Evidence That the Stalk of Drosophila Kinesin Heavy Chain Is an α-Helical Coiled Coil

Margaret de Cuevas,* Terence Tao,$§ and Lawrence S. B. Goldstein*

*Department of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts 02138; ‡Department of Muscle Research, Boston Biomedical Research Institute, Boston, Massachusetts 02114; and § Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115

Abstract. Kinesin is a mechanochemical enzyme composed of three distinct domains: a globular head domain, a rodlike stalk domain, and a small globular tail domain. The stalk domain has sequence features characteristic of α-helical coiled coils. To gain insight into the structure of the kinesin stalk, we expressed it from a segment of the Drosophila melanogaster kinesin heavy chain gene and purified it from Escherichia coli. When observed by EM, this protein formed a rodlike structure 40–55 nm long that was occasionally bent at a hingelike region near the middle of the molecule. An additional EM study and a chemical cross-linking study showed that this protein forms a parallel dimer and that the two chains are in register. Finally, using circular dichroism spectroscopy, we showed that this protein is ∼55–60% α-helical in physiological aqueous solution at 25°C, and ∼85–90% α-helical at 4°C. From these results, we conclude that the stalk of kinesin heavy chain forms an α-helical coiled coil structure. The temperature dependence of the circular dichroism signal has two major transitions, at 25–30°C and at 45–50°C, which suggests that a portion of the α-helical structure in the stalk is less stable than the rest. By producing the amino-terminal (coil 1) and carboxy-terminal (coil 2) halves of the stalk separately in E. coli, we showed that the region that melts below 30°C lies within coil 1, while the majority of coil 2 melts above 45°C. We suggest that this difference in stability may play a role in the force-generating mechanism or regulation of kinesin.

Intracellular motility in eukaryotic cells depends upon the action of molecular motors. One such molecular motor is kinesin, which is a microtubule-dependent motor protein that is found in the cytoplasm of most eukaryotic cells. Recent studies have implicated kinesin in several cellular processes involving organelle transport (Vale et al., 1985; Schroer et al., 1988; Pfister et al., 1989) and ER extension (Dabora and Sheetz, 1988).

Although the in vivo function and force-generating mechanism of kinesin remain unknown, much progress has been made towards understanding its structural organization. Native kinesin appears to be a tetramer composed of two heavy chains and two light chains (Bloom et al., 1988; Kuznetsov et al., 1988). The kinesin heavy chain gene from Drosophila melanogaster (Yang et al., 1989) and from squid (Kosik et al., 1990) have been cloned and sequenced. Analyses of the deduced protein sequence, together with microtubule-binding analyses of truncated polypeptides (Yang et al., 1989) and immunoelectron microscopic studies (Amos, 1987; Ingold et al., 1988; Hirokawa et al., 1989; Scholey et al., 1989), suggest that kinesin heavy chain is composed of three distinct domains: a globular amino-terminal head domain that contains the ATP and microtubule binding sites, a rodlike middle domain, and a small globular carboxy-terminal tail domain that may interact with the light chains and possibly with organelles.

In addition to the three-domain organization of kinesin heavy chain, the deduced protein sequence suggests a more detailed structure for the middle domain. Analyses of the sequence of this domain revealed many of the features that are diagnostic of proteins that have an α-helical coiled coil conformation (Yang et al., 1989; Kosik et al., 1990). In particular, the sequence predicts a largely unbroken α-helix that shows a characteristic heptapeptide-repeat pattern a,b,c,d,e,f,g, with strong enrichment of hydrophobic residues at positions a and d. The sequence also shows a weak periodicity of negatively and positively charged residues, similar to that found in the α-helical coiled coil regions of other filamentous proteins, e.g., myosin, keratin, and tropomyosin (McLachlan and Karm, 1983). Interestingly, there is also an interruption near the middle of the proposed α-helical coiled coil region. This segment is unlikely to be α-helical because of the presence of a proline and several glycines in a short stretch of amino acids, and it may correspond to the bend seen near the middle of the stalk region in electron micrographs of porcine (Amos, 1987) and bovine (Hirokawa et al., 1989; Hisanaga et al., 1989) kinesin. Taken together, these data suggest that the middle domain of kinesin heavy chain...
forms the bent, rodlike stalk of native kinesin. Direct evidence for this view, however, as well as for the function of the stalk, has been lacking.

To gain insight into the structure, and ultimately the function, of the kinesin stalk, we have investigated the structure of kinesin heavy chain from Drosophila melanogaster using molecular biological, biochemical, and biophysical techniques. In this paper, we report our analyses of proteins expressed in Escherichia coli from the proposed stalk domain of the gene. Collectively, our analyses confirm that the middle domain of the gene forms the stalk of kinesin heavy chain and that the stalk is an α-helical coiled coil. In addition, our studies using circular dichroism (CD) spectroscopy suggest that the α-helical structure in coil 1 of the stalk is less stable than in coil 2. In view of this disparity, we suggest that the coil 1 region of the stalk may play an important role in the force-generating mechanism or regulation of kinesin.

### Materials and Methods

#### Plasmid Construction

The plasmids pET-STK (2B) and pET-STK were constructed in the translation vector pET-5c (Rosenberg et al., 1987). To construct pET-STK (2B), the unique BamHI site in pET-5c was digested and blunted with the Klkenow fragment of DNA polymerase I. A PvuII fragment from pBSI-1, a plasmid that contains the full-length kinesin heavy chain cDNA clone 1 (Yang et al., 1989), was inserted into this site: the fragment contains sequence encoding residues 448–863 of kinesin heavy chain. Because the BamHI site is located downstream from the protein start point in pET-5c, the novel sequence MASMTGGQQMGRI is added to the beginning of the kinesin heavy chain portion of the protein. The STK (2B) protein also has the novel sequence DPNS encoded by the vector appended to its carboxy end.

pET-STK was constructed identically to pET-STK (2B). During its construction, however, 2 bp was inadvertently deleted from the BamHI site in the vector. Because of this deletion, the protein expressed from pET-STK begins at an internal methionine, residue 470, and is missing 35 residues (13 of vector and 22 of kinesin sequence) from its amino-terminal end; this end was verified by protein sequencing (see below). The protein is otherwise identical to the STK (2B) protein. A high level of protein expression is possible from pET-STK because there is a fortuitous Shine-Dalgarno sequence located 6 bp upstream from the internal ATG encoding residue 470.

The plasmids pET-STK.9A and pET-STK 1 were constructed from pET-STK and pET-STK (2B), respectively. To construct pET-STK.9A, an EcoRI fragment encoding 1,280 amino acids of Drosophila α-spectrin was inserted in frame into the EcoRI site at the end of the kinesin heavy chain portion of pET-STK. To construct pET-STK 1, the unique EcoRI and AflII sites in pET-STK (2B) were digested and blunted with the Klkenow fragment of DNA polymerase I, and the plasmid was reclosed. The remaining sequences in the plasmid encode residues 448–595 of kinesin heavy chain. In addition, the STK 1 protein has the novel sequence ILEDERAS encoded by the vector appended to its carboxy end.

To construct pET-STK 2, the unique BamHI site in the translation vector pET-5b (Rosenberg et al., 1987) was digested and blunted with the Klkenow fragment of DNA polymerase I. An AflII–PvuII fragment from pBSI-1, containing sequences encoding residues 594–863 of kinesin heavy chain, was inserted into this site. This protein has the novel sequence MASMTGGQQMGRID at its amino end and DPNS at its carboxy end.

All plasmids were transformed into E. coli strain BL21 (DE3) for expression (Rosenberg et al., 1987).

For protein sequencing, samples of the STK protein were electrophoresed to PVDF membrane and subjected to automated Edman degradation on a gas phase protein sequencer (model 470A; Applied Biosystems, Inc., Foster City, CA). The resultant phenylthiohydantoin amino acid fractions were identified using an on-line HPLC (model 120A, ABI).

---

1. Abbreviations used in this paper: CD, circular dichroism; NbS₂, 5,5'-dithiobis-2-nitrobenzoate.
Cross-linking Experiments

A stock solution of 5,5'-dithiobis-2-nitrobenzoate (NB$_2$S$_2$; Sigma Chemical Co., St. Louis, MO) was prepared as described by Lehrer (1975). Purified protein samples were dialyzed against 20 mM phosphate, pH 7.5, 0.1 M NaCl, and 1 mM EDTA just before the addition of NB$_2$S$_2$. The purified protein (~30 µg/ml) was reacted with NB$_2$S$_2$ (1.0 mM) for 1 h at room temperature. For the reaction in SDS, 1% SDS was added to the protein sample 5 min before the addition of NB$_2$S$_2$. All samples were then treated with 0.02 M iodoacetamide for 30 min at room temperature. For gel electrophoresis, the samples were concentrated by precipitation in 10% TCA, re-suspended in SDS-PAGE sample buffer without any reducing agent, and neutralized by vapor phase addition of NH$_4$OH until the indicator dye turned blue. The prepared samples were run on nonreducing 7.5% SDS-polyacrylamide gels and stained with Coomassie blue.

Circular Dichroism Spectroscopy

To ensure that the cysteines in the proteins were reduced, purified protein samples were incubated in 20 mM Tris-HCl, pH 7.0, 10 M NaCl, 5-7.5 mM DTT, and 1 mM EDTA at room temperature for 1 h. The samples were then dialyzed against PBS buffer (10 mM phosphate, pH 7.0, 150 mM NaCl, 0.5 mM DTT, 1 mM EDTA) in preparation for CD spectroscopy. The CD spectra were recorded in a 1-mm pathlength cuvette at a sample concentration of 0.01-0.10 mg/ml. The percent α-helix was calculated from the spectra using the program PROSEC (Aviv Assoc. Inc., Lakewood, NJ), a protein secondary structure estimator that uses an algorithm and reference spectra from Yang et al. (1986). The temperature dependence of the CD signal at 222 nm was followed in a 1-cm pathlength cuvette at a sample concentration of 0.01-0.10 mg/ml. All studies were performed in a CD spectropolarimeter (Aviv model 60 DS, Aviv Assoc. Inc.) with a Peltier temperature control unit (Model 89100 A, Hewlett-Packard Co., Palo Alto, CA). Protein concentration was determined either by amino acid analysis or by Bradford (Bio-Rad Laboratories) assay, using as a standard a stalk protein sample whose concentration was determined by amino acid analysis. For amino acid analysis, samples were hydrolyzed in 6 N HCl at 110°C in vacuo for 16-20 h and analyzed either on an amino acid analyzer (Model 7300, Beckman Instruments, Inc., Palo Alto, CA) or on a derivatizer (Model 420A, Applied Biosystems, Inc.) with subsequent analysis by on-line HPLC (Model 130A, ABI).

Results

Expression and Purification of a Fragment of Kinesin Heavy Chain

To examine the structure of the middle domain of kinesin heavy chain, we constructed a plasmid, pET-STK (Fig. 1). This plasmid was constructed by inserting a 1.25-kb PvuII fragment from the Drosophila kinesin heavy chain gene into the translation vector pET-5c (Rosenberg et al., 1987). The protein expressed by pET-STK, whose expression begins at the first ATG in the PvuII fragment (see Materials and Methods), contains 361 residues or 95% of the predicted α-helical coiled coil region plus 33 residues of the predicted carboxy-terminal tail domain. In addition to the kinesin heavy chain sequence, the expressed protein contains four residues from the vector at its carboxy terminus. Synthesis of the pET-STK protein is controlled by a phage T7 promoter; in E. coli strain BL21(DE3), which was used as the host for pET-STK, synthesis of T7 RNA polymerase is controlled by the lacUV5 promoter (Rosenberg et al., 1987).

After induction with isopropylthiolgalactoside, pET-STK expresses a 46-kD polypeptide at high levels in E. coli, most of which is soluble upon lysis. Fig. 2 shows a Coomassie blue-stained SDS-polyacrylamide gel of total cell lysates and samples at various stages of purification. The protein was purified from cell lysates initially by ammonium sulfate precipitation and boiling in high salt. When the ammonium sulfate fraction that contains the 46-kD protein was placed in a boiling water bath for 2-5 min, most of the bacterial proteins precipitated. Most of the 46-kD protein, however, remained in the supernatant, together with a few lower molecular weight proteins and most of the nucleic acids. For EM, chemical cross-linking, and some CD experiments, the 46-kD protein was further purified by gel filtration chromatography on a Bio-Gel A5M column, which does not remove nucleic acid contaminants. For other CD experiments, the 46-kD protein was purified instead by DE-52 and MonoQ
ion exchange chromatography, to remove both protein and nucleic acid contaminants. Both preparations gave similar results when analyzed by CD.

**EM Characterization**

Initially, we used EM to determine whether the protein expressed by pET-STK corresponds to the stalk seen in electron micrographs of native mammalian brain kinesin (Amos, 1987; Hirokawa et al., 1989). Purified samples of protein were low angle rotary shadowed (Tyler and Branton, 1980) and observed by EM. The molecules exhibited several consistent morphological features, as can be seen in the low magnification field in Fig. 3. Most of the molecules were rod shaped and between 45 and 55 nm long. While the majority of the replicas was relatively straight or gently curved, a few of them appeared to be sharply bent at a hinge-like region near the middle of the molecule. These different features are shown at a higher magnification in Fig. 4. The overall appearance of the expressed protein, including its rod-like shape, dimensions, and the location of the occasional bend, is consistent with the appearance of the stalk of native mammalian brain kinesin observed by EM. From these observations we conclude that the middle domain of kinesin heavy chain forms the extended stalk of the molecule.

The length of the stalk molecules and the position of the hinge were measured. The overall length of the stalk was 47 nm (SD = 7.5 nm, n = 125; data not shown). This length is consistent with, though slightly shorter than, the length of the stalk of a similarly sized kinesin heavy chain from porcine brain, which was measured to be 50-70 nm (Amos, 1987). This length is also slightly shorter than the 54-nm length that is predicted from the length-per-residue relation seen in two other α-helical coiled coil proteins, the myosin heavy chain tail and tropomyosin, which have 1.50 Å of length per residue (Fraser and MacRae, 1973). To determine the location of the hinge, we used only those molecules that contained a sharp, well-defined kink or bend. We measured the length of each “arm” of the molecule from the bend to its tip. The lengths of the short and long arms were 20 nm (SD = 4.4 nm) and 29 nm (SD = 4.1 nm), respectively (n = 58; data not shown). These measurements place the hinge, when its position is expressed as a percentage of total stalk length, ~41% from one end of the stalk. This position is consistent with the position of proline 587, which lies within the 23–amino acid–long disruption in the heptapeptide-repeat pattern (Yang et al., 1989) that is located ~37% of the way from the amino end of the stalk. We conclude from this finding that the disruption in the heptapeptide-repeat pattern forms a flexible hinge in the stalk of kinesin heavy chain. Because this hinge region is apparently conserved in evolution (Kosik et al., 1990), it may play an important role in kinesin function.

We also determined the effect of different sample preparation procedures on the percentage of molecules that were sharply bent. Contrary to a study on native adrenal medulla kinesin (Hisanaga et al., 1989), we did not find that the salt concentration dramatically affected the percentage of extended molecules. Approximately 12% of stalk molecules were bent in samples that had been dissolved in high salt buffer (0.1 M KCl + 1.0 M ammonium acetate; data not shown). In low salt buffer (10 mM KCl), however, the percentage of bent molecules increased only to 20–25%. None of the samples we observed showed a majority of stalk molecules in the bent conformation.

It is not possible to determine if the stalk purified from E. coli is a monomer or a dimer simply by inspecting images such as those shown in Figs. 3 and 4. To solve this problem, we constructed a new expression plasmid, pET-STK.9A (Fig. 5 A, a), that would enable us to distinguish between monomeric and dimeric stalk molecules by EM. The plasmid was made by inserting a fragment of the Drosophila α-spectrin gene in frame at the 3’ end of the stalk coding region in pET-STK. After induction with isopropylthiogalactoside, pET-STK.9A expresses in E. coli a 200-kD fusion protein (STK.9A) that is comprised of the kinesin stalk with 1,280 residues of α-spectrin attached to its carboxy end. This piece of α-spectrin probably has an extended α-helical conformation, and it appears in EM as an elongated molecule with a length of 58 ± 4 nm (Dubreuil et al., 1991). It is also unlikely to be dimeric, based on EM observations (Dubreuil et al., 1991; our results, below). We reasoned that, in EM, STK.9A could have one of three conformations. If the kinesin stalk were a monomer, then STK.9A would appear in EM as a long extended molecule, ~105 nm in length, composed of the rod-like stalk attached to a single extended piece of spectrin (Fig. 5 A, b). If the stalk were an antiparallel dimer, STK.9A would appear as a longer linear molecule, ~163 nm in length, with a piece of spectrin extending off each end of the stalk dimer (Fig. 5 A, c). If the stalk were a parallel dimer, however, each rod-like stalk structure would have two ex-
tended pieces of spectrin attached to one end of it. This dimeric chimera would appear in EM as a distinctive Y-shaped structure (Fig. 5 A, d).

The STK.9A fusion protein was expressed and purified from *E. coli* by ammonium sulfate precipitation and gel filtration chromatography on a Sepharose CL-4B column; purified samples are shown on a Coomassie blue-stained SDS-polyacrylamide gel in Fig. 6. These samples were then
prepared for EM by low angle rotary shadowing in the same manner as the stalk. The predominant conformation displayed by the STK.9A fusion protein, which is shown in Fig. 5 B, is a Y-shaped structure. Each chimeric molecule is composed of a shorter, rodlike "base" that corresponds in appearance and length, 44 nm (SD = 6.4 nm), to the stalk alone with two longer "arms" attached to one end of it. The length of these arms, 59 nm (SD = 5.9 nm), is consistent with the length of the spectrin piece alone. None of the other structures that were seen in EM corresponded consistently with either of the conformations diagrammed in Fig. 5 A, b and c. We occasionally saw long unbranched extended molecules, but their lengths were varied and inconsistent; it is possible that they were pieces of nucleic acid, which remained with the fusion protein in our purification procedure. We conclude from these observations that the kinesin stalk is a parallel dimer.

**Chemical Cross-linking Analysis**

As further evidence that the kinesin stalk forms a dimer, we conducted a cross-linking study using the aromatic disulfide NbS2. This chemical catalyzes the formation of disulfide bonds, by a two-step disulfide exchange process, between two cysteines whose thiol groups are in close proximity. The protein expressed by pET-STK contains two of the three cysteine residues, Cys 632 and Cys 695, that are located in the predicted coiled coil region of the kinesin stalk. Previous work on tropomyosin (Lehrer, 1975) and myosin subfragment 2 (Lu and Lehrer, 1984), molecules known to dimerize into coiled coils, demonstrated the ability of NbS2 to form disulfide cross-links between two parallel chains within the same molecule. Thus we reasoned that, if the expressed protein forms a parallel dimer and the cysteine residues in one subunit are close to those in the other subunit, then NbS2 should be able to form cross-links between the subunits.
The results of our experiment are shown on a Coomassie blue-stained nonreducing SDS-polyacrylamide gel in Fig. 7. In the absence of NbS₂, a sample of reduced protein appears as a band corresponding to the expected monomer size of 46 kD. After reaction with NbS₂, however, the majority of the protein has been converted to dimers (lane 2). In the presence of NbS₂, the majority of the protein remains monomeric (lane 3). After reaction with 1.0 mM NbS₂, the majority of the proteins in SDS-polyacrylamide gels (Griffith, 1972), each band probably corresponds to one of the three possible cross-linked species. It is not known why additional faint bands were occasionally seen in the cross-linked species, as in Fig. 7. In contrast to these results, no cross-linked species was formed in protein that was denatured in 1% SDS before the addition of NbS₂. Thus, NbS₂ probably reacts with both pairs of cysteines in the stalk molecule and cross-links the subunits only if they are in their native state, before denaturation and dissociation by SDS. This finding demonstrates that the stalk forms a dimer in its native state. In addition, since cross-linking by NbS₂ requires the close proximity of two thiol groups, it strongly suggests that the two chains in the dimer are in register.

**Circular Dichroism Analysis**

To confirm that the stalk of kinesin heavy chain forms an extended α-helix, we used CD spectroscopy to determine the α-helical content of the stalk protein produced in *E. coli*. Based on analyses of its predicted protein sequence, 90% of the residues in the protein expressed by pET-STK are predicted to form an α-helical coiled coil structure and therefore to be α-helical. The remaining residues do not show a strong tendency to form α-helix (Yang et al., 1989).

As shown in Fig. 8, a and b, the CD spectrum of the expressed protein has two minima at 208 and 222 nm and a maximum at 195 nm, all of which are characteristic of α-helices. When analyzed by the program PROSEC, a protein secondary structure estimator that is based on an algorithm and reference spectra from Yang et al. (1986), these spectra show that the expressed protein is ∼55–60% α-helical at 25°C and 85–90% α-helical at 4°C, with 0% β-turn or β-sheet at either temperature. Differences in protein concentration (within the 0.01–0.10 mg/ml range) and purification method (either fast performance liquid chromatography or gel filtration chromatography; see Materials and Methods for details) did not appear to affect the spectrum of the protein. These findings confirm that the stalk protein forms an extended α-helix. In addition, they suggest that a significant portion of the α-helical structure in the stalk protein is melting out at near physiological temperature.

To determine the temperature dependence of the CD signal, we measured the molar ellipticity of the stalk protein at 222 nm between 4 and 80°C. As shown in Fig. 8 c there are two major transitions in the melting curve. The midpoints of the transitions were obtained by taking a first derivative of the melting curve, which is shown in the inset in Fig. 8 c. The midpoint of the second major transition is at 45–50°C, which is consistent with the CD melting transitions of other stable coiled coil structures, e.g., paramyosin and the myosin rod (Privalov, 1982). The extent of the transition indicates that ∼50% of the α-helical structure is melting out at this temperature. The midpoint of the first transition, however, is at 25–30°C, which indicates that the remaining portion of the α-helical structure in the expressed protein is relatively unstable. Hence at 4°C the molar ellipticity is considerably lower than at 25°C, consistent with the spectra.

To determine in which portion of the stalk the region of instability lies, we constructed two new plasmids, pET-STK 1 and pET-STK 2, that each express only a fragment of the stalk (Fig. 1). To construct the plasmids, we took advantage of an *AfI* II site located within the region predicted to encode...
Figure 8. CD studies of stalk proteins. (a) CD spectrum of the STK protein at 25°C. The two minima at 208 and 222 nm and the maximum at 195 nm are characteristic of α-helices. When analyzed by the PROSEC (protein secondary structure estimator) program, the spectrum shows that the protein is 55–60% α-helical at this temperature. This spectrum was recorded at a protein concentration of 0.089 mg/ml in PBS buffer, using fast performance liquid chromatography-purified protein. (b) CD spectrum of the STK protein at 4°C. At this temperature, the STK protein is 85–90% α-helical. The spectrum was recorded at a protein concentration of 0.037 mg/ml in PBS buffer, using protein purified by gel filtration. (c–f) Temperature dependence of the CD signal at 222 nm for different stalk proteins. The midpoint of the transition(s) in each melting curve was obtained by taking a first derivative, which is shown in the inset in each panel. The melting curves of the STK protein (c) and STK(2B) protein (d) are similar: each has two major transitions with midpoints at 25–30°C and 45–50°C, respectively. The melting curve in c was recorded at a protein concentration of 0.014 mg/ml in PBS buffer, using fast performance liquid chromatography-purified protein; (d) was recorded at a protein concentration of 0.10 mg/ml. The melting curve of the STK 1 protein (e) has one transition with a midpoint at 20–25°C and was recorded at a protein concentration of 0.10 mg/ml. The melting curve of the STK 2 protein (f) has one transition whose midpoint is at 45–50°C and was recorded at a protein concentration of 0.035 mg/ml. The melting curves in d–f were recorded using protein purified by gel filtration chromatography.

Because the protein expressed by pET-STK 1 contains additional vector and coil 1 sequences at its amino end that are not contained in the protein expressed by pET-STK, we constructed a third plasmid, pET-STK(2B) (Fig. 1). This plasmid expresses a 50-kD protein that consists of the full length STK protein with the additional coil 1 and vector residues at its amino end. The CD spectrum of the STK(2B) protein indicates that it is ~85% α-helical at 4°C (data not shown), and its melting curve, which is shown in Fig. 8 d, has two major transitions, at 25–30°C and at 45–50°C. These findings show that the STK(2B) and STK proteins behave consistently.

Discussion

We have presented here an investigation of the structure of the stalk of kinesin heavy chain by EM, chemical cross-linking, and CD analysis. Our results demonstrate that (a)
the middle domain of the kinesin heavy chain gene, whose predicted protein sequence contains features characteristic of α-helical coiled coils, forms a rodlike stalk 47 ± 7.5 nm in length with a hinge near its middle; (b) the stalk forms a parallel dimer with both chains in register; and (c) the stalk is predominantly α-helical. Taken together, these results provide strong evidence that the stalk of kinesin heavy chain forms an α-helical coiled coil. In addition, our CD studies suggest that the α-helical structure in coil 1 of the stalk is less stable than in coil 2, contrary to what is predicted from the sequence of squid kinesin heavy chain (Kosik et al., 1990). The question that arises is what function the stalk of kinesin might have, and whether the differential stability of coils 1 and 2 has an influence on that function.

There are three possible functions for the α-helical coiled coil stalk of kinesin heavy chain that are suggested by its structure. (a) A portion of the stalk might function in generating force for motility. It is tempting to suggest that the stalk might contribute to force production by a mechanism similar to the helix-coil transition model proposed for another molecular motor, myosin heavy chain, whose structural organization and behavior are strikingly similar to those of kinesin (Harrington, 1971; Warrick and Spudich, 1987). In particular, for such a mechanism one would expect the segment of the stalk between the head and the hinge to be relatively unstable. The finding that the coiled coil structure is less stable in coil 1 than in coil 2 is consistent with this model. (b) Some regions of the stalk might regulate the motile or binding properties of kinesin. This possibility arises from a quick-freeze, deep-etch EM study of bovine brain kinesin (Hirokawa et al., 1989), in which the protein appeared to be only about one-third as long when cross-linking microtubules to latex microspheres as when free in solution, suggesting that a portion of the stalk may be lying along the surface of the microsphere or the microtubule. Similarly sized cross-bridges were also observed between microtubules and vesicles in vivo. Binding of the stalk to microtubules or vesicles might also stabilize the α-helical conformation of coil 1, which is likely to be significantly unfolded at 25°C (near the physiological temperature for Drosophila) when kinesin is free in solution. This stabilization might be an important feature of kinesin regulation. (c) The stalk might act as a passive link between the head and the small globular tail of the heavy chain. This final possibility is supported in part by the observation that the kinesin head and only 11 residues of the stalk are sufficient to move microtubules in vitro (Yang et al., 1990). Thus, although the stalk may contribute to force production or regulation in vivo, it is apparently not essential for motility in vitro, which is similar to the situation for myosin (Toyoshima et al., 1987; Lovell et al., 1988). Further experiments will be needed to determine whether any portion of the stalk is essential for movement or regulation in vivo.

We thank Ron Dubreuil for a clone expressing the α-spectrin cDNA fragment in E. coli; Russell Stewart for assistance with FPLC; René Lu and Anna Wong for doing some of the amino acid analyses; Renée Robinson, Mary Gordy, and William Lane of the Harvard Microchemistry Facility (Cambridge, MA) for protein sequencing and additional amino acid analyses; Norio Ueno for assistance with the PROSEC program; and Dan Branton for the use of his electron microscope. We also thank Sam Lehrer for useful discussions about disulfide cross-linking and CD studies.

This work was supported by a National Science Foundation predoctoral fellowship to Margaret de Cuebas, National Institutes of Health grants GM35252 to Lawrence S. B. Goldstein and AR12673 to Terence Tao, and an American Cancer Society Faculty Research Award to Lawrence S. B. Goldstein.

Received for publication 28 January 1991 and in revised form 25 October 1991.

References
Amos, L. A. 1987. Kinesin from pig brain studied by electron microscopy. J. Cell Sci. 87:105–111.
Bloom, G. S., M. C. Wagner, K. K. Pfister, and S. T. Brady. 1988. Native structure and physical properties of bovine brain kinesin and identification of the ATP-binding subunit polypeptide. Biochemistry. 27:3409–3416.
Dabora, S. L., and M. F. Sheetz. 1988. The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts. Cell. 54:27–35.
Dubreuil, R. R., E. Brandin, J. H. Sun Reisberg, L. S. B. Goldstein, and D. Branton. 1991. Structure, calmodulin-binding, and calcium-binding of recombiant alpha spectrin polypeptides. J. Biol. Chem. 266:7189–7193.
Franz, R. B., D. B., and T. F. MacRae. 1973. Conformation in Fibrin Proteins and Related Synthetic Polypeptides. Academic Press Inc., New York. 628 pp.
Griffith, I. P. 1972. The effect of cross-links on the mobility of proteins in dodecyl sulphate-polyacrylamide gels. Biochem. J. 126:553–560.
Harrington, W. F. 1971. A mechanoechemical mechanism for muscle contraction. Proc. Nat. Acad. Sci. USA. 68:685–689.
Hirokawa, N., K. K. Pfister, H. Yorifuji, M. C. Wagner, S. T. Brady, and G. S. Bloom. 1989. Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration. Cell. 56:867–878.
Hisanaga, S., H. Murofushi, K. Okuhara, R. Sato, Y. Masuda, H. Sakai, and N. Hirokawa. 1989. The three-dimensional structure of adrenal medulla kinesin. Cell Motil. Cytoskeleton. 12:264–272.
Ingold, A. L., S. A. Cohn, and J. M. Scholey. 1988. Inhibition of kinesin-driven microtubule motility by monoclonal antibodies to kinesin heavy chains. J. Cell Biol. 107:3657–3667.
Kosik, K. S., L. D. Orecchio, R. Schnapp, H. Inouye, and R. L. Neve. 1990. The primary structure and analysis of the squid kinesin heavy chain. J. Biol. Chem. 265:3278–3285.
Karnavov, S. A., E. A. Vaisberg, N. A. Shamina, N. N. Magretova, V. Y. Chernyak, and V. I. Gelfand. 1988. The quaternary structure of bovine brain kinesin. EMBO (Eur. Mol. Biol. Organ.) J. 7:353–356.
Lehrer, S. S. 1975. Intramolecular crosslinking of tropomyosin via disulfide bond formation: evidence for chain register. Proc. Natl. Acad. Sci. USA. 72:3377–3381.
Lovell, S. G., T. Karr, and W. F. Harrington. 1988. Suppression of contractile force in muscle fibers by antibody to myosin subfragment 2. Proc. Natl. Acad. Sci. USA. 85:1849–1853.
Lu, R. C., and S. S. Lehrer. 1984. Effects of interchain disulfide cross-links on the trypsin cleavage pattern and conformation of myosin subfragment 2. Biochemistry. 23:5975–5981.
McClellan, A. D., and J. Karn. 1983. Periodic features in the amino acid sequence of nematode myosin heavy and light chains. J. Biol. Chem. 258:1453–1463.
Privatov, P. L. 1982. Stability of proteins. Adv. Protein Chem. 35:1–104.
Rosenberg, A. H., B. N. Lade, D. Chui, S.-W. Lin, J. J. Dunn, and F. W. Studier. 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene (Amst.) 56:125–135.
Scholey, J. M., and J. Heuser, J. T. Yang, and L. S. B. Goldstein. 1989. Identification of globular mechanochanical heads of kinesin. Nature (London.). 338:357–361.
Schroer, T. A., B. J. Schnapp, T. S. Reese, and M. P. Sheetz. 1988. The role of kinesin and other soluble factors in organelle movement along microtubules. J. Cell Biol. 107:1785–1792.
Toyoshima, Y., T. J. Kron, E. M. McNally, K. R. Niewling, C. Toyoshima, and J. A. Spudich. 1987. Myosin subfragment 1 is sufficient to move actin filaments in vitro. Nature (London.). 328:536–539.
Tyler, J. M., and D. Branton. 1980. Rotary shadowing of extended molecules dried from glycerol. J. Ultrastruct. Res. 71:95–102.
Wale, D. B., T. S. Reese, and M. P. Sheetz. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell. 42:39–50.
Warrick, H. M., and J. A. Spudich. 1987. Myosin structure and function in cell motility. Annu. Rev. Cell Biol. 3:379–421.
Yang, J. T., C.-S. C. Wu, and H. M. Martinez. 1986. Calculation of protein conformation from circular dichroism. Methods Enzymol. 130:208–269.
Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three-domain structure of kinesin heavy chain is revealed by DNA sequence and microtubule binding analyses. Cell. 56:879–889.
Yang, J. T., W. M. Saxton, R. J. Stewart, E. C. Raff, and L. S. B. Goldstein. 1988. The head of kinesin is sufficient for force generation and motility in vitro. Science (Wash. DC). 242:49–47.