The kinesin family member BimC has a highly positively charged domain of ~70 amino acids at the N terminus of the motor domain. Motor domain constructs of BimC were prepared with and without this extra domain to determine its influence. The level of microtubules needed for half saturation of the ATPase of BimC motor domain constructs is reduced by ~7000-fold at low ionic strength upon addition of this extra N-terminal extension. Although the change in microtubule affinity is less at higher salt, addition of the N-terminal domain still produces a 20-fold increase in affinity for microtubules in 200 mM potassium acetate. A fusion protein of the N-terminal domain and thioredoxin binds tightly to MTs at low salt, consistent with the increased affinity of motor domain constructs (which contain the N-terminal domain) being due to the additional binding of the N-terminal domain to the microtubule. Hydrodynamic analysis indicates that the N-terminal extension is in a highly extended conformation, suggesting that it may be intrinsically disordered. Fusion of the N-terminal extension of BimC onto the motor domain of conventional kinesin produces a similar large increase in microtubule affinity without significant reduction in $k_{cat}$ or velocity in an in vitro motility assay, suggesting that the N-terminal extension can act in a modular manner to increase the microtubule affinity of kinesin motor domains without a decrease in velocity.

BimC is the founding member of the BimC/Eg5 or N-2 class of motor proteins in the kinesin superfamily (see Refs. 1 and 2). The motor domain is at the N terminus, followed by an extended region with a number of predicted coiled-coil domains. BimC was first discovered in Aspergillus nidulans (3), and members of this class are widely distributed from yeast to higher organisms. The native molecule is a homotetramer that is believed to facilitate cross-linking of microtubules (MTs) in the overlap region of the spindle. The motor domains are at each end of the tetramer and are attached to different microtubules that are capable of producing the sliding that pushes apart the poles (5). Because of its role in mitosis, human Eg5 is an attractive target for drug development, and monastrol is a reversible inhibitor of Eg5 that blocks mitosis (6–8). Monastrol is highly selective for Eg5 from higher organisms and does not even inhibit the closely related A. nidulans BimC (8).

Detailed studies of the kinetics and structural properties of BimC motor domains from lower organisms have not been performed, but the related Eg5 from higher organisms has been studied more extensively. The crystal structure of the motor domain of human Eg5 (9) indicates that the overall folding of the motor domain is similar to that of other kinesins, but the neck linker is in a novel conformation. Image reconstruction of decorated MTs indicates that the attached head of an Eg5 dimer is in a similar position to that of other kinesins with an additional detached density whose position is less sensitive to nucleotide than to conventional kinesin (10). Initial kinetic characterization (11) established that the ATPase mechanism of Eg5 was similar to that of conventional kinesin except that it was slower, with ADP release being at least partially rate-limiting and with non-hydrolyzable ATP analogs producing tighter binding to MTs. Unlike conventional kinesin, however, there are indications that Eg5 dimers are not processive (12). Recent work has indicated how the kinetics and state of oligomerization vary with inclusion of increasing regions of the neck coil (7, 8).

Although BimC is classified as an N-terminal motor because of its grouping with other N-terminal motors by similarity of sequence of the motor domains, BimC from A. nidulans actually has a significant domain of ~70 amino acids appended to the N terminus of the motor (3). This N-terminal extension (Nte) is highly positively charged and has weak sequence similarity to part of the proline-rich region of MAP2 that is involved in MT binding (Fig. 1). These features suggest that the Nte of BimC may constitute a second MT-binding site that could influence the kinetics of BimC and its affinity for MTs. We report here that the Nte binds independently to MTs and that attachment of the Nte to the BimC motor domain greatly increases the affinity for MTs. Furthermore, the Nte can act in a modular manner, as attachment of the Nte to the motor domain of conventional kinesin also results in a large increase in affinity for MTs, without significant inhibition of $k_{cat}$ or sliding velocity.

**EXPERIMENTAL PROCEDURES**

**Construction of Expression Plasmids**—The BimC constructs indicated in Figs. 1–3 were derived from the partial cDNA clone and the overlapping genomic clone of Enos and Morris (3). The cDNA and genomic clones were fused to generate a full-length construct using the DdeI site at position 559. PCR was then used to generate a series of motor domain constructs that were cloned into pET21 (Novagen) for expression. BC72M is a nonfuson protein that contains the core motor domain and the region homologous to the neck linker of conventional kinesin but lacks the Nte of BimC. It was obtained by converting Ile72 of BimC into an initiation methionine and introducing a stop codon **Received for publication, August 25, 2003, and in revised form, September 29, 2003.**

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after Lys⁴²⁸. Although BC72M expresses well, constructs beginning at residue 1 and containing the Nte did not express protein at detectable levels. The 5′ coding region is highly enriched in GC base pairs, and a new primer with the sequence GCCATGGTGTGCGGACGTCG-TACTTATGG was used to reduce the number of GC base pairs and to replace codons with unfavorable usage by Escherichia coli. This new construct had detectable, but low, levels of expression as a non-fusion protein. The amount of protein that could be isolated, however, was not enough for extensive biochemical characterization, and this construct was subsequently subcloned into pET32 (Novagen) as a fusion protein with thioredoxin (TT-BC1M), which expressed at a high level.

The DNA sequence of the derived constructs did not agree completely with the published sequence (GenBank™ accession number M32075; Ref. 5), suggesting that at least one of the constructs contained a shorter 5′ genomic region than that reported originally designed to match the published sequence. Ser⁹ is near the N terminus of thioredoxin and contain Tyr at position 9 instead of Ser because the 5′ primer was originally designed to match the published sequence. Ser⁹ is near the N terminus in a region that is likely to be unstructured and outside of the region of sequence similarity with MAP2. Consequently the change of Ser to Tyr at position 9 is not likely to significantly affect the MT binding properties. As a control, constructs containing the wild-type Ser⁹ were also generated as indicated in Fig. 3, and their ATPase and hydrodynamic properties were indistinguishable for those containing the SOY substitution (see “Results”).

TT-BKinM and TT-BKin412GS contain the same N-terminal region of BimC fused to thioredoxin as described for TT-BC1M(Tyr⁹), but the motor domain of BimC is replaced by the motor domain of conventional kinesin as indicated in Figs. 1–3. DKKH412GS and BKin412GS in addition are fused at the C terminus to cytoplasmic gelsolin derived from A. nidulans.

**Protein Expression and Purification**—General procedures for induction and purification on phosphocellulose were carried out as described (14). MgATP was maintained at ≥0.1 mM during the purification of all constructs containing the motor domain. DKKH412GS and BC72M were expressed as non-fusion proteins and purified essentially as described for conventional kinesin motor domains (14, 15). The TT series of thioredoxin fusions (Fig. 3) contains a His tag, and they were purified by both phosphocellulose and Ni-NTA metal affinity chromatography. The fusion proteins were cleaved with thrombin, and the released motor domains were separated from thioredoxin and thrombin by chromatography on phosphocellulose at pH 6.5. TM-Nte does not contain a His tag and was purified by chromatography on phosphocellulose and gel filtration on Sephacryl S-300. Final preparations were dialyzed against 50% glycerol in A25 buffer with 50 mM KC1, 2 mM diethiothreitol and 0.1 mM MgATP (for motor domain constructs) and stored at ~8 °C in small aliquots.

Taxol-stabilized MTs were prepared from phosphocellulose-purified tubulin as described (16), except that the source of the tubulin was pig brain rather than cow brain. MT concentrations are reported as the concentration of tubulin heterodimers.

**Hydrodynamic Characterization**—Gel filtration and velocity sedimentation in a sucrose gradient were used to estimate the D_{20,w} and s_{20,w} values. Standards proteins were bovine catalase, bovine serum albumin, bovine carbonic anhydrase, and bovine cholinesterase with s_{20,w} values of 11.3, 4.4, 3.7, 3.0, and 1.9 S and D_{20,w} values of 4.4, 6.1, 7.4, 9.3, and 12.7 × 10^{-13} cm²/s, respectively. Most values are from Ref. 17, but in some cases, the literature values are not self-consistent, and minor adjustments have been made so that each set of s_{20,w}, D_{20,w}, and M, values obeys the Svedberg equation and yields an [f] versus [M] plot with a plateau (see “ Results”). Chromatography on phosphocellulose and gel filtration on Sephacryl S-300 (Amersham Biosciences). Fractions were analyzed by SDS-PAGE to determine the peak positions. Cytochrome c is retarded on the column at low ionic strength and was not included in the regression in determinations in the absence of added NaCl. Ultracentrifugation was performed on 5-ml linear gradients of 90% sucrose in an MLS-60 rotor (Beckman) that were prepared at 10% sucrose in A25 buffer, with partial specific volume of 0.73 cm³/g. The buffer used was A25 buffer (25 mM potassium ACES, pH 6.9, 2 mM magnesium acetate, 2 mM K-EGTA, 0.1 mM K-EDTA and 1 mM β-mercaptoethanol) with 25 mM KC1 and was supplemented with 200 mM NaCl, as indicated. For motor domains, the centrifugation was for 18 h at 44,000 rpm with catalase, serum albumin and carbonic anhydrase as standards. For the smaller TM-Nte constructs, centrifugation was for 36 h at 50,000 rpm with ovalbumin, carbonic anhydrase and cytochrome c as standards. A partial specific volume of 0.73 cm³/g was used in all calculations.

**Kinetic and Binding Measurements**—All reactions and binding experiments were conducted at 25 °C in A25 buffer supplemented with KCl and NaCl as indicated. The coupled enzyme system of pyruvate kinase and lactate dehydrogenase were used to monitor ATP hydrolysis in the presence of 2 mM P-enolpyruvate and 1 mM MgATP as described (15, 18). Determination of k_{cat} and K_{M,ATP}, values were typically performed in 5 or more concentrations of MTs in duplicate, with error bars indicated. The data were fit to the Michaelis-Menten equation to the full quadratic expression for mutual depletion (19) using the Solver routine of Excel (Microsoft). When it was not possible to go to a high enough MT concentration to accurately determine k_{cat} and K_{M,ATP}, the k_{cat}/K_{M,ATP} value was reported. At high ionic strength when the maximum MT concentration was much less than K_{M,ATP}, the k_{cat}/K_{M,ATP} value was determined from the linear increase in rate with increasing MT concentration.

**Motility**—Short F-actin filaments were capped with complexes of kinesin-gelsolin fusion proteins and labeled with rhodamine phalloidin essentially as described by Yajima et al. (20). The complexes with F-actin were formed in 100 mM KCl in A25 buffer without EDTA or EGTA and containing 1 mM MgATP and 1 mM CaCl₂. Immediately before loading into a flow cell for observation, the complexes were diluted 1:100 into the same buffer but without CaCl₂ and with 25 mM KCl and 0.4 mg/ml casein. Movement of these complexes along salt-washed sea urchin sperm axonemes was observed by fluorescence microscopy with an Olympus XI71 microscope using excitation by a 10 milliwatt laser at 532 nm (Beta Electronics) and a 560–610 nm band-pass filter for emission. A 60 × 1.45 numerical aperture objective was used with an additional 4× projection lens. Images were acquired with a PicIII intensified camera (Instrutech) at 30 frames/s, recorded on S-VHS tape, digitized, and analyzed with NIH-Image. In some cases, a rolling average of 2 or 4 video frames was used. Motility was only measured for spots that exhibited continuous movement for > 1 s. Motility was difficult to observe with BKin412GS because most of the short actin filaments rapidly became tethered to the surface of the coverslip through one end only, with the other end moving freely in solution. This was also observed with DKKH412GS, but to a much reduced extent. Pretreatment of the coverslip with the basic protein lysozyme reduced the amount of surface binding, suggesting that the binding was enhanced by the interaction of the positive Nte with anionic sites on the coverslip. For all of the measurements reported here, the coverslips were washed with detergent, blocked with lysozyme at 2 mg/ml for 5 min, thoroughly rinsed with water, and air dried before assembly of a flow cell using two strips of double-sided tape. Axonemes were flowed through the cell to allow attachment, and then the cell was flushed with buffer containing 0.4 mg/ml casein to further block the surface before introduction of the motor-actin complexes.

**Results**

**Properties of BimC Motor Domain**—Previous work (21) indicated that DKH346 (residues 1–346 of Drosophila kinesin as indicated in Fig. 1A) is a good model for a motor domain of conventional kinesin that contains the core ATPase domain of Drosophila kinesin plus a complete neck linker. DKKH346 is monomeric because it lacks the neck coiled-coil region that is required for dimerization. BC72M (see Figs. 1, 2, and 3) is an analogous BimC construct that begins and ends on positions that are equivalent in a sequence alignment to positions 3 and 345 of Drosophila kinesin. Hydrodynamic analysis (Table I) indicates that BC72M is also monomeric. The basal ATPase rate in the absence of MTs is ~0.1 s⁻¹ for all of the BimC motor domain constructs studied here. The dependence of the ATPase rate of BC72M on the concentration of MTs over a range of KCl concentrations is given in Fig. 4A, and kinetic parameters are summarized in Table II. Even in low salt buffer, saturation is far from complete at the highest MT concentration. The k_{cat} value for the maximum ATPase rate at saturating levels of MTs and the K_{M,ATP} value for the concentration of MTs producing half the maximum rate can only be estimated at ~31
s⁻¹ and ~17 μM, respectively, in 3 mM KCl. At higher ionic strength, the ATPase rate is strongly inhibited, as is also observed with other kinesin family members. Separate estimation of kcat and kₐₐ₉ values is not feasible at higher salt, but the bimolecular rate kₐₐ₉/ATPase = kcat/Kₐ₉ can still be determined, and the dependence of kₐ₉ on ionic strength is indicated in Fig. 5. For comparison, the ATPase rate of the motor domain of conventional kinesin (DKH346) was determined at 3 and 25 mM KCl as indicated in Fig. 4B. The kcat and Kₐ₉ values of ~86 s⁻¹ and ~5 μM in 3 mM KCl for DKH346 indicate that BimC is slower than an equivalent construct of conventional kinesin and has weaker affinity for MTs in the presence of ATP.

Influence of Nte—BC1M contains the full Nte domain of BimC in addition to the motor domain and neck linker of BC72M as indicated in Figs. 1–3. The D₂₀,ᵥ value of BC1M could not be obtained by gel filtration because it binds to the column, even in the presence of 200 mM NaCl, but the sedimentation coefficient of 3.3 S (Table I) is similar to that of BC72M and indicates that BC1M is also a monomer under these conditions. Inclusion of the Nte in BC1M produces a striking increase in the affinity of the motor for MTs during ATP hydrolysis as indicated in Fig. 6. At 3 mM KCl in Fig. 6A, the activation by MTs is close to stoichiometric, even at a BimC concentration of only 8.3 nM. Exact determination of the Kₐ₉ value under these conditions is complicated by the tight binding with Kₐ₉ < Kᵥ and the possibility that the affinity for BimC may change as the MT lattice approaches saturation with bound BimC. However, the 7000-fold decrease in Kₐ₉ on addition of the Nte (~0.0025 μM for BC1M versus ~17 μM for BC72M) clearly indicates a dramatic increase in MT affinity. The kₐ₉ value of 24 s⁻¹ for BC1M is similar to the value of ~33 s⁻¹ for BC72M, indicating that addition of the Nte does not prevent catalytic turnover at close to the normal rate. The kₐ₉ of BC1M is also strongly dependent on ionic strength (Fig. 6), and the dependence is even greater than for BC72M, as indicated in Fig. 5. BC1M with wild-type Ser at position 9 has kcat and Kₐ₉ values of 24.2 s⁻¹ and 0.118 μM, respectively, in 75 mM KCl. These values are essentially identical to the corresponding values of 24.5 s⁻¹ and 0.107 μM for BC1M with Tyr at position 9 (Table II); thus, this substitution has no influence on kₐ₉ or MT affinity.

BimC contains a region with a high concentration of mixed negative and positively charged groups that immediately provides an additional domain of ~70 amino acids at its N terminus that is not found in other kinesins. This N-terminal extension (Nte) can be subdivided into a region with a high concentration of mixed charge that is immediately adjacent to the motor domain and a highly positively charged region that is similar to a part of MAP2, as indicated in panels A and B. This region of BimC is designated the MAP2 similarity region (MSR). The lines linking panels A and B and panels B and C indicate the locations of the corresponding regions. B, sequence alignment of the N-terminal regions of rat kinesin (43), Drosophila kinesin (44), BimC (3) (with the sequence corrections described under \("Experimental Procedures\), and part of the proline-rich regions of MAP2 (45). GenBank™ accession numbers are P56536, P17210, M32075, and P20357, respectively. The alignment of the motor domains is anchored by the start of the tubulin-binding repeats (see Ref. 46).
The strength of 0.24. These rates with 200 mM potassium acetate of complexes of dimeric kinesin-gelsolin with short fluorescent F-actin filaments was determined with and without fusion to the BimC Nte. Drosophila kinesin (DKH412GS) had a velocity of 0.485 ± 0.084 (19) µm s⁻¹, which is similar to the value of 0.45 µm s⁻¹ obtained in the same buffer with DKH960 adsorbed to glass (23). The velocity of the corresponding construct with the Nte (BKin412GS) was also similar at 0.448 ± 0.034 (12) µm s⁻¹.

Conformational Analysis of the Nte Domain—As indicated in Table I, a fusion protein of thioredoxin and BimC residues 1–71 (TM-Nte) is predominantly monomeric under these conditions, and thus the Nte does not have a significant tendency to dimerize. These experiments were performed in buffer supplemented with 200 mM NaCl, because TM-Nte interacts with the column at lower ionic strength. With 1000 mM NaCl, the $D_{20,w}$ value was 8.3 × 10⁻⁷ cm²/s, indicating that 200 mM NaCl is sufficient for overcoming ionic interaction with the column. In addition, the Nte seems to be in an extended conformation, as indicated by the large shift in migration of TM-Nte relative to the globular standard proteins between gel filtration and sedimentation (Fig. 8). On gel filtration, TM-Nte (20.0 kDa for monomer) elutes well before carbonic anhydrase (29 kDa) and just after ovalbumin (45 kDa), indicating that TM-Nte is a monomer.

Modularity—To test the generality of the BimC Nte to increase the affinity of motor domains for MTs, the Nte of BimC was fused to the N terminus of the DKH346 motor domain of Drosophila kinesin as indicated in Figs. 1–3 for BKinM. The ATPase of this construct also saturates at extremely low MT concentrations at low ionic strength (Fig. 7), as observed for BC1M. The $k_{cat}$ value of 65 s⁻¹ for BKinM is similar to that of 85 s⁻¹ DKH346 alone (Fig. 4).

Motility—The velocity of sliding along axonemes for complexes of dimeric kinesin-gelsolin with short fluorescent F-actin filaments was determined with and without fusion to the BimC Nte. Drosophila kinesin (DKH412GS) had a velocity of 0.485 ± 0.084 (19) µm s⁻¹, which is similar to the value of 0.45 µm s⁻¹ obtained in the same buffer with DKH960 adsorbed to glass (23). The velocity of the corresponding construct with the Nte (BKin412GS) was also similar at 0.448 ± 0.034 (12) µm s⁻¹.

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Hydrodynamic characterization

| Constructs | $S_{20,w}$ (S) | $D_{20,w}$ | $M_a$ (Svedberg) | $M_b$ (Peptide) | Oligomeric state |
|------------|----------------|-----------|------------------|----------------|-----------------|
| BC72M      | 3.22 ± 0.11 (4) | $7.89 ± 0.07$ (3) | 36.8            | 39.3           | Monomer         |
| BC1M       | 3.30 ± 0.03 (4) | ND        | 46.9            | 20.0           | Monomer         |
| TM-Nte     | 1.69 ± 0.06 (3) | $7.94 ± 0.04$ (2) | 19.2            | 12.2           | Monomer         |
| TM-S       | 1.79 (1)        | ND        | —               | —              | —               |

$^a$ Calculated by the Svedberg equation.

$^b$ Calculated from the amino acid sequence.

$^c$ ND, not determined.

**DISCUSSION**

Full-length members of the BimC family have been classified as having low ATPase rates and sliding velocities compared with conventional kinesin. They are also characterized as binding tightly to MTs even in the presence of ATP and requiring high salt for elution (see Ref. 4). Although the oligomeric state and other factors likely also contribute to the properties of the full-length enzyme, motor domain constructs of Eg5 are significantly slower than conventional kinesin and bind more tightly to MTs in the presence of ATP ($k_{cat}$ values of $2–14\ s^{-1}$ for Eg5 (7, 8, 11) compared with $–80\ s^{-1}$ for kinesin and lower $K_{cat, MT}$ values of 0.3 $\mu M$ for Eg5 and 8 $\mu M$ in A25 buffer (8) versus $–5\ \mu M$ for DKH346 in the same buffer). The motor domain from *A. nidulans* BimC that is studied here, however, has properties more similar to those of the motor domain of conventional kinesin with a $k_{cat}$ value of $–25\ s^{-1}$ and with binding to MTs in the presence of ATP that is even weaker, not stronger, than that of conventional kinesin ($K_{cat, MT}$ values of $–17$ and $–5\ \mu M$ for *A. nidulans* BimC and kinesin, respectively, in 3 mM KCl).

Inclusion of the Nte to form BC1M, though, produces a dramatic 7000-fold increase in MT affinity at low ionic strength without a significant decrease in $k_{cat}$. The Nte binds directly to MTs in a salt-dependent manner that parallels the influence of the Nte on the binding of the motor domain to MTs (Fig. 9). This finding suggests that the increased binding affinity of the motor with attached Nte domain likely represents the combined effect of the binding of both the motor domain and the Nte to the MT. With kinesin (21, 25) and Eg5 (7, 8), truncation into the neck linker region also produces an increase in $k_{cat}$ and a decrease in $K_{cat, MT}$, but these changes are comparatively modest and likely represent an abnormal situation as discussed previously (16).

Although the increase in affinity is greatly reduced at high ionic strength, addition of the Nte still results in a 20-fold increase in $k_{cat, ATPase}$ in 200 mM potassium acetate and thus is likely to be of physiological significance. Increased affinity of BimC for MTs would facilitate cross-linking of MTs to help stabilize the spindle. The dynamic nature of the spindle, however, requires that the cross-linking cannot be too stable, or it would inhibit remodeling and extension. The Nte fulfills both requirements as the lack of a decrease in $k_{cat}$ or sliding velocity upon the addition of the Nte indicates that the increase in MT
affinity is achieved without a decrease in the dynamic behavior of the motor along the MT.

The Nte can be divided into two main regions. The region immediately adjacent to the motor domain (65–75) is highly charged, but it contains both positive and negative groups. It has relatively little effect on \( K_{0.5(MT)} \) compared with the full Nte. The rest of the Nte has a high net positive charge (nine arginines and two lysines, but only two glutamic acids and no aspartic acids between positions 2 and 62) and has weak sequence similarity to part of the proline-rich domain of MAP2, as indicated in Fig. 1. These two charged subregions are separated by two hydrophobic residues (Val63 and Leu64). The region of MAP2 that is similar to the MSR has mixed charge (nine arginines and two lysines, but only two glutamic acids and no aspartic acids between positions 2 and 62) and has relatively little effect on \( K_{0.5(MT)} \) compared with the full Nte (Table II). The rest of the Nte has a high net positive charge (nine arginines and two lysines, but only two glutamic acids and no aspartic acids between positions 2 and 62) and has weak sequence similarity to part of the proline-rich domain of MAP2, as indicated in Fig. 1. These two charged subregions are separated by two hydrophobic residues (Val63 and Leu64). The large excess of positive charge in the MAP2 similarity region (MSR) is likely responsible for the extremely high dependence on ionic strength with a slope of almost 10 for a plot of log(\( k_{cat} \)) versus log(I) for BC1M in Fig. 5. The MSR is also enriched in threonine and proline (12 and 7, respectively). Phosphorylation of a Thr or Ser residue in this region is an attractive possibility for reducing the MT affinity, as has been observed with the second MT-binding region of Kid (26).

A number of kinesins have a region with mixed charge immediately adjacent to the N terminus of the motor domain, but none have a region with sequence similarity to the MSR of BimC, not even other members of the BimC class. Other fungal BimCs, however, do have a smaller region at the N terminus with excess positive charge that may be functionally equivalent. Eg5 from higher organisms does not have any N-terminal extension and may utilize other features such as surface loops on the motor domain to increase affinity for MTs in analogy to the K-loop of Ki61A (27, 28). Other examples of regions outside the motor domain influencing MT affinity are the charged region of the neck coil of kinesin (29) and the second MT-binding sites of Kid (30), Ncd (24), CENP-E (31), and kinesin (32–34). The region of MAP2 that corresponds to the MSR has less net positive charge and potentially does not bind as tightly to MTs. It is unclear, even, if the observed weak sequence similarity is not just the result of both regions needing to be extended and to have an excess of positive charge. It has been shown recently (35) that the MT-binding repeat region of MAPs (see Fig. 1C) actually binds on the inside surface of the MT, and thus many previous studies of the binding of MAPs to intact MTs may represent nonspecific binding to the outer surface. The region of MAP2 that is similar to the MSR, however, is outside the repeat region and is expected to interact with the
outside surface of the MT, even in the revised model for binding of MAPs (35).

Large hydrophobic residues are under-represented in the MSR (only five total for leucine, isoleucine, and valine, and no tryptophan, phenylalanine, tyrosine, or methionine). This pattern of a large excess of groups with a single charge type and a low hydrophobicity is characteristic of natively unfolded peptides that are usually in an extended conformation (36, 37), particularly at low ionic strength where the repulsion between groups of like charge is greater. The hydrodynamic characterization of the Nte fused to thioredoxin (Fig. 8 and Table I) indicates that the Nte region is in fact highly extended, even in the presence of 225 mM salt. Although the MSR contains a number of prolines, it is not as highly enriched in prolines as is typical for peptides in the extended PPII helical conformation (38, 39), and the extent to which the PPII helix contributes to the extended conformation of the Nte is unknown.

The high $k_{\text{bi(ADP)}}$ values of $-10^4 \mu M^{-1} s^{-1}$ for BC1M at lower ionic strength are in large excess of the value of $20-30 \mu M^{-1} s^{-1}$ estimated for the maximum diffusion controlled rate of association of a motor domain with an MT in the absence of specific effects (40). Some of this acceleration is likely because of an increased association rate with the MT, leading to productive ADP release ($k_{\text{bi(ADP)}}$) that is caused by favorable charge interactions at low ionic strength. However, some of the high $k_{\text{bi(ADP)}}$ value can also be caused by an increase in kinetic processivity in which multiple ATP molecules are hydrolyzed per productive encounter with an MT, as discussed previously (41). Determination of the number of ATP molecules hydrolyzed per encounter requires measurement of the molecular rate of MT-stimulation of ADP release ($k_{\text{bi(ADP)}}$). The usual method for determination of $k_{\text{bi(ADP)}}$ employs mantATP, which is a good analog of ATP for most kinesins. However, mantATP is a poor analog of ATP for BimC because mantADP binds tightly to BimC, with a slow rate of release even in the presence of MTs. In fact, mantATP behaves as a classical slow-tight inhibitor of the ATPase of BimC. Further work with other methods for measurement of $k_{\text{bi(ADP)}}$ will be required for the determination of the relative contributions of these factors to the total acceleration.

The Nte of BimC produces a similar decrease in $K_{0.5,\text{MT}}$, when fused onto the motor domain of conventional kinesin. Furthermore, the Nte has little effect on the $k_{\text{cat}}$ at saturating MTs or on the sliding velocity, indicating that the principal effect is to increase binding to the MT. A similar lack of inhibition was observed with the second MT-binding site of Kid (30) and with variation in the amount of positive charge in loop 12 (K-loop) of Kif1A and kinesin (27), although Rogers et al. (42) did observe an $-2$-fold reduction in $k_{\text{cat}}$ upon introduction of a K-loop into KIF1D and kinesin. The additional binding of the Nte to the MT must only tether the motor domain to the MT while still allowing any conformational changes of the motor domain that are needed to produce rapid motility. The extended and likely flexible conformation of the Nte would facilitate such tethering, without producing rigid coupling. In addition, the binding of the Nte must be sufficiently weak so that its dissociation does not introduce an internal load on the motor

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**Fig. 7.** MT-ATPase kinetics of BKinM. KCl concentration is 25 (●) and 75 (■). Values of $k_{\text{cat}}$ and $K_{0.5,\text{MT}}$ are summarized in Table II. Fitting and theoretical plots are as in Fig. 6.

**Fig. 8.** Hydrodynamic analysis of TM-Nte. TM-Nte was analyzed by gel filtration on Sephacryl S-300 (A) or sedimentation on a sucrose gradient (B). Sedimentation analysis of control TM-S is shown (C). Analysis was performed in A25 buffer with 25 mM KCl and 200 mM NaCl. Fractions were analyzed by SDS-PAGE. Proteins are designated as bovine serum albumin (BSA), catalase (Cat), ovalbumin (OA), carbonic anhydrase (CA), Nte of BimC fused to thioredoxin (TM-Nte), thioredoxin without the Nte (TM-S), and cytochrome c (Cy).

**Fig. 9.** Binding of TM-Nte to MTs. MTs and TM-Nte were mixed in A25 buffer with KCl and centrifuged at 250,000 × g for 25 min and 25 °C or at 6000 × g for 60 s as indicated in B for “low speed spin.” KCl concentration was 1 mM in A and B and as indicated in C. Unspun samples (U), supernatants (S), and pellet (P) fractions were analyzed by SDS-PAGE at equivalent loadings. The location of tubulin subunits are indicated by “Tub” and TM-Nte by “Nte.” TM-Nte and MTs were at 4 μM each for all KCl concentrations in C. Note that MT concentration is expressed as concentration of tubulin heterodimers. Gel images are compressed vertically for compact presentation.

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a D. D. Hackney, unpublished observations.
that might reduce the sliding velocity. The apparent modular behavior of the Nte is consistent with its location at the N terminus of the motor domain, which is unoccupied in N-terminal kinesin motors. Flexibility in the Nte would also facilitate the ability of the Nte to function with other motor domains in a modular manner because precise coordination is not required. These properties and the modular action of the Nte suggest that it may be useful in nanotechnology applications in which tight binding of these and other kinesin family members, without inhibition of sliding velocity, could be advantageous.

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