Formation of the Compact Conformer of Kinesin Requires a COOH-terminal Heavy Chain Domain and Inhibits Microtubule-stimulated ATPase Activity*

(Received for publication, December 9, 1998, and in revised form, January 29, 1999)

Maryanne F. Stock, Jennifer Guerrero†, Brian Cobb, Christopher T. Eggers, Ting-Guang Huang‡, Xun Li, and David D. Hackney¶

From the Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

Full-length Drosophila kinesin heavy chain from position 1 to 975 was expressed in Escherichia coil (DKH975) and is a dimer. The sedimentation coefficient of DKH975 shifts from 5.4 S at 1 mM NaCl to −6.9 S at <0.2 mM NaCl. This transition of DKH975 between extended and compact conformations is essentially identical to that for the heavy chain dimer of bovine kinesin (Hackney, D. D., Levitt, J. D., and Suhan, J. (1992) J. Biol. Chem. 267, 8696–8701). Thus the capacity for undergoing the 7 S/5 S transition is an intrinsic property of the heavy chains and requires neither light chains nor eukaryotic post-translational modification. DKH960 undergoes a similar transition, indicating that the extreme COOH-terminal region is not required. More extensive deletions from the COOH-terminal (DKH945 and DKH937) result in a shift in the midpoint for the transition to lower salt concentrations. DKH927 and shorter constructs remaining extended even in the absence of added salt. Thus the COOH-terminal ~50 amino acids are required for the formation of the compact conformation. Separately expressed COOH-terminal tail segments and NH2-terminal head/neck segments interact in a salt-dependent manner that is consistent with the compact conformer being produced by the interaction of domains from these regions of the heavy chain dimer. The microtubule-stimulated ATPase rate of DKH975 in the compact conformer is strongly inhibited compared with the rate of extended DKH894 (4 s⁻¹ and 35 s⁻¹, respectively, for kcat at saturating microtubules).

Kinesin is an ATP-dependent motor protein that is involved in movement of membranous vesicles along MTs¹ (see Refs. 1 and 2). The NH2-terminal ~540 amino acids of the heavy chain forms a globular motor domain (head) that has MT-stimulated ATPase activity (see Fig. 2B). The motor domain is followed by a long central coiled-coil stalk region and a small nonhelical domain at the COOH-terminal. The central region contains several positions at which the coiled-coil propensity is low and these likely represent hinges in the stalk. The first coiled-coil region that extends from the motor domain is designated the neck region. Constructs that contain the head and the COOH-terminal part of the coiled-coil neck form dimers (3). There is a likely hinge at position −400 that marks the boundary between the neck and the stalk. Peptides from the neck (4, 5) and stalk (6) have been demonstrated to interact in a coiled-coil manner by several criteria. The crystal structure of a dimeric head plus neck construct has been recently determined (7) and it directly demonstrates the coiled-coil interactions in the neck region.

Native kinesin is a heterotetramer composed of a dimeric heavy chain core with two light chains attached in the COOH-terminal region (8, 9). At high salt kinesin exist in an extended conformation with an s20,w value of ~6 S, but adopts a more compact conformation at low salt concentration with an s20,w value of ~9 S. This global conformational transition is readily reversible and can be observed by electron microscopy (10, 11). A species lacking detectable light chains is also obtained from bovine brain and undergoes a corresponding salt-dependent transition between extended (~5 S) and compact (~7 S) conformations (11). We report here that Drosophila heavy chain dimers also undergo a 7 S/5 S transition. Furthermore, specific regions of the tail and head/neck interact in a salt-dependent manner that is consistent with this interaction being responsible for producing the compact conformation.

Isolated head domains have a high MT-activated ATPase activity when prepared by limited proteolysis (12) or as fusion proteins (13). The rate of ~40 s⁻¹ at saturating MT concentrations that is observed with dimeric head constructs (see Ref. 3) is consistent with tight coupling of 1 ATP per step during motility (40 s⁻¹ per head × 2 heads per dimer × 8 nm per tubulin dimer along protofilament predicts a sliding rate of ~600 nm/s that is equal to the observed sliding rate of single molecules, see Ref. 14, for discussion). The maximum ATPase rate of bovine kinesin in the compact conformer, however, is only ~1 s⁻¹ for the 9 S heterotetramer and ~7 s⁻¹ for the 7 S dimer (15) and this suggests that the inhibition of full-length kinesin versus free head domains is due to interaction of the heads with tail domains in the compact conformer. Myosin from smooth muscle and nonmuscle cells undergoes a similar conformational transition at low salt concentration that produces a compact form with a highly inhibited rate of actin-stimulated ATP hydrolysis (16, 17).

It has not, however, been possible to directly test the inhibition of ATPase of kinesin in the compact versus the extended conformers because the transition could only be produced at high ionic strength. MT-stimulated ATPase and MT-stimulated ADP release are strongly inhibited by even moderate salt concentrations (18, 19) and it would be difficult to separate the
direct inhibitory effect of salts from their possible secondary activation linked to the 7 S/S S transition. We report here that removal of a small COOH-terminal region blocks formation of the compact conformation. This allows comparison of the MT-stimulated ATPase rate of full-length kinesin in the compact conformation to that of this slightly shorter extended construct at low salt where both are potentially active. Under these conditions, the extended construct has the same high ATPase activity as short dimers of heads, whereas the compact construct is strongly inhibited.

MATERIALS AND METHODS

All reactions were performed at 25 °C in A25 buffer as described previously (3). Centrifugation on sucrose density gradients and gel filtration on Bio-Gel A-5m were performed using standard proteins as described previously (11). SDS-PAGE was performed by the method of Laemmli (20) using a 4% stacking gel and a 11% separating gel, except for the separation of the long constructs (Fig. 1B) which used a 6% separating gel.

Construction of Expression Plasmids—All kinesin constructs were derived from the original Drosophila cDNA clone of Yang et al. (21). pGST864–975 encodes the tail domain region between positions 864 and 975. This construct was synthesized by insertion of the NcoI fragment of kinesin that contains the COOH-terminal into the Smal site of pGEX-2T (Pharmacia). pGST864–893 was obtained by cleavage of pGST864–975 with NcoI and EcoRI, treatment with DNA polymerase (Klenow), and religation. This procedure removes the coding region between amino acids 894 and the COOH terminus and results in a fusion protein with an extension of EFIVTD beyond position 910. pGST893–975 was obtained by insertion of the NcoI/EcoRI fragment of kinesin that contains the COOH-terminal into a modified pGEX-2T (Pharmacia) that contains an NcoI site following the BamHI site of the multiple cloning site. The resulting construct contains pGEX-2T sequence through the thrombin cleavage site terminating in pGEX-2T (Pharmacia). pGST864–893 was obtained by cleavage of pGST864–975 with NcoI and EcoRI, treatment with DNA polymerase (Klenow), and religation. This procedure removes the coding region between amino acids 894 and the COOH terminus and results in a fusion protein with an extension of EFIVTD beyond position 910. pGST893–975 was similarly obtained by insertion of the NcoI/EcoRI fragment of kinesin that was not cut at the internal NcoI site at 910. Constructs containing the extreme COOH terminus are highly susceptible to proteolysis during isolation and the particular preparations used here have some proteolytic cleavage as indicated in Fig. 1. For the GSTase fusions, the largest species is the size expected for a full-length construct in each case (Fig. 1A). The proteolytic cleavages are not likely to have occurred in the GSTase domain, because GSTase itself is resistant to proteolysis and because all of the proteolytic fragments continue to bind tightly to glutathione-Sepharose. Also, in preparations that have been more extensively proteolysed, the ladder of fragments shifts down toward the mass for GSTase, but not as expected for proteolytic removal of the kinesin-derived COOH-terminal domain (Fig. 1A). The longer fragment (PF-I) results from cleavage at XhoI as this fragment of GST893–975 comigrates slightly faster than uncleaved GST893–960. The short fragment (PF-II) results from cleavage at ~940 as this fragment of GST893–975 and GST893–960 is slightly larger than GST893–937. In more extensively proteolyzed preparations such as that used in Fig. 5, the fragment terminating at ~940 is the major species. Constructs lacking the 940–975 region are not significantly proteolysed.

Full-length DKH975 is also partially proteolyzed to species corresponding to cleavage at the PF-I and PF-II sites as indicated in Fig. 1B. Proteins obtained from inclusion bodies were used for size standards as they have likely precipitated before undergoing proteolysis. Although there is some unproteolyzed protein, the major band in a typical preparation of DKH975, as indicated in Fig. 1B, corresponds to cleavage at the PF-I site. There is also a significant band corresponding to cleavage at the PF-II site. Some preparations of DKH975 are more highly proteolyzed with the major band corresponding to PF-II and these preparations have not been included in the work presented here. Preparations of DKH960 show much less proteolysis and appear predominately as a single band of the expected size. Although it cannot be excluded that some proteolysis of DKH960 has occurred to produce the PF-I product, which is approximately the same size as DKH960, there is no major accumulation of the PF-II product as observed with DKH975. All of the shorter constructs (DKH894-DKH945) migrated as single bands at the same position as the corresponding proteins isolated from inclusion bodies.

RESULTS

Salt Dependence of Sedimentation of Long Heavy Chain Constructs—The sedimentation coefficients of the full-length Drosophila kinesin heavy chain construct, DKH975, and truncated DKH960 are 5.4 S in A25 buffer with 25 mM KCl and 1 mM NaCl, but shift to 6.7–7.0 S with lower salt concentrations, with a midpoint for the transition of ~0.5 mM NaCl (Fig. 2A). The behaviour of these long Drosophila constructs is essentially identical to that of the bovine kinesin heavy chain dimer (11). The elution position of DKH975 and DKH960 during gel filtration on Bio-Gel A-5m at high and low salt (not shown), as well, is essentially identical to that of the bovine heavy chain dimer (11) with an
increase in Stokes radius at high salt. The similarity of the sedimentation and diffusion properties to those of bovine dimer indicates that DKH975 and DKH960 are also dimers that undergo a transition from a compact conformation at low salt to an extended, highly asymmetric, conformation at high salt concentration. A possible model for this transition is presented in Fig. 2B for discussion.

The sedimentation coefficient of DKH894, DKH910, and DKH927 remain \(\approx 5.6\) S (Fig. 2A), even at low salt and thus removal of the COOH-terminal 48 amino acids inhibits formation of the compact dimer. Deletions of intermediate length, DKH945 and DKH937, still form the compact conformation at low salt, but the midpoint for the transition is shifted to lower salt concentration. The midpoints for DKH945 and DKH937 are similar, but DKH937 is shifted slightly to lower concentrations.

In the preparations of DKH975 that were used, the major component was a proteolysis fragment, PF-I, of approximately the same size as DKH960 (see "Materials and Methods"). Thus the observed sedimentation behavior of DKH975 preparations is dominated by the PF-I species and does not necessarily represent the behavior of true full-length DKH975. At 300 mM NaCl, DKH960 should be in the compact conformation, but DKH945 and shorter should be mainly extended. Chromatography of DKH975 preparations at 300 mM NaCl indicates that full-length DKH975 comigrates with the PF-I species at the position expected for the compact dimer, and thus it is likely that full-length DKH975 does behave similarly to DKH960. The smaller PF-II species elutes earlier at the position expected for the extended conformer, consistent with its length of \(-940\).

Localization of the Site of Interaction in the NH\(_2\)-terminal Region—The inability of DKH894-DKH927 to form the compact dimer at low ionic strength suggests that formation of the compact dimer results from interaction of the COOH-terminal region with regions closer to the head domain. A GSTase fusion protein containing the kinesin heavy chain from amino acids 864–975, labeled GST864–975, was used to determine if it could interact with head domains in a salt-dependent manner. GST864–975 was loaded onto a column containing glutathione-Sepharose and head domains were passed down the column in low salt buffer to determine if binding could occur. As indicated in Fig. 3A, DKH365, DKH381, and DKH392 were retained by the column, whereas DKH340 and DKH357 passed through. Subsequent elution of the columns with high salt buffer released the bound DKH365, DKH381, and DKH392.

Localization of the Site of Interaction in the COOH-terminal Region—In order to further localize the site of interaction in the tail region, GSTase fusions containing smaller parts of the COOH-terminal region were tested for interaction with DKH392. As indicated in Fig. 3B, the region between 893 and 975 was insufficient for salt-dependent interaction, but the region between 864 and 893 was not sufficient for tight interaction. Column binding tests were also performed with GSTase fusions containing kinesin tail sequences from 893 to 937 and 893 to 960 as indicated in Fig. 4. Under these conditions, none of the three head domains binds to a GSTase control, GST893–910 (not shown), or GST893–937. Extension of the tail domain

**Fig. 1.** SDS-PAGE analysis of protein preparations. A, SDS-PAGE of GSTase tail fusion proteins. M, molecular weight standards; G, GSTase; A, GST864–975; B, GST893–975; C, GST893–960; D, GST893–937 and E, GST893–910. Arrows indicate position for GST864–975 of the two major proteolytic fragments (PF-I and PF-II) formed by cleavage of the extreme COOH-terminal region. B, SDS-PAGE of non-fusion constructs. Proteins from inclusion bodies are indicated as xxxx, where xxx is the length of the construct. Only the region corresponding to the full-length species is shown. The long constructs differ only slightly in relative size and tall gels (Hoefer SE280) with 4% acrylamide were required for even partial separation. Also the time for electrophoresis was increased past the time required to elute the tracking dye so that the long constructs migrated closer to the bottom of the gel. The proteins were loaded at a low level that was just detectable by sensitive staining with colloidal Coomassie G-250 (31). Even moderate increases in loading produced bands that were too wide for resolution and migrated faster as illustrated by the two loadings of DKH960 at the right of the gel.

**Fig. 2.** A, dependence of \(s_{20,w}\) on salt concentration for heavy chain dimers. Sucrose density centrifugation was performed in A25 buffer with 0.05–0.1 mM MgATP. The values at the lowest ionic strength are for A25 buffer alone. Higher ionic strengths were obtained by supplementing A25 buffer with 25 mM KCl and 0–1 M NaCl. Variable aggregation to species larger than the dimer was observed at low ionic strength and high concentration, especially for Lys927. The concentration dependence of all of the constructs (except for DKH975) was investigated at low ionic strength and the reported \(s_{20,w}\) values at less than 100 mM ionic strength were obtained at very low initial protein concentration (<0.02 mg/ml) where aggregation was not a problem. DKH975, solid circles; DKH960, open circles; DKH945, squares; DKH937, open diamonds; DKH927, open triangles; DKH910, filled triangles; and DKH894, filled diamonds. B, model for conformational transition. The shaded ovals represent head domains and the smaller solid ovals represent the COOH-terminal non-helical domains. The specific interaction that is indicated for the compact 7 S conformation has part of the COOH-terminal non-helical domain interacting with the motor domain and the adjacent potentially helical region of the tail interacting with the coiled-coil neck. This detailed interpretation is speculative, but is consistent with the localization of the regions that are required for formation of the compact conformer as developed under “Discussion.”
to 960 (GST893–960) does produce salt-dependent binding of DKH365 and DKH405, but not DKH346. However, the binding of DKH405 is stronger than that of DKH365, as DKH365 is rapidly eluted by 150 mM salt, whereas higher concentrations of salt are required to rapidly elute DKH405.

Comigration of Head and Tail Regions during Centrifugation at Low Salt Concentration—Sucrose density gradient centrifugation of DKH392 alone in the absence of added salt (Fig. 5A) gives a sedimentation coefficient of 5.9 S compared with 5.2 S observed previously in 25 mM KCl (22). The self-aggregation of DKH392 is more extensive at lower salt (3) and this shift in $s_{20, w}$ value likely results from some reversible aggregation under these conditions. Sedimentation of GST893–975 alone gives a sedimentation coefficient of 4.4 S that is consistent with a dimeric protein of its subunit molecular weight and is similar to the value of 4.4 S obtained for the parent dimeric GSTase (not shown). The preparation of GST893–975 used here is more extensively proteolyzed than the sample of GST893–975 in Fig. 1A. The proteolytic fragment PF-II is the most abundant species and only a minor amount of PF-I is present, but considerable full-length GST893–975 is still present.

When GST893–975 is mixed with an excess of DKH392 before sedimentation, full-length GST893–975 comigrates with DKH392 at a higher sedimentation coefficient of 7 S (Fig. 4C). Most of the major proteolytic fragment (PF-II) of GST893–975 does not migrate more rapidly in the presence of DKH392, but some of the proteolytic fragment does shift as indicated by streaking into fractions further down the gradient. Weaker interaction of PF-II with DKH392 is consistent with the size of the COOH-terminal truncation to 940 (Fig. 1B) and the shift in the midpoint for DKH937 and DKH945 that is observed in Fig. 2. The parent GSTase protein shows no shift in sedimentation coefficient when centrifuged under these conditions in the presence of excess DKH392 (not shown).

The interaction observed between GST893–975 and DKH392 is likely to be weak and reversible under these conditions and is only observed in Fig. 5C because DKH392 was in excess. Sedimentation of GST893–975 with excess DKH392 in buffer with 25 mM KCl results in only a partial shift of GST893–975 (not shown), indicating that even low concentrations of added

---

**Fig. 3.** Binding of head domains to GSTase tails. Columns of glutathione-Sepharose (0.2-ml bed volume) were loaded with GSTase fusion proteins (0.3 mg) and washed with A25 buffer containing 1 mM NaCl to remove unbound fusion protein. Following equilibration with A25 buffer containing 0.05 mM MgADP, head constructs were loaded (0.1 ml of 0.13 mg/ml) and washed with a total of 0.8 ml of the same buffer. The combined eluents were pooled as the low salt run-through fraction. The columns were then washed with 0.35 ml of additional A25 and eluted with 0.6 ml of A25 with 1 mM NaCl as the high salt elution fraction. The low salt run through fraction and the 1 mM NaCl elution fractions were analyzed by SDS-PAGE as shown. Recovery of the head constructs in the combination of the low and high salt fractions was essentially complete. A, binding of different head constructs to columns containing bound GST64–975. B, binding of DKH392 to columns containing different GSTase tail fusions or control GSTase.

**Fig. 4.** Binding of heads to GST893–937 and GST893–960. Columns of glutathione-Sepharose (1 ml bed volume) were loaded with GSTase fusion proteins (2.5 mg). A mixture of DKH346, DKH365, and DKH405 was loaded on the columns (1 ml of 0.2 mg/ml each) in A25 buffer with 10 mM NaCl and washed with a total of 1.5 ml of buffer with 10 mM NaCl. The combined eluents were pooled as the run-through fraction (RT). The column was then washed sequentially with increasing salt concentrations of 10, 150, and 1000 mM NaCl (2.5 ml total for each). Fractions were analyzed by SDS-PAGE. Loading of each fraction was adjusted so that the protein concentration would equal that of the preload if the recovery was 100% in that fraction.

**Fig. 5.** Gradient centrifugation of mixture of DKH392 and GST893–975. SDS-PAGE of fractions from sucrose gradient centrifugation of DKH392 and GST893–975 with standard proteins in A25 buffer without added salt. Centrifugation was for 18 h at 4 °C in an SW41 rotor (Beckman) at 40,000 rpm. A, DKH392; B, GST893–975; and C, both DKH392 and GST893–975. BSA, bovine serum albumin; C.A., carbonic anhydrase; 893, full-length GST893–975; P.F., proteolytic fragment PF-II of GST893–975; and 392, DKH392.
The indicated sequence is that for Drosophila. The strong conservation observed at the beginning of this region extends into the area on the NH₂-terminal side of position 881, but there is no strong conservation on the COOH-terminal side of position 950. Positively and negatively charged residues are indicated by + and −, respectively, on the line above the sequence. The heptad repeat positions a and d are also indicated above the sequence for heptad frames 7N-3 and 7N. B, prediction of coiled-coil tendency for the sequence. The heptad frames are indicated schematically by solid ovals (both the COOH-terminal heavy chain regions and the light chains in native kinesin) is designated by an open square. Under physiological conditions kinesin is likely to be mainly in the compact conformer I (11) with K<sub>3</sub> < 1. Kinesin can potentially bind to the membrane receptor (dappled) as either in the extended conformer (via K<sub>2</sub>) or as the compact conformer (via K<sub>3</sub>). If the affinity of the tail domain for receptors (K<sub>t</sub>) is greater than the affinity of the tail domain for heads (1/K<sub>f</sub>), then binding of free kinesin to receptors would shift the equilibrium toward the active extended state IV. The relative values of K<sub>t</sub> versus K<sub>f</sub> and of K<sub>t</sub> versus K<sub>3</sub> are not known, but binding of the heads to the tail in I could potentially interfere with binding of the tail to the receptor to form III via K<sub>3</sub> or K<sub>4</sub>.

DISCUSSION

The heavy chain dimer of bovine kinesin has previously been shown to undergo a transition from an extended conformer at high salt concentration to a more compact conformer at low salt concentration (11). The bovine dimer, however, likely results from proteolysis of the light chains during isolation of the native bovine heterotetramer (11, 23) and the heavy chain dimer could potentially still contain small light chain domains that are not easily detected by SDS-PAGE. The ability of the Drosophila heavy chain dimer obtained by expression in E. coli to form the compact conformer at low ionic strength indicates that the heavy chain itself possesses the capability to form a compact conformation without the involvement of light chains or eukaryotic post translational modification.

Two types of mechanisms could produce the compact conformer. One possibility is that the stalk is bent back on itself at the likely hinge at position ~600 and that the interaction of the front and back half of the stalk along their lengths provides the major stabilization of the compact conformer. In the other type of model, as indicated in Fig. 2B, formation of the compact conformer is driven by a favorable interaction between a domain in the NH₂-terminal head region and a domain in the COOH-terminal tail region that can occur because there is sufficient flexibility in the stalk for the two ends of kinesin to come into contact. The break in the predicted coiled-coil of the stalk around position 600 is a candidate hinge for either model. The inability of DKH927 to form the compact conformer at low salt (Fig. 2A) indicates that the COOH-terminal region between 927 and 960 is required and, conversely, that the stalk domain itself cannot produce a stable compact conformation. This suggests that the compact conformer is not produced by interactions within the stalk, but rather by interaction between specific segments in the NH₂- and COOH-terminal regions as indicated in Fig. 2B. Such a direct interaction is supported by the observation that separately expressed tails and heads interact in a salt-dependent manner (Figs. 3–5). This does not totally preclude involvement of antiparallel interactions between the front and back half of the stalk domain in the

**Fig. 6.** MT-stimulated ATPase. The MT-stimulated ATPase was determined for 50 μM kinesin heavy chain in A25 buffer with 50 mM KCl, 1 mM MgATP, 2 mM potassium phosphoenolpyruvate, and 0.3 mM NADH with pyruvate kinase and lactate dehydrogenase. Squares, DKH894; diamonds, DKH975. The reactions were not stirred after initial mixing.

**Fig. 7.** Analysis of tail sequences that are required for formation of the compact conformation. A, results of a multiple sequence alignment performed by ClustalW (MacVector, Oxford Molecular Group) on conventional kinesins (Drosophila (32), human (33), human neuronal (34), mouse (GeneBank, X61435), squid (35), and uchchin (36)). The indicated sequence is that for Drosophila with a "#" indicating absolute conservation in all 5 species and a "," indicating only conservation of a weak interaction that requires high levels of DKH392 to comigrate with DKH392 in the absence of added salt when the concentration of DKH392 is reduced (not shown), indicative of a weak interaction that requires high levels of DKH392 to maintain a significant fraction of the high levels of DKH392 as the complex.

**ATPase Rates**—The initial rates for ATP hydrolysis was determined for compact DKH975 and extended DKH894 in A25 buffer with 50 mM KCl (Fig. 6). The k<sub>cat</sub> and K<sub>0.5(ATP)</sub> values were 35 and 3.8 s⁻¹ and 1.9 and 0.27 μM, respectively, for DKH894 and DKH975.

**Fig. 8.** Model for regulation of kinesin. The two head domains of kinesin are indicated schematically by solid ovals and the tail region (both the COOH-terminal heavy chain regions and the light chains in native kinesin) is designated by an open square. Under physiological conditions kinesin is likely to be mainly in the compact conformer I (11) with K<sub>3</sub> < 1. Kinesin can potentially bind to the membrane receptor (dappled) as either in the extended conformer (via K<sub>2</sub>) or as the compact conformer (via K<sub>3</sub>). If the affinity of the tail domain for receptors (K<sub>t</sub>) is greater than the affinity of the tail domain for heads (1/K<sub>f</sub>), then binding of free kinesin to receptors would shift the equilibrium toward the active extended state IV. The relative values of K<sub>t</sub> versus K<sub>f</sub> and of K<sub>t</sub> versus K<sub>3</sub> are not known, but binding of the heads to the tail in I could potentially interfere with binding of the tail to the receptor to form III via K<sub>3</sub> or K<sub>4</sub>.
compact conformation, but does indicate that any such interactions within the stalk are not the major energetic driving force for formation of the compact conformation.

Analysis of the tendency for coiled-coil formation indicates (Fig. 7) that the region between 850 and 910 is likely to be coiled-coil in heptad frame 7N with a weak area around Pro363. The region between 910 and 930 has a weaker predicted tendency for coiled-coil formation in heptad frame 7N-3. Detailed inspection (Fig. 7) indicates that there is a reasonable heptad repeat in both heptad frames 7N-3 and 7N, that could facilitate a 4-helix or antiparallel coiled-coil interaction between the ~883 and 930 region of the tail and the helical region of the neck.

The ability of DKH937 to form the compact conformer at low salt (Fig. 2A) and the ability of GST893–960 to bind to DKH365 (Fig. 4) indicate that the tail region from 893–937 and the head region of 1–365 are sufficient for tight interaction, although interaction of additional regions may provide further stabilization. The NH$_2$-terminal half of the coiled-coil neck is likely to play a critical role as even partial deletion (DKH357 versus DKH365) strongly inhibits interaction with the tail domains. These regions of both the neck and tail are highly charged (16 charged for 340–365 and 15 charged for 912–936) and this could account for the high salt dependence of the interaction. The whole region between 883–936, however, has an abundance of charged groups and is not possible to definitely predict the alignment of the tail and neck regions in the complex. Although the NH$_2$-terminal half of the neck is likely to be important for the binding of the tail domain, several constructs containing the neck and adjacent regions, in the absence of complete head domains, have been tested for binding to the tail domain and none have been found to bind strongly. It is thus likely that strong binding to the tail domain requires interaction over a more extended region than just the NH$_2$-terminal half of the coiled-coil neck. Although the ability of DKH937 to form a compact conformation at low salt indicates that the region beyond 937 is not absolutely required, the shift in the midpoint for the transition with longer constructs indicates that the region beyond 937 can stabilize the interaction. If the region of ~345–365 interacts with ~910–930 in an antiparallel configuration as in Fig. 2B, then the region of the tail beyond 930 would be juxtaposed to the head domain and in a position for potential interaction. Conventional kinesins show strong sequence conservation in this region up to position 950 (Fig. 7) and the conserved region (940–950) surrounding the IAK at positions 942–944 is thus a candidate for this additional area of interaction with the head. This antiparallel alignment would also juxtapose the COOH-terminal half of the neck with the region between ~883 and 910 and interaction of these regions could be responsible for the stronger interaction of DKH405 versus DKH365 with tails (Fig. 4).

The highly charged NH$_2$-terminal half of the coiled-coil neck has a gap in the hydrophobic heptad repeat and this region was postulated to be capable of reversibly uncoiling, while the dimer remained anchored by the stronger interactions in the better heptad repeats of the COOH-terminal half of the neck (24). Recent work with peptides from the neck region has established that such uncoiling of the NH$_2$-terminal half of the neck can occur (4, 5). Although the exact oligomeric state of the complex between the tail and head/neck is not known, the interaction between monomeric DKH365 and GST893–975 suggests that formation of a 4-helix bundle is not necessary, at least for the NH$_2$-terminal half of the neck. An alternative possibility is that strand displacement may occur with uncoiling of the NH$_2$-terminal half of the neck to allow formation of a pair of antiparallel coiled-coils with the region from ~910 to 930 of the tail. The relatively poor prediction for a parallel coiled-coil interaction of this region of the tail with itself may be reflective of an actual role in antiparallel interaction with the neck.

Fungal kinesins are relatives of conventional kinesin (25) that have lost the highly charged nature of both the neck and tail regions that are homologous to those implicated above in formation of the compact conformation. Either these regions of fungal kinesins do not interact, or both regions have co-evolved to maintain an interaction that is not as highly dependent on charge. Interestingly, the IAK region is, however, conserved between conventional and fungal kinesins.

Entropic effects are likely to be the major cause of the weaker apparent interaction of domains on separate constructs relative to domains that are covalently linked. For example, DKH975 remains predominantly in the compact conformation at 0.3 m salt, whereas interaction of GST893–975 and DKH392 during cosedimentation is weak and readily reversible even in the absence of added salt. When the domains are on separate constructs, they are free to diffuse away from each other following dissociation, whereas the dissociated domains of DKH975 are restrained to remain near each other because they are covalently linked through the stalk. The failure of DKH405 or DKH365 to bind to GST893–937 in the column binding assay (Fig. 4) while DKH365 can still form the compact conformer, likely also reflects the more stringent requirements for association of fragments that are not covalently linked. Additive contributions from the entire extended binding region including the IAK region that is present in GST893–960 are apparently required for binding to be tight enough to be observed under these more stringent assay conditions. Entropic considerations also predict that the column binding experiments (Figs. 3 and 4) can better detect weak interactions compared with cosedimentation experiments (Fig. 5). The sedimentation velocity of an equilibrating mixture is directly related to the fraction associated; but in the column binding assay, head domains must interact sequentially with many bound GST-tail fusions before they can be eluted.

The analysis of ATPase rates of heavy chain constructs that are close to full-length is complicated by the tendency for formation of asters of MTs in the presence of ATP as originally reported by Urrutia et al. (26). The heavy chain constructs used here can also produce such asters during the course of an ATPase assay at high concentration as verified by electron microscopy. The ATPase results in Fig. 6 were obtained as initial rates at low kinesin concentration (30 nM) in order to minimize the contribution from kinesin molecules in asters. Extended DKH894 has a high k$_{cat}$ at saturating MT concentration that is similar to that of fully active shorter constructs (3). The k$_{cat}$ of DKH975, however, is 10-fold lower, indicating inhibition in the compact conformer. The true rate of DKH975 in the compact conformer may be even lower than the observed rate of 3.4 s$^{-1}$ as the K$_{D(MT)}$ of 0.27 μM is unexpectedly low and part of the observed activity likely represents catalysis by the contaminating PF-II species which has a high ATPase rate and a low K$_{D(MT)}$ value. With bovine kinesin, inhibition of MT-stimulated ATPase activity results from inhibition of the ability of MTs to stimulate ADP release from the compact conformer that is present at low ionic strength (15). This inhibition could result directly from steric blockage by the tail of the MT interaction site on the head or indirectly by a conformational change in the head or neck that is induced by binding of the tail.

---

2 M. Stock and D. Hackney, unpublished observations.

3 D. Hackney, unpublished observations.
domains.

If the site on the tail domain to which the head domain bound was overlapping with the site on the tail to which the vesicle receptor bound, then competition between these two binding modes would provide a natural basis for regulation by mass action (11) as illustrated by the model of Fig. 8. Even if the receptor-binding site is on the light chains, the binding of tails to heads versus receptors could be still be competitive as long as steric constraints prevented both interactions from occurring at the same time. When free in solution, kinesin would be in the compact conformer and be inhibited, while binding of the membrane receptor to the tail would release the heads in their active form. If the binding of the tail to the receptor was stronger than binding of the tail to the heads and if kinesin was in excess, then all the receptors would be occupied with active kinesin and any excess kinesin would be in the compact conformer and thus inhibited. Such binding-induced activation of kinesin has been reported with nonphysiological surfaces (27–29) and is likely to also be responsible for the high motor activity of adsorbed kinesin in motility assays, but activation by physiological receptors remains to be demonstrated. The occurrence of receptor-induced activation by induction of the extended conformer would not preclude additional or alternative regulation by other means such as phosphorylation (30).

Acknowledgments—We thank the Drug Synthesis and Chemistry Branch of the National Cancer Institute for Taxol, L. S. B. Goldstein for providing Drosophila kinesin cDNA, and S. Admarral and J. Negy for assistance in cloning and preliminary experiments.

REFERENCES
1. Vale, R. D., and Fletterick, R. J. (1997) Annu. Rev. Cell Dev. Biol. 13, 745–777
2. Hirokawa, N. (1998) Science 279, 519–526
3. Jiang, W., Stock, M., Li, X., and Hackney, D. D. (1997) J. Biol. Chem. 272, 7626–7632
4. Mori, H., Takenawa, T., Arisaka, F., and Shimizu, T. (1997) Biochemistry 36, 1933–1942
5. Tripet, B., Vale, R. D., and Hodges, R. S. (1997) J. Biol. Chem. 272, 8846–8856
6. deCuevas, M., Tao, T., and Goldstein, L. S. B. (1992) J. Cell Biol. 116, 957–965
7. Kozlelski, F., Saek, S., Marx, A., Thormahlen, M., Schonbrunn, E., Biou, V., Thompson, A., Mandelkow, E. M., and Mandelkow, E. (1997) Cell 91, 985–994
8. Kuznetsov, S. A., Vaisberg, E. A., Shantina, N. A., Magretova, N. N., Chernyak, V. Y., and Gelfand, V. I. (1988) EMBO J. 7, 353–356
9. Bloom, G. S., Wagner, M. C., Pfister, K. K., and Brady, S. T. (1988) Biochemistry 27, 3409–3416
10. Hisanaga, S., Murofushi, H., Okuhara, K., Sato, R., Masuda, Y., Sakai, H., and Hirokawa, N. (1989) Cell Motil. Cytoskeleton 12, 264–272
11. Hackney, D. D., Levitt, J. D., and Suhan, J. (1992) J. Biol. Chem. 267, 8696–8701
12. Kuznetsov, S. A., Vaisberg, E. A., Rothwell, S. W., Murphy, D. B., and Gelfand, V. I. (1989) J. Biol. Chem. 264, 589–595
13. Stewart, R. J., Tahler, J. P., and Goldstein, L. S. B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5209–5213
14. Huang, T-G., and Hackney, D. D. (1994) J. Biol. Chem. 269, 16493–16501
15. Hackney, D. D., Levitt, J. D., and Wagner, D. D. (1991) Biochem. Biophys. Res. Commun. 174, 810–815
16. Trybus, D. M., Huiatt, T. W., and Lawey, S. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6151–6155
17. Ikebe, M., Hinkins, S., and Hartshorne, D. J. (1983) Biochemistry 22, 4580–4587
18. Wagner, M. C., Pfister, K. K., Bloom, G. S., and Brady, S. T. (1989) Cell Motil. Cytoskeleton 12, 195–215
19. Cheng, J-Q., Jiang, W., and Hackney, D. D. (1998) Biochemistry 37, 5288–5295
20. Laemmli, U. K. (1970) Nature 227, 680–685
21. Yang, J. T., Saxton, W. M., and Goldstein, L. S. B. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1864–1868
22. Huang, T-G., Suhan, J., and Hackney, D. D. (1994) J. Biol. Chem. 269, 16502–16507
23. Hackney, D. D. (1991) Methods Enzymol. 196, 175–181
24. Hackney, D. D. (1994) J. Biol. Chem. 269, 16508–16511
25. Steinberg, G., and Schliwa, M. (1995) Mol. Biol. Cell 6, 1605–1618
26. Urrutia, R., McNiven, M. A., Albanesi, J. P., Murphy, D. B., and Bachar, B. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6701–6705
27. Jiang, M. Y., and Sheetz, M. P. (1995) Biophys. J. 68, 283a–285a
28. Coy, D. L., and Howard, J. (1996) Biophys. J. 70, A36
29. Moraga, D. E., and Murphy, D. B. (1997) Mol. Biol. Cell 8, 258a
30. Lee, K., and Hollenbeck, P. J. (1995) J. Biol. Chem. 270, 5600–5605
31. Neuhoff, V., Arold, N., Taube, D., and Ehrihahrt, W. (1988) Electrophoresis 9, 255–262
32. Yang, J. T., Laymon, R. A., and Goldstein, L. S. B. (1989) Cell 56, 879–889
33. Navone, F., Niclas, J., Bodenheer, N., Sparks, L., Bernstein, H. D., McCaffrey, G., and Vale, R. D. (1992) J. Cell Biol. 117, 2663–2675
34. Niclas, F., Navone, F., Bomb-Bodden, N., and Vale, R. D. (1994) Neuron 12, 1059–1072
35. Kosik, K. S., Orecchio, L. D., Schnapp, B. J., Inouye, H., and Neve, R. L. (1990) J. Biol. Chem. 265, 3278–3283
36. Wright, B. D., Henson, J. H., Hedaman, K. F., Willy, P. J., Morand, J. N., and Scholey, J. M. (1991) J. Cell Biol. 113, 817–833
37. MacKnight, A. E. (1994) The Diversity of Myosin-like Proteins, Cambridge University, Cambridge
38. Lupas, A. N. (1996) Methods Enzymol. 266, 513–525