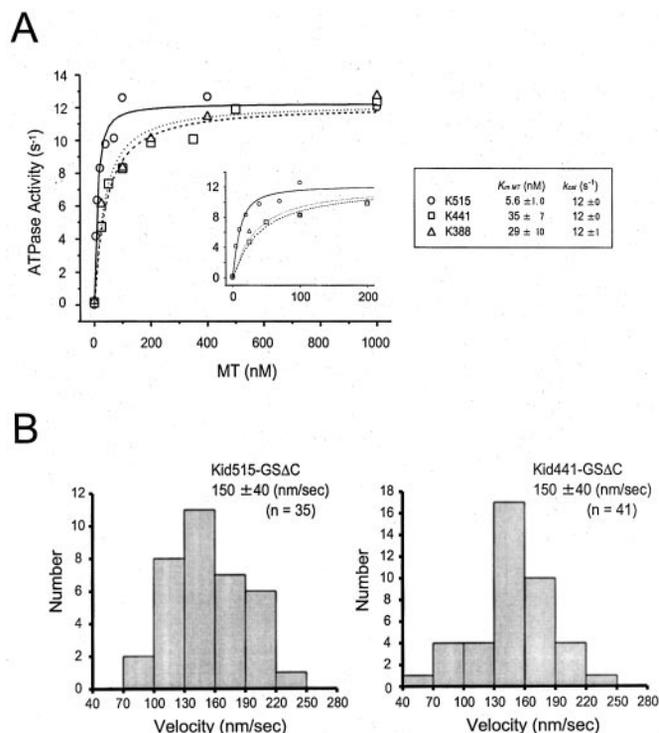


FIG. 4. ATPase activity and motility. *A*, the MT-stimulated ATPase activities of Kid515, Kid441, and Kid388. Each dot is an averaged value of three or four independent measurements. Solid (Kid515), broken (Kid441), and dotted (Kid388) lines are the fitting curves by the Michaelis-Menten equation. The small graph inside shows the range of MT concentrations up to 200 nM. The inset indicates the $K_{m,MT} \pm \text{S.E.}$ and $k_{cat} \pm \text{S.E.}$ *B*, distributions and averaged velocities $\pm \text{S.D.}$ of Kid515-GS Δ C and Kid441-GS Δ C.

(140–1000 $\mu\text{M}^{-1} \text{s}^{-1}$) (42) and KIF1A (6900 $\mu\text{M}^{-1} \text{s}^{-1}$) (28), suggesting the possibility of Kid as a processive motor although it is not clear that ATP hydrolysis and the movement of Kid are tightly coupled or not. KIF1A is shown to move processively with diffusion along an MT in a monomeric form, and the mechanism is explained by interaction between the K-loop in the motor domain of KIF1A and the E-hook of MT (43). Kid is a monomer and is distinct from the members in the Unc104/KIF1 subfamily that have the K-loop but has the second microtubule-binding domain outside its motor domain. It would be interesting to analyze the single-molecule behavior of Kid to determine whether it is a processive motor.

Kid has been reported to show very important roles in cell division. In this report, the property of the Kid molecule was elucidated *in vitro*. The high affinity of Kid for MTs is thought to be appropriate property for alignment of chromosomes on the metaphase plate without detaching from a spindle. Although the role of Kid is unknown when Kid localizes around the centromere, spindle pole-proximal side on the chromosomes at the anaphase, Kid may contribute to anchor chromosomes to spindles with its high affinity for MTs. The study of localization of Kid fragments and mutants will reveal the *in vivo* function of the second microtubule-binding site.

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