JOURNAL OF STRUCTURAL BIOLOGY **122,** 30–41 (1998)
ARTICLE NO. SB983986

The Coiled-Coil Helix in the Neck of Kinesin

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Received January 14, 1998, and in revised form March 17, 1998

Kinesin is a microtubule-dependent motor protein. We have recently determined the X-ray structure of monomeric and dimeric kinesin from rat brain. The dimer consists of two motor domains, held together by their α-helical neck domains forming a coiled coil. Here we analyze the nature of the interactions in the neck domain (residues 339–370). Overall, the neck helix shows a heptad repeat $(abcdefg)_n$ typical of coiled coils, with mostly nonpolar residues in positions a and d. However, the first segment (339–355) contains several nonclassical residues in the a and d positions which tend to weaken the hydrophobic interaction along the common interface. Instead, stabilization is achieved by a hydrophobic "coat" formed by the a and d residues and the long aliphatic moieties of lysines and glutamates, extending away from the coiled-coil core. By contrast, the second segment of the kinesin neck (356–370) shows a classical leucine zipper pattern in which most of the hydrophobic residues are buried at the highly symmetrical dimer interface. The end of the neck reveals the structure of a potential coiled-coil "trigger" sequence.

Key Words: **X-ray crystallography; microtubules; motor proteins; motility; coiled coil.**

INTRODUCTION

Coiled coils of α-helices are a well-recognized structural element in proteins, found in elongated or fiber-forming proteins such as myosin, α-keratin, tropomyosin, and kinesins, but also in proteins which are not elongated. Over 20 protein structures have been determined to high resolution that contain coiled-coil elements, some of them very short (Lupas, 1996, 1997; Kohn *et al*., 1997). The α-helices interacting with other domains within a protein molecule or with other protein molecules are often recognized by their heptad signature where blocks of seven residues (termed abcdefg) occur repeatedly such that the residues in position a and d are nonpolar (often Leu or Ile; Cohen and Parry, 1990). Since these residues are roughly aligned on one side of the α-helix this generates a hydrophobic "seam" which can act as a glue to other proteins or protein domains.

In the absence of external interactions α-helices tend to be nearly straight, with a helical pitch of 0.54 nm, corresponding to 3.65 residues per turn. If one side of the helix is hydrophobic and the other side is hydrophilic, such amphipathic helices can lean against other parts of the protein, e.g., surface α-helices positioned over a core β-sheet ("ridges into grooves" packing; Lupas, 1996). If two amphipathic α-helices interact directly with one another they wind around one another and form a coiled-coil dimer (crossover repeat around 14 nm, diameter ~2 nm). Here the helical repeat decreases to 0.51 nm or 3.5 residues per turn along the coiled-coil axis. The packing has been likened to "knobs-into-holes" (Crick, 1953).

The stability of a coiled coil depends partly on the propensity of the sequence to form an α-helix (since this is the building block; e.g., high propensities are observed for Glu, Met, Ala) and, more importantly, on the nature of the amino acids in the a and d positions conforming to the necessity for hydrophobic interaction in the buried interface. Leucines are generally preferred at d positions in two-stranded parallel coiled coils and branched chain amino acids such as isoleucine or valine at the a position. However, other factors also influence the stability. Attractive or repulsive interactions between the two helices have been detected between charged residues at the g position of one and at the e position of the next heptad (Alber, 1992). In addition, elongated side chains of charged residues pointing toward the solvent protect the hydrophobic core from solvent interactions.

Since the majority of coiled coils are characterized by their heptad pattern, coiled-coil proteins can be detected by scanning for its presence. Several algorithms are available for predicting the coiled-coil probability of amino acid sequences, such as COILS (Lupas *et al*., 1991), PAIRCOIL (Berger *et al*., 1995), or MATCHER (Fischetti *et al.*, 1993). These programs generally rely on the probability of a given residue to occur on certain positions within coiled coils. The probability of forming an α-helix as such can be predicted from the helix propensities of the constituent residues

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(Zhou *et al*., 1994; Monera *et al*., 1995) and can be calculated by secondary structure prediction programs (e.g., Chou and Fasman, 1978; Rost and Sander, 1993).

Many motor proteins interacting with actin filaments or microtubules occur as dimers containing two motor domains (which interact with the track filament and hydrolyze ATP) coupled by coiled-coil necks or tails, myosin or kinesin being examples. In the case of myosin, the long C-terminal portion of the coiled coil (the light meromyosin domain) serves to assemble many myosin molecules into a rod (the thick filaments of muscle). The neck helix attached to the motor domain is covered with regulatory proteins (light chains), it does not form a coiled coil with its partner molecule, and is thought to mediate the power stroke of myosin independently of its partner (Rayment *et al*., 1993; for review see Holmes, 1997). By contrast, kinesin does not form filaments, and the advantage for having a dimeric coiled-coil structure is not a priori obvious. One possible explanation is that kinesin moves in a processive fashion (Berliner *et al*., 1995; Gilbert *et al*., 1998); i.e., one head stays attached to the microtubule while the other changes position. This "hand-over-hand" mechanism would require a tight coordination between the heads at the level of the neck coiled coil, with the possibility of opening and closing of the coiled coil periodically during motion.

The structure of the motor domain of kinesin from human brain (a plus-end directed microtubule motor) has recently been determined (Kull *et al*., 1996), as well as that of the related retrograde motor ncd from *Drosophila* (Sablin *et al*., 1996). These motors show striking similarities between each other and to other nucleotide-binding proteins such as G-proteins or myosin (reviewed by Fletterick and Vale, 1997). The two studies revealed the core motor domains but lacked the neck domains which were disordered. This problem has now been solved for the case of rat brain kinesin whose structure has been solved in the monomeric and dimeric state (rK354 and rK379, Sack *et al*., 1997; Kozielski *et al*., 1997a). Since in both cases the neck domains are mostly ordered and visible we have now the chance of analyzing the neck α-helix by itself (in the monomer) or in the coiled-coil state. The structural results show that (a) the neck helix of the monomer is straight and has the standard parameters of an α-helix mentioned above; (b) the two neck helices of the dimer enter a coiled-coil relationship with the expected structural parameters. (c) However, this coiled coil shows two clearly distinguishable segments. Segment II (residues 356–370) forms a classical "leucine zipper," where hydrophobic residues at the a and d positions are juxtaposed at the interface to generate hydrophobic interactions. Segment I (residues 339–355) shows large deviations from the standard picture, with hydrophilic or even charged residues at the interface, and nonstandard orientations of the interface residues. These observations will be discussed within a model assuming that segment I can open up when a kinesin dimer interacts with its target microtubule, setting the heads free to perform movement.

MATERIALS AND METHODS

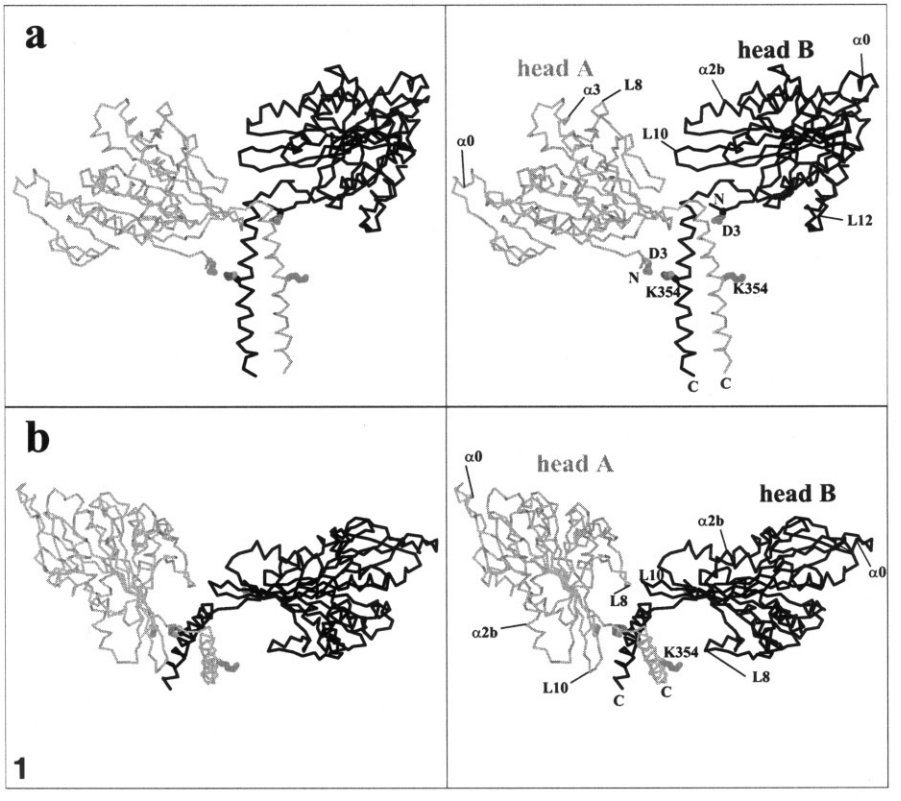
The present studies are largely based on the rat brain kinesin constructs rK354 and rK379, containing the motor domain in the strict sense (~335 residues) and the α-helical neck (starting at Ala339). The greater length of rK379 leads to dimerization, while rK354 remains monomeric in solution. The generation of the constructs and their expression in *Escherichia coli* is described elsewhere (Kozielski *et al*., 1997b), as well as their X-ray crystal structures (Sack *et al*., 1997; Kozielski *et al*., 1997a). Complete data sets of the monomer and dimer crystals were obtained at 1.9 Å (monomer) and 3.0 Å (dimer) resolution [available at the Brookhaven Data Bank (PDB Access Codes 2½ in. and 3½ in)].

RESULTS

Orientation and Order of the Monomeric and Dimeric Neck Helices

Based on gross structural parameters the kinesin heavy chain can be subdivided into several separate domains. The N-terminal motor or core domain encompassing residues up to the end of helix α6 (Lys325 in the rat sequence) is linked to the neck helix (aa 339–370) through the head–neck junction (linker residues Thr326–Thr338). The neck helix is separated from the coiled-coil stalk (starting around residue 406) by some 35 amino acids with no predicted secondary structure ("hinge region"). The stalk is also predicted to be interrupted by nonhelical segments.

The crystal structures offer three independent views of the motor and neck domains, one of the monomer (rK354) and two of the dimer (heads A and B of rK379; Fig. 1). We can therefore ask whether the dimerization has an influence on the extent of the neck helix, its orientation, or its internal structure. The extent and orientation are remarkably similar in the monomer and dimer structures. In both cases the α-helical conformation starts at the same residue, Ala339, an a position in the heptad repeats. This start occurs one helical turn later than expected, since the preceding Leu335 which occupies a potential d position in this case has an extended conformation (part of strand β10). Second, the orientation of the neck helix relative to the motor domain is comparable in the three cases; the helix runs


a
head A
head B
α0
α3
L8
α2b
L10
α0
N
D3
L12
D3
N
K354
K354
C
C
b
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head B
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L10
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L8
α2b
K354
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C
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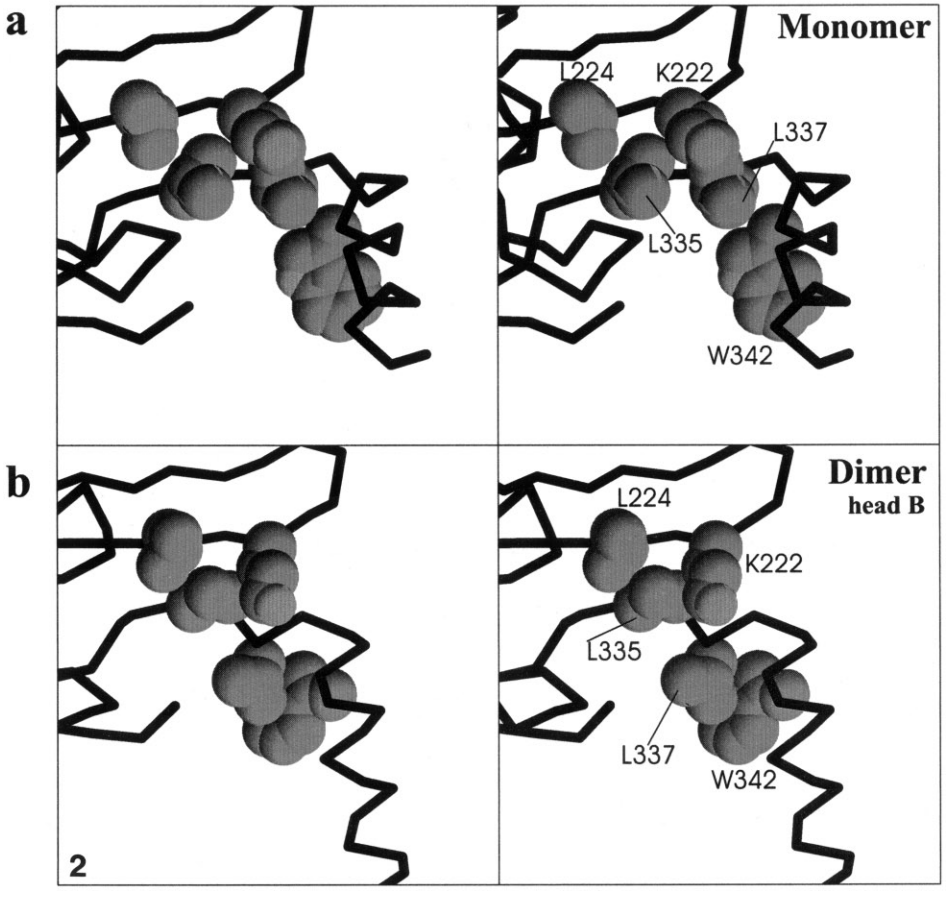
1


a
Monomer
L224
K222
L337
L335
W342
b
Dimer
head B
L224
K222
L335
L337
W342
2

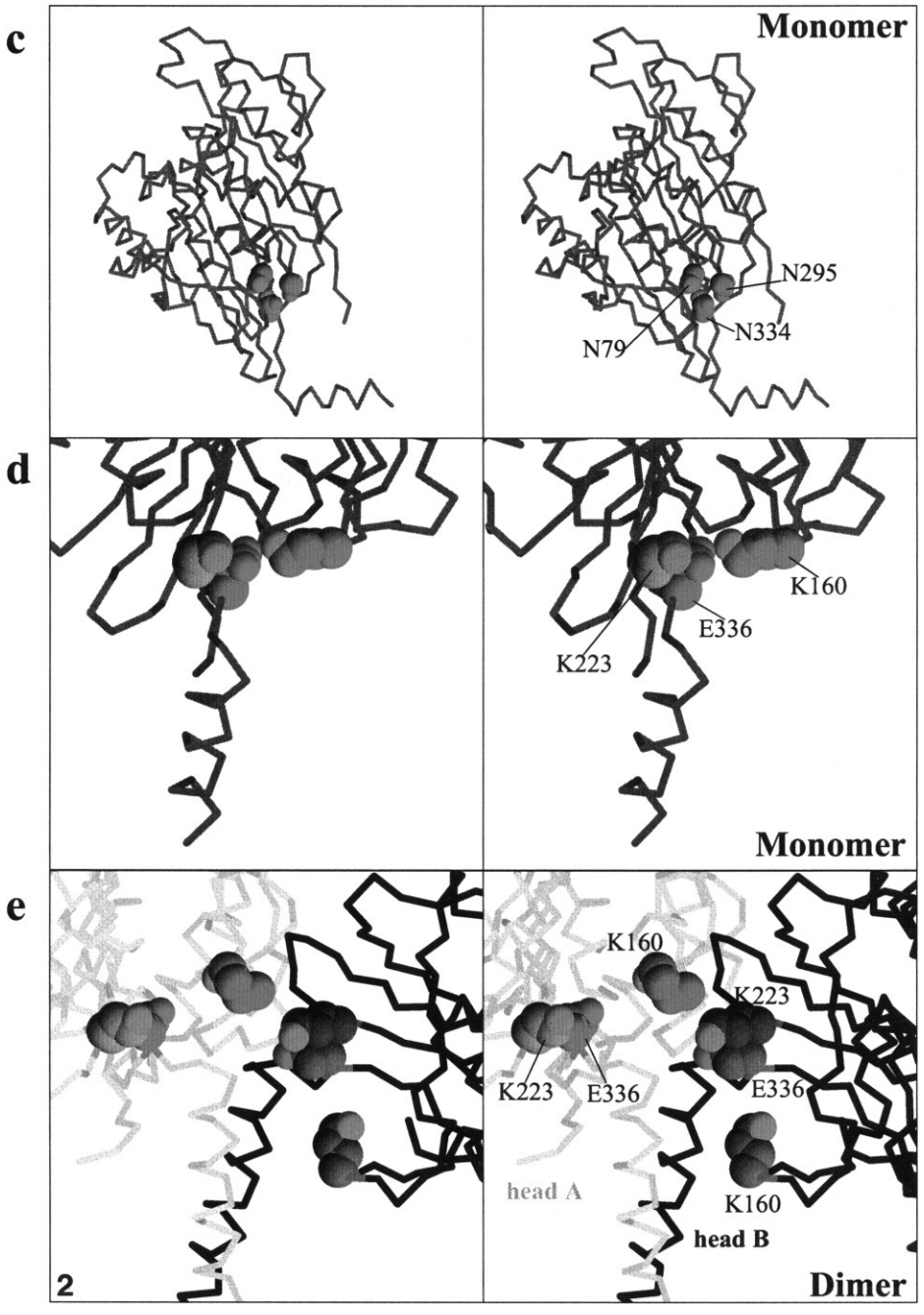


FIG. 2. Stereo diagrams showing interactions of residues situated in the head-neck junction. (a) In the rK354 monomer residues Leu224, Leu335, the aliphatic side chain of Lys222, and Leu337 form a hydrophobic cluster. Trp342 is oriented at an angle which does not allow an interaction with this cluster (instead there is an interaction with Tyr346, see Fig. 6a). This cluster is likely to be among the main determinants of the orientation of the linker vs the motor domain. (b) In the rK379 dimer Trp342 and Leu337 have moved toward each other, Leu337 no longer has contact with Lys222. For clarity, only head B is drawn here. (c) Cluster of Asn334, Asn295, and Asn79. This structure is similar in the monomer and in the dimer (not shown). Asn334 is in a position to form a hydrogen bond with either Asn295 or Asn79. This might function as an intramolecular sensor. (d) In the monomer an ion pair can be detected between Glu336 and Lys160 (loop 8). Running parallel to Glu336, but interacting mainly along its aliphatic side chain, is Lys223 (β7). (e) In the dimer the interaction between Glu336 and Lys223 is still observed, but the ion pair between Glu336 and Lys160 is no longer present in either head. In the figure head A (light gray) has an orientation roughly similar to that of the monomer.

FIG. 1. (a) Stereo diagram of the kinesin dimer backbone showing the two heads (labeled A and B) and their connection via a coiled-coil helix. The coiled coil is roughly parallel to the plane of the paper with the C-terminus pointing toward the bottom. Asp3 and Lys354 are drawn as sticks to show the relative distances between them. Whereas Asp3 of head A and Lys354 of head B come close together (~0.6 nm), the corresponding Asp3 of head B and Lys354 of head A are much more distant from each other(~2 nm). (b) The motor domains of the two molecules are related by a ~120-degree rotation around an axis pointing into the plane of the paper. In this arrangement the two heads cannot bind onto a microtubule surface in equivalent positions. The rotational axis is close to, but not coincident with, the axis of the coiled coil.

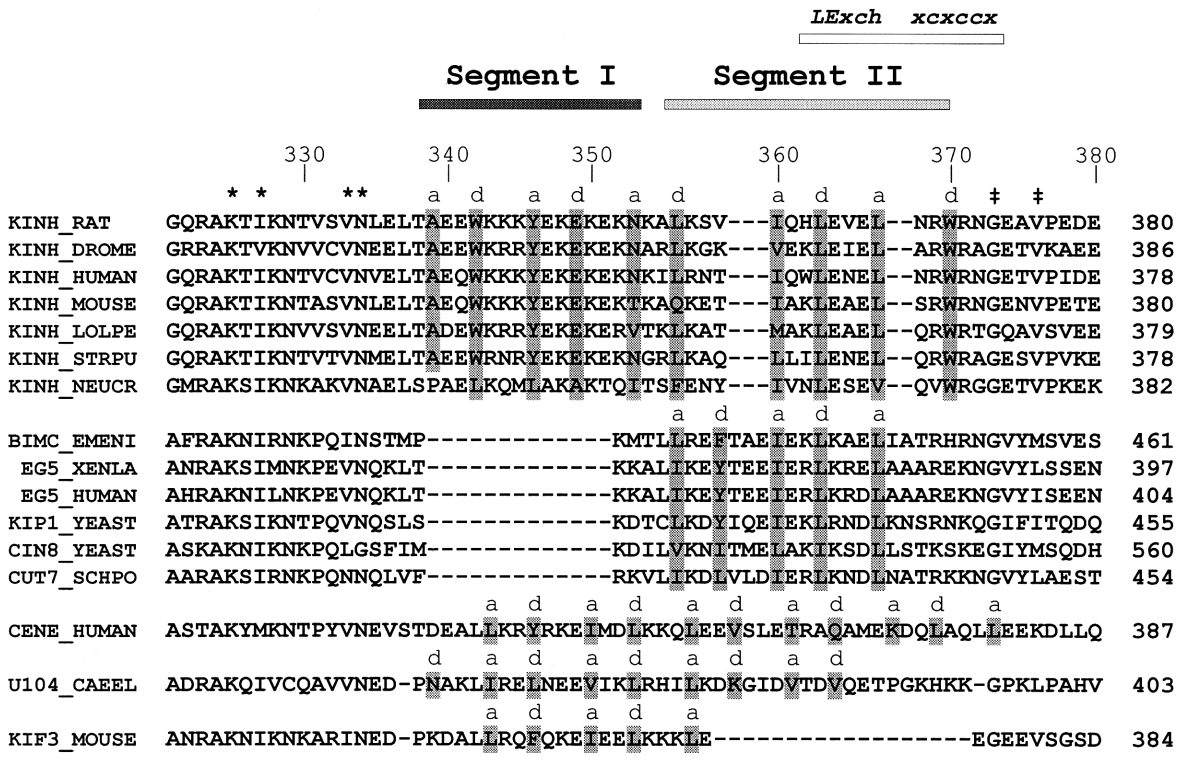


FIG. 3. Alignment of the sequences adjacent to the kinesin motor domain (forming the kinesin neck). Bars indicate the location of segment I (dark gray) and segment II (light gray); a and d positions are highlighted. The asterisks mark the residues implicated by Case *et al.* (1997) to be involved in determining plus-end directionality. A region homologous to segment I (i.e., highly charged) is lacking from the motors not belonging to the khc subfamily. Interestingly, the coiled coil is followed by a motif Gxxh (h, hydrophobic), which is also conserved in almost all N-terminal motors (marked by ‡). The open bar indicates the consensus for the coiled-coil nucleation sequence from cortexillin (Steinmetz *et al.*, 1998).

roughly in the same plane as the core β-sheet of the motor. If one aligns the monomer, head A and head B on the basis of their core motor domains, the initial orientations of neck helices A and B differ by ~30°, and the neck helix A and the monomer neck helix differ by only ~8°, suggesting that the head–neck arrangement is largely intrinsic to the monomer. The divergence in orientation occurs roughly around Asn334 which can therefore be regarded as a hinge. Finally, the internal order of the neck domains is similar as well, since they all form α-helices starting at the same residue (Ala339). Apart from this overall similarity there are variations in detail. The most conspicuous one is the variation in the angle and rotation between the head and its neck which leads to two types of pseudo-symmetrical transformations in the dimer: The neck helices are related by a twofold axis, whereas the two heads are related by a ~120° rotation (Fig. 1b) about an axis that is close to but not coincident with the coiled-coil axis (~25° difference between the two axes). One consequence is that the symmetry of the kinesin dimer revealed by the crystal structure is not compatible with the symmetry of the microtubule lattice.

Interactions between Neck Helices and Motor Domains

In our standard view of the kinesin dimer (Fig. 1a) the coiled coil appears roughly midway between the two heads. Because of the two distinct symmetries relating the heads or the neck helices (Fig. 1b), the two neck helices interact differently with residues from the motor domains. The transition from a ~180° relationship between the neck helices to a ~120° relationship between the heads must be accommodated by the spatial arrangement of amino acids within the head-neck junction. This junction is formed by two short β strands β9 (K328-V331) and β10 (N334-E336). While the position of β9 is defined by its interaction with the N-terminal strand β0, β10 interacts with the core of the motor domain at β7 (Sack *et al.*, 1997). This general feature is found in the monomer and for both heads of the dimer. However, there are subtle differences, due to the fact that the necks emanate from the different heads at slightly different angles (from Asn334 onward). There is a hydrophobic interaction (within each chain) between Leu335 of β10 and Leu224 and the aliphatic

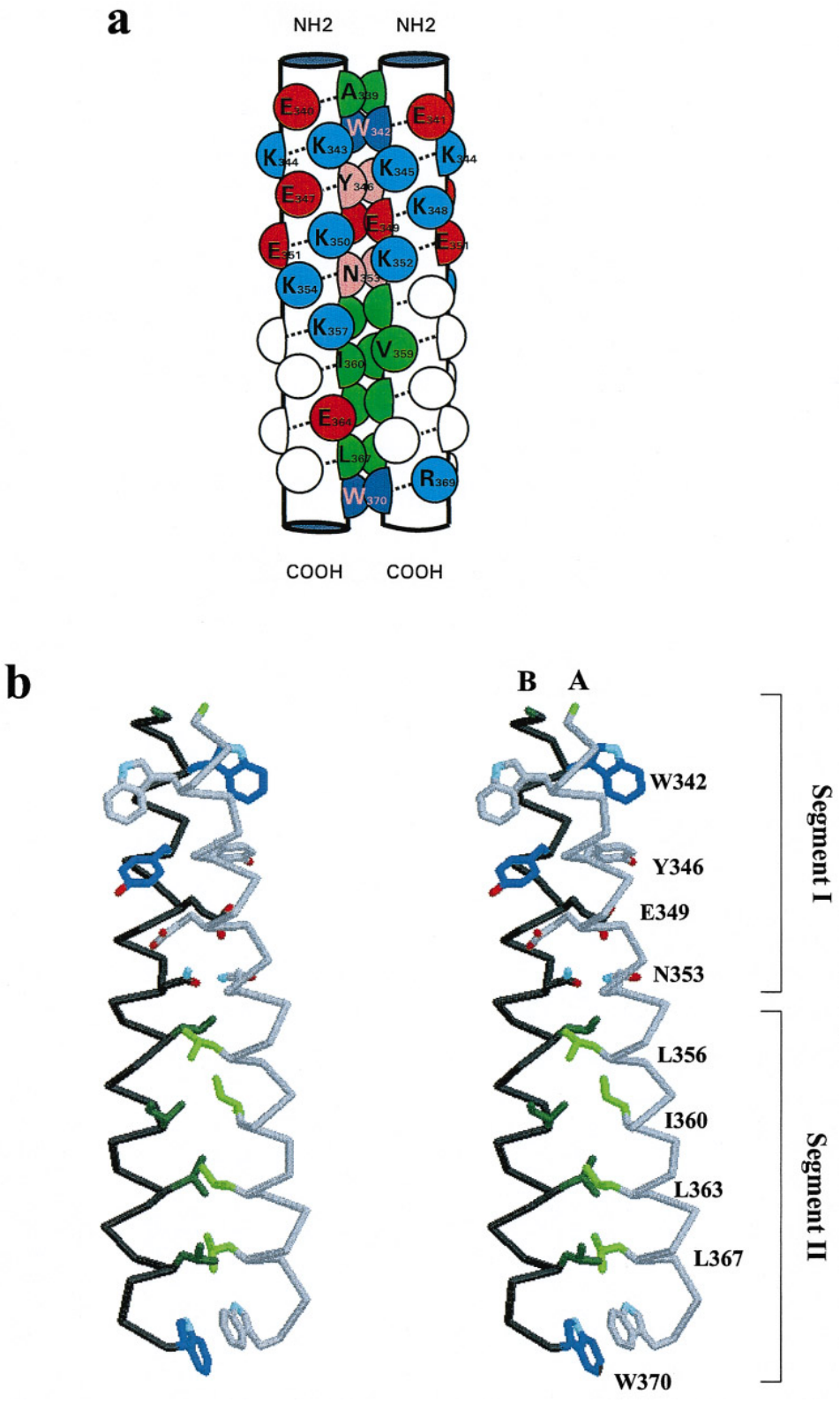


FIG. 4. (a) Model showing the arrangement of amino acids within the hydrophobic core of the coiled coil, as well as the distribution of charged amino acids. Residues that are negatively charged are colored red; residues that are positively charged are light blue. Aliphatic residues close to or in the hydrophobic core are green. Coloring in light red depicts polar residues Tyr346 and Asn353. Note the clustering of charged residues (most of which are lysines) at the N-terminus of the sequence. (b) Overview of the kinesin neck structure, showing only side chains for the a and d positions. The α-carbon backbones are shown in gray (for molecule A) and black (for molecule B, cf. Fig. 1). In the C-terminal part (lower half, residues 356–370) the interface between the two α-helices resembles the structure known from other coiled coils such as GCN4, where leucine side chains from the two molecules interact with each other in the interior of the structure. By contrast, the N-terminal part (upper half, residues 339–355) appears to be held together by an entirely different mechanism. Here, for the length of four turns of the helix, there is no interaction between the side chains of the a or d positions coming from the two helices (Ala339, Trp342, Tyr346, Glu349). Instead these side chains point away from each other at approximately 180° angles and therefore do not contribute to the stability by their mutual interaction. This unconventional conformation corresponds to the observation that a peptide containing only the N-terminal part of the kinesin neck has only a very weak tendency to dimerize (Morii *et al.*, 1997).

portion of Lys222 of β7 (Figs. 2a and 2b). There is also an intrachain interaction between Glu336 and Lys223. In the monomer and in head B this contains both hydrophobic and ionic features (between the aliphatic side chains and the charged head groups, respectively), while the ionic interaction appears to be absent in head A; instead there is an apposition of the γ-carboxyl group of Glu336 and the hydroxyl group of Ser225 (intrachain). Asn334 is close to Asn295 and to Asn79 (distances between 3.1 and 3.6 Å, depending on the head), allowing formation of a hydrogen bonding with either residue and thus potential switching (Fig. 2c). However, the most remarkable feature is an ionic intrachain interaction between Lys160 (loop 8) and Glu336 (strand β10) present only in the monomer and not in the dimer (Figs. 2d and 2e). This is interesting because both Lys160 and Glu336 (together with Leu and Thr/Ser in position 337–338) are conserved in ubiquitous and neuronal kinesins from all species, suggesting a similar three-dimensional arrangement. In the monomer Leu337 forms a very close contact to Lys222 of loop 10; this contact is not present in the dimer even if the residues are close enough, judged by the position of their C_α atoms. Instead, Leu337 appears to be involved in linking the head to the neck by covering the part of the indole ring of Trp342 that is not shielded within the coiled coil itself (see below).

In addition to the contacts described above there is a possible interchain interaction between the two molecules in the dimer, involving the neck helix of one head and the N-terminus of the other (Fig. 1a). Lys354 of head B approaches Asp3 of head A (0.59 nm between charged groups), suggestive of a possible salt bridge (on the left side of the neck in Fig. 1a). The corresponding pair of Lys354 of head A and Asp3 of head B are much more distant from each other (2.0 nm between their charged groups). Thus it is conceivable that rearrangements of the heads during the mechanochemical cycle could be supported by alternating between salt bridges Lys354(B)–Asp3(A) or Lys354(A)–Asp3(B). However, neither of these salt bridges is strictly observed in the actual crystal structure. Instead the two heads seem to be suspended on the head–neck junction (residues 334–338). Interestingly, though, all kinesins that possess the conserved highly charged neck sequence of segment I (see below, and Fig. 3) also have an aspartic acid or glutamic acid close to their N-terminus, arguing for a potential role in molecular switching.

Contacts between the Coiled-Coil Neck Helices

The neck helix starts with Ala339 both in the monomer and in the dimer. In the electron density data of the dimer it can be traced up to Trp370 (Fig. 4b). After that it becomes disordered up to the end (Asp379), in agreement with the low α-helical propensity of the sequence and the absence of a heptad repeat. The coiled-coil conformation is found along the entire length of the helix. However, a closer look at the side chain interactions within the interface between the two helices reveals important structural differences between the amino-terminal and the carboxy-terminal half of the coiled coil (called segments I and II, respectively).

The N-terminal part of the kinesin neck helix (residues Ala339–Ala355, segment I, Figs. 5a and 5b) is largely α-helical in the monomer (Fig. 5a, the last three residues 351–354 are disordered). In the dimer, segment I contains nonclassical residues in the a and d positions which weaken the coiled-coil interaction, compared to a classical leucine zipper (Fig. 5b). Trp342 is bulky, Tyr346 is bulky as well as polar, and Glu349 is charged. This generates a coiled-coil interface where the residues at the a and d positions are not buried in the interior but stretch out sideways from the coiled-coil interface and contribute to a hydrophobic collar, in concert with the extended aliphatic side chains of lysines and glutamates surrounding the coiled-coil backbone. In contrast, the C-terminal portion of the kinesin neck (segment II, Fig. 5c) shows a typical leucine zipper pattern, found in proteins such as the yeast transcriptional activator GCN4 (Alber, 1992). Here most of the hydrophobic residues are buried at the highly symmetrical dimer interface, and the "handshaking" between the branched apolar side chains of Leu356, Leu363, and Leu367 is clearly visible (Fig. 5c, green).

The pairwise orientation of the two different tryptophanes 342 and 370 in the neck helix provides an interesting contrast. The C-terminal pair of Trp370 are aligned roughly in parallel, suggesting a stacking interaction at the coiled-coil interface. By contrast, the N-terminal pair of Trp342 are facing toward the perimeter of the coiled coil and appear to lean against the side chain of the Lys343 of the second helix, without interacting directly. In fact, it appears that Trp342 changes its orientation upon dimerization. In the structure of the monomer Trp342 and Tyr346 are found roughly on the same side of the α-helix (Fig. 5a) where a partial interaction between the two hydrophobic rings is achieved. In the dimer structure, Trp342 and Tyr346 are found on opposite sides of the α-helix where they are shielded from the solvent by interacting both with side chains of their parental helix and with side chains of its coiled-coil partner.

More detailed stereo views of selected contact sites are provided in Fig. 6. Figure 6a shows the environment of Trp342 in the coiled coil. Trp342 of chain A (gray) is shielded both by Lys345 of chain A and by

Ala339, Lys343, and Tyr346 of chain B (black). The indole ring is embedded in a groove formed by the aliphatic chains of the lysines, the C_β of Ala339, and the ring of Tyr346, with all charged or polar atoms directed toward the solvent. The environment of Tyr346 (Fig. 6b) shows a similar structure. Tyr346 of chain B is surrounded by side chains both of chain B (Lys350) and of chain A (Trp342, Lys345, Lys348, and Glu349). The side chains of Glu349 also point toward the perimeter, where they interact with side chains of both α-helices. In this setting, Glu349 of head A seems to prevent repulsive interactions between Lys350 of head B and Lys348/Lys352 of head A. Thus, Glu349 might have a stabilizing, rather than a destabilizing, effect. This is a special case, as ion pairs are generally believed to be important if they can form between e and g positions between chains (see Alber, 1992; Tripet *et al.*, 1997).

In contrast to segment I, the hydrophobic residues that provide the coiled-coil interaction in segment II conform to a regular "knobs-into-holes" structure. As an example we show Leu363 of chain B (a d position), surrounded by residues of chain A located at a, d, e, and a positions (Fig. 6c). The base of the hole is formed by the backbone atoms of Leu363 and of Glu364 of chain A.

Thus, while segment II forms a regular zipper structure of interacting residues at heptad positions, segment I could be considered an "inverted zipper" where spikes from long and bulky side chains both intercalate and project outward at the same time. This hydrophobic collar provides some stabilization of the coiled coil, in spite of the high overall density of charged residues. The arrangement is reminiscent of a cylindrical micelle containing apolar groups on the inside and shielded by polar groups on the outside.

Independently of the "micellar" structure, direct charge–charge interactions between residues from the two helices can have either stabilizing or destabilizing effects when they approach each other. As seen in Fig. 4a, the region between residues 343 and 352 is dominated by a cluster of positive charges on the lysine side chains. One might expect that this would lead to strong repulsive intra- and interchain interactions. However, Figs. 6a and 6b show that the direct interaction between most lysines is prevented by the presence of the phenolic hydroxyl group and of the γ-carboxyl group of Glu349. In any case, whereas in a molecule such as GCN4 the coiled coil is also stabilized by attractive ionic forces (Alber, 1992), no similar mechanism can be detected in the kinesin neck. One exception is a possible Glu364–Arg371 interaction; however, this is not detectable in our structure, because Arg371 is not ordered.

DISCUSSION

Structure and Interaction Motifs within the Kinesin Coiled Coil

The main features of the neck structure can be summarized as follows: The α-helical conformation extends from residues 339–370. This is broadly in agreement with the heptads observed in the sequence but one turn shorter than expected (at the beginning). The start of the α-helix is independent of dimerization since it occurs in the monomer as well, even though this part of segment I has a lower helical content in model peptides (see below). The connection to the motor domain is made via a transition region, particularly the strand β10 which connects up to the central β sheet of the motor domain (Sack *et al.*, 1997). This junction determines the general head–neck orientation which is surprisingly reproducible between the monomer and heads A and B of the dimer, yet is sufficiently flexible to allow different symmetries.

Compared to the monomer, the backbone atoms in the dimer helices show small deviations, compatible with the change from a straight single helix to a coiled double helix. More significantly, there are also changes in the side chain positions. The most striking is the conserved potential ion pair Glu336–Lys160 which changes from 2.8 Å distance in the monomer to 6.8 and 12.5 Å, respectively, in the dimer. Further changes involve the orientations and interactions of Leu337 and Trp342 which move into positions where they can interact with partners from the partner coil.

In the initial half of the neck (segment I) the two helices interact with each other, but not in the standard fashion of a coiled coil where hydrophobic residues in a and d positions interact with one another, generating a knobs-into-holes packing. All unusual amino acids point to the perimeter of the structure. Instead of a hydrophobic core, a "hydrophobic coat" is formed. This coincides with an unusual amino acid composition. The polar Tyr346 and the charged Glu349 do not appear to have a destabilizing effect per se; on the contrary, they might act to lessen repulsive forces expected from the clustering of lysines 343, 345, 348, and 350. By contrast, the second half of the neck (segment II) is a regular coiled coil reminiscent of a typical leucine zipper (e.g., the GCN4 structure, Alber, 1992). The stacking of Trp370 is an elegant way of terminating the coiled coil, as no knobs-into-holes environment is required for achieving a high stability.

Coiled-Coil Segment Structure vs Stability

Since the crystal structures of rat brain kinesin show the molecule both in the monomeric and in the

dimeric states, we can now compare them to recent circular dichroism and self-association studies of model peptides derived from the neck region (Morii *et al*., 1997; Tripet *et al*., 1997). For example, Morii *et al*. (1997) determined the dissociation constants of peptides corresponding to residues 332–349 (peptide I), 350–379 (peptide II), and 332–369 (peptide III) of human kinesin. In our sequence they correspond to residues 334–351, 352–381, and 334–371, i.e., roughly equivalent to our segment I, segment II, and segments I + II which is the entire neck region. Although all three peptides had a high α-helical content, peptides I and II had only a moderate tendency to dimerize (K_d of 9.6 mM and of 60 μM, respectively). However, they seemed to act together synergistically, as the dissociation constant of peptide III was only 62 nM, a value that corresponds to the K_d of dimeric kinesin constructs (Correia *et al*., 1995; Jiang *et al*., 1997). This peptide was also kinetically stable and the chains did not exchange. Similar peptides from human kinesin (numbering of rat kinesin = number in human kinesin plus 2) were analyzed by Tripet *et al*. (1997), i.e. K1 (hK316–355 = rK318–357), K2 (hK330–369 = rK332–371), K3 (hK344–383 = rK346–385), K4 (hK316–369 = rK318–371), and K5 (hK330–383 = rK332–385). In the light of the X-ray structure these peptides contained sequences before or after the actual neck helix. CD spectra showed that K2 to K4 formed α-helical dimers. However, loss of the two C-terminal heptads from K4 led to formation of a β-stranded structure in the remaining peptide (K1). Interestingly, K1 underwent a β-strand to α-helix transition in the presence of 50% trifluoroethanol, indicating the capacity of this region for structural rearrangements. The authors suggested that the presence at a or d positions of Tyr346, Glu349, and Asn353 has a destabilizing effect on the coiled coil. Furthermore, most of the electrostatic interactions within the coiled coil would be expected to be repulsive (e.g., K345-K350, K352-K357) and thus further decrease the coiled-coil stability. In general, the results on the model peptides are in agreement with the observed X-ray structure. The most notable difference is that peptides corresponding to our segment I and the head–neck transition region had little α-helical structure, whereas the neck helix is observed even in the monomer structure. This illustrates that the formation of helices can be influenced by other domains, in this case the head domain. By the same token, one could speculate that the order or disorder of the helical conformation could be influenced by other partners, for example, tubulin, which could therefore alter the interaction between the neck domains in a kinesin dimer.

Implications for Kinesin Motility

Based on the weak interaction between the N-terminal half of the two neck helices in the dimer one could speculate that during stepping of the kinesin molecule, the neck helices could separate or even partly melt to allow movement of the temporarily unattached head to its next binding site (Tripet *et al*., 1997). This movement could be accompanied by loss of α-helix in segment I. This model agrees very well with our recent observations that the two heads of a kinesin dimer bind to two separate subunits of β-tubulin, probably along the same protofilament (Thormählen *et al*., 1998; Hoenger *et al*., 1998). The result implies that the kinesin neck must open up substantially upon binding to microtubules and perhaps even lose some of its α-helical conformation. Structurally one could envisage such a loss of coiled-coil structure if the micellar collar of segment I with its positively charged outside was broken up by the interaction with another charged molecule, and tubulin with its high negative charge would be an obvious candidate.

Given the possible scenario of an uncoiling of kinesin's neck helix during motility it is interesting to note that the end of the neck contains a sequence that has been implicated in nucleating coiled-coil interactions (Fig. 3). Such an element would be ideally located for closing the coiled coil again, either transiently during motility or after dissociation from the microtubule. The consensus sequence comprises 1.5 heptads of a conserved sequence Leu^{d}-Glu^{e}-X^{f}-charged^{g}-hydrophobic^{a}-X^{b}-charged^{c}-X^{d}-charged^{e}-charged^{f}-X^{g} (Steinmetz *et al*., 1998). If the underlined residues at the g and subsequent e positions have opposite charges they can strengthen the interaction between the two helices and place them in register. In the rat kinesin neck sequence this conserved region corresponds to residues 363–373 (L-E-V-E-L-N-R-W-R-N-G). There are two leucines in heptad positions, providing for strong hydrophobic interaction, and the two underlined residues Glu366 and Arg371 indeed have opposite charges and could thus supply additional binding energy. Whether the ionic bond actually exists cannot be ascertained at present since the residues beyond Trp370 are disordered in the crystal structure and thus not visible.

In summary, the X-ray structure of the kinesin dimer opens up a novel view of a coiled-coil structure. In the crystal, the heads contain bound ADP and therefore bind to microtubules only weakly (Crevel *et al*., 1996). Thus it is likely that the crystal structure represents the kinesin dimer in its free state. Knowledge of the microtubule-bound state of the kinesin dimer (at low resolution) comes from electron microscopy and image reconstruction studies (Thormählen *et al*., 1998; Hoenger *et al*., 1998),

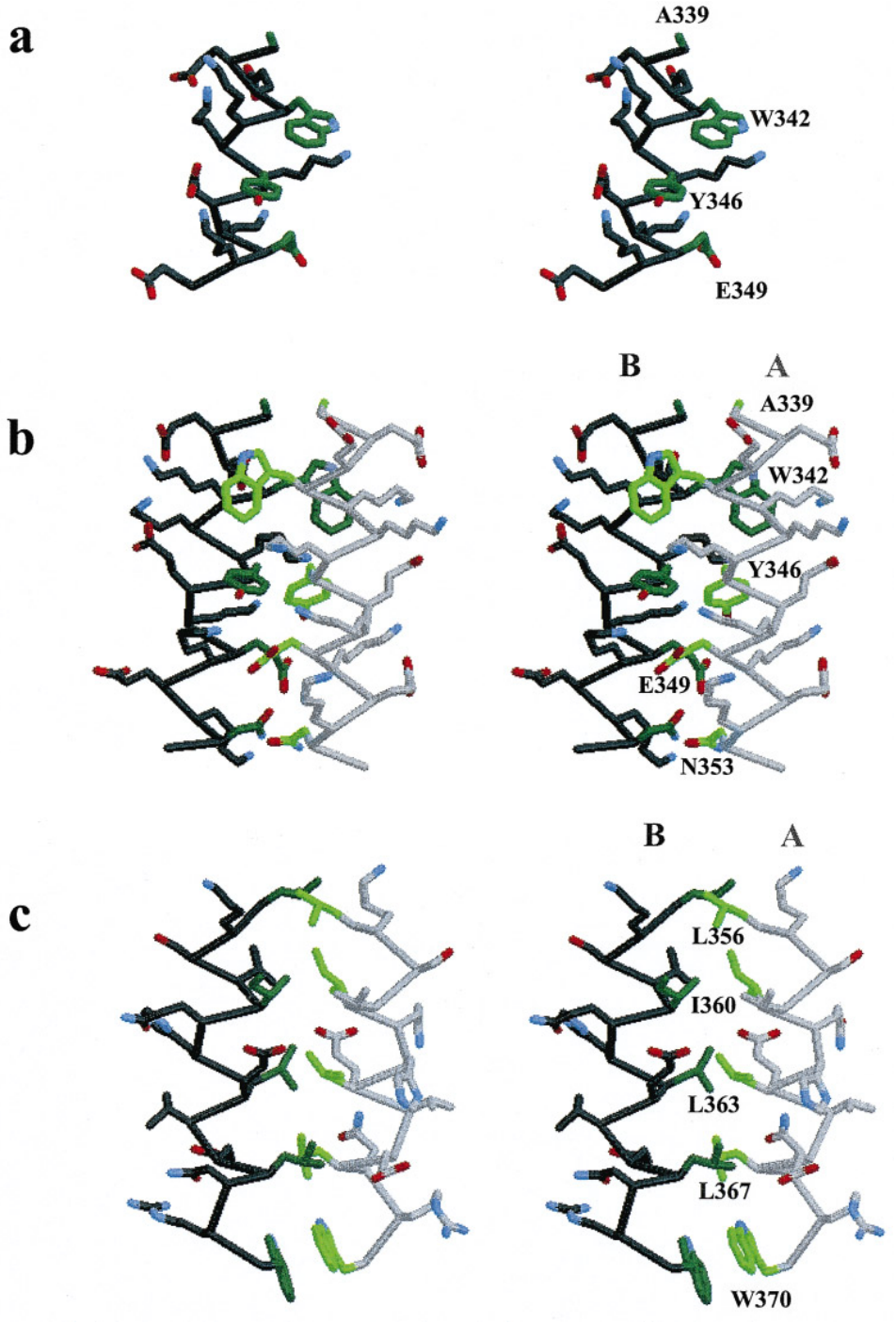


FIG. 5. (a) Neck helix of the monomer containing residues Ala339 to Glu351. Residues in a and d positions are shown in green. The side chains of Ala339, Trp342, and Tyr346 lie roughly within a vertical plane on the same side of the helix, whereas in the dimer, the indole ring of Trp342 side chain moves away from Tyr346 so that the side chains point in different directions, enabling them to make contact with the neighboring helix (see Fig. 5b). (b) Segment I in stereo view. Residues in a and d positions are shown in green; they point alternatingly to the front or to the back. Segment I does not contain amino acids typically found in a coiled-coil interface (i.e., leucines, isoleucines, valines). (c) Segment II in stereo view. The formation of the coiled coil is due to the presence of leucines 356, 363, and 367, of Ile 360 and of Trp370. The two Trp370 show a partial overlap, suggesting a stacking interaction.

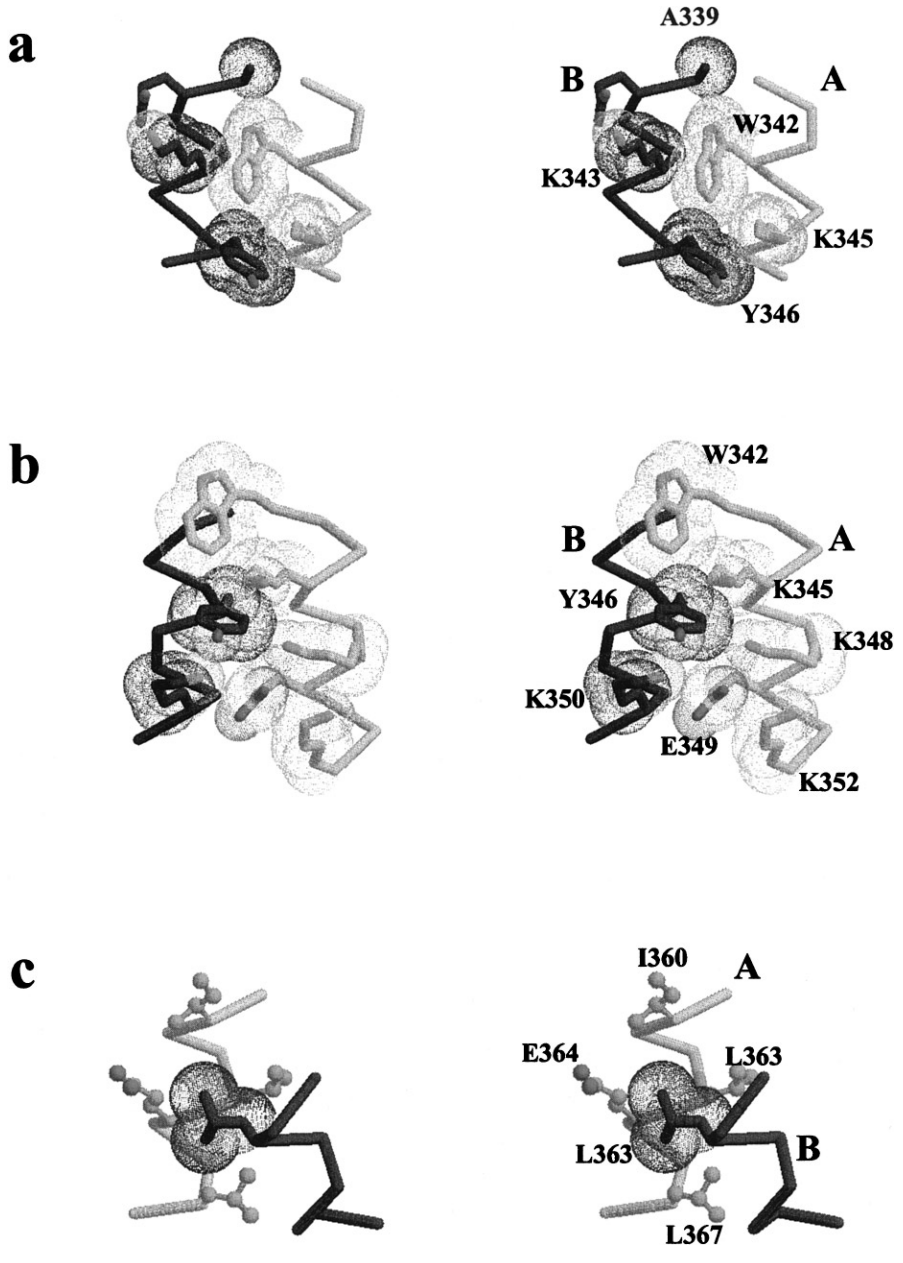


FIG. 6. Close-up views of selected residues. For reasons of clarity only those residues have been drawn that interact with amino acids at a or d positions which would be lying on the side of the helix facing the reader. (a) Environment of Trp342. Chain A is shown in gray; chain B is in black. Trp342 is protected from the solvent by interacting with Ala339, Lys343, and Tyr346 of the neighboring chain and also by being shielded by Lys345 of its parental chain. The shielding is only partial, as one side of the indole ring is left unprotected in the coiled coil; it is instead shielded by Leu337 in the head–neck junction (see text). In contrast to what would be expected for a hydrophobic residue at a d position, there is almost no interchain interaction of the two tryptophanes. All charged or polar atoms point toward the solvent. (b) Close-up view of Tyr346 and Glu349. The hydrophobic benzyl rings of Tyr346 are not juxtaposed at the dimer interface, but point radially away from the helical axis. They are shielded from the solvent by the presence of the phenolic hydroxyl group and by the neighboring aliphatic chains of Lys345, Lys348, and Lys350 and by Glu349. Glu349 is centered between Lys348 and Lys352 of the parental and Lys350 of the partner chain, seeming to prevent repulsive interactions between the charged amino groups. (c) Environment of Leu363 from chain B creating the knobs-into-holes interaction postulated by Crick (1953). The leucine side chain points into a hole formed by the side chains of Ile360, Leu363, Glu364, and Leu367 of chain A. The backbone between residues 363 and 364 forms the base of the hole.

and the structures in the two states are clearly different. Although the transition between the structures is presently not known, the structure of the neck helix offers intriguing insights, in particular, the rearrangements that must take place in segment I and the potential nucleating sequence at the end of segment II. As the structure of kinesin's partner tubulin has been solved recently (Nogales *et al.*, 1998), it should become possible to propose more detailed models of interaction and to test them experimentally.

ACKNOWLEDGMENTS

We thank Jens Müller, Frank Kozielski, and Eva-Maria Mandelkow for their contributions to the crystal structures of kinesin on which this study is based and for critical discussions and the Deutsche Forschungsgemeinschaft for support.

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