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Kinesin–ADP: whole lotta shakin' goin' on

Margot E. Quinlan, Joseph N. Forkey and Yale E. Goldman

A mobile state of the kinesin–ADP head is discovered using multi- and single-molecule fluorescence polarization.

Since the 1985 discovery of kinesin in extracts of nervous tissue[1–3] and sea urchin eggs[4], this molecular motor has repeatedly been a source of astonishment. Over 250 isoforms of kinesin-like proteins, grouped into at least 10 subfamilies, have been deposited into the amino acid sequence databases (*http://www.blocks.fhcrc.org/~kinesin/*). These proteins carry out myriad functions in cell biology including vesicle and organelle transport, spindle and chromosome motion during mitosis and microtubule dynamics. Although *in vitro* motility assays, optical traps, cryo-electron microscopy (cryo-EM), X-ray crystallography and recombinant structure-function studies have provided considerable details about the mechanism of chemical-to-mechanical transduction, the dynamics of structural changes that lead to motion have been elusive. On page 540 of this issue of *Nature Structural Biology*, Sosa *et al.*[5] report the application of a novel spectroscopic technique to individual kinesin molecules that reveals a new facet of the interaction between kinesin and its cytoskeletal tracks, microtubules.

Conventional brain kinesin, the most studied isoform, is a ~350 kDa molecular motor with two N-terminal, globular ~40 kDa heads, an α-helical coiled coil stalk and two light chains that presumably determine cargo specificity. The two heads cooperate to perform a repetitive hand-over-hand action that maintains attachment to the microtubule, making it possible for kinesin to 'walk' processively (Box 1). Kinesin's mechanochemical cycle differs from that of other classical motors in that attachment to its track is weaker in the presence of ADP than with ATP[6], the opposite of dynein and myosin. Brain kinesin and many other isoforms move in the direction of the plus end of a microtubule, which is the fast growing end and usually points toward the periphery of the cell. It was a surprise that some family members have C-terminal motor domains[7,8] and that these often move in the opposite direction[9,10]. Another unexpected discovery was that KIF1A, a single-headed kinesin, is also processive[11]. This processivity is thought to be mediated by a sequence insertion that provides an extra microtubule binding site in the KIF1A head[12].

The structure of the microtubule–kinesin complex has been determined by cryo-EM. The highest resolution electron density maps (15 Å) have been obtained with KIF1A, in part because it binds very tightly to microtubules. Fig. 1 shows the crystal structure of the motor domain from brain kinesin docked into the cryo-EM map of the KIF1A head bound to a microtubule. The atomic model of brain kinesin fits into the map uniquely and remarkably well except where KIF1A has extra sequence insertions (marked by asterisks on the wire-frame)[12]. Two binding sites between the kinesin and the microtubule, marked MB1 and MB2, are located along the microtubule axis. This presumably stabilizes the complex and limits axial rotations (in the plane of the diagram) when kinesin bears a mechanical load, such as a vesicle.

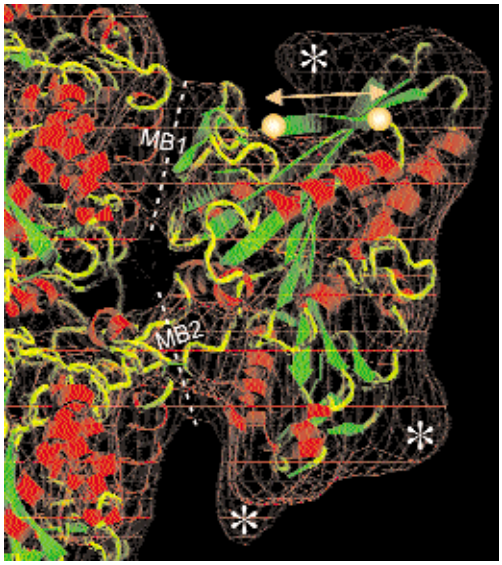


Fig. 1 Atomic models of tubulin and conventional kinesin fit into a wire-frame electron density image of a microtubule decorated with KIF1A calculated from cryo-EM data[12]. The microtuble axis is vertical with the plus end up. Ribbon diagrams of α-tubulin (lower left) and β-tubulin (upper left) are placed within the wire-frame of the microtubule. A ribbon representation of the conventional kinesin crystal structure is shown within the wire-frame on the right side. Orange balls indicate residues 169 and 174. The double-headed arrow shows the approximate orientation of a bifunctional fluorophore bound at these sites in Sosa *et al.*[5]. MB1 and MB2 are kinesin's microtubule binding sites and the density in the cryo-EM map near the dashed lines represents the protein contacts. Asterisks indicate extra electron density of the KIF1A map, which is presumably caused by sequence insertions not present in conventional kinesin (ribbon diagram). The figure was modified from an image kindly supplied by M. Kikkawa, University of Tokyo. The crystal structure of KIF1A and its fit into this cryo-EM map will appear in *Nature*[20].

Cryo-EM provides limited information concerning the angular distribution and dynamic motions during active energy transduction. Spectroscopic approaches, such as the fluorescence polarization method used by Sosa *et al.*[5], probe structural changes at a much higher time resolution. Fluorescence polarization is principally sensitive to orientation[13]. Sosa *et al.*[5] labeled a single-headed construct, derived from conventional kinesin, at cysteine residues engineered into the positions indicated by the orange balls in Fig. 1. They cross-linked the two residues with a bifunctional fluorophore, which results in a fluorescent probe with an average orientation expected to lie along the line linking the cysteines. This known orientation is a major benefit of bifunctional probes[14] and, in the present case, places the probe nearly perpendicular to the microtubule axis in a position sensitive to axial rotations. Polarized fluorescence was measured on ensembles of labeled, microtubule-bound kinesin heads in the absence of nucleotide, with the ATP analog AMP-PNP, and with ADP-AlF_4, an analog of the ATP hydrolysis products. Under these conditions, the kinesins were well ordered, and therefore, nearly immobilized on the microtubule. However, polarization measurements in the presence of ADP indicated a surprisingly broad orientational distribution.

With such a broad angular distribution, fluorescence measurements on ensembles generally cannot distinguish dynamic motions from disorder of a static population. The difficulty arises because in a steady-state distribution of rotating molecules, motions of an individual molecule, on the time scale much longer than the fluorescence lifetime (typically 5–50 ns), are averaged out in the total signal (Fig. 2). The polarized fluorescence, then, is identical to that from a static ensemble with the same overall distribution.

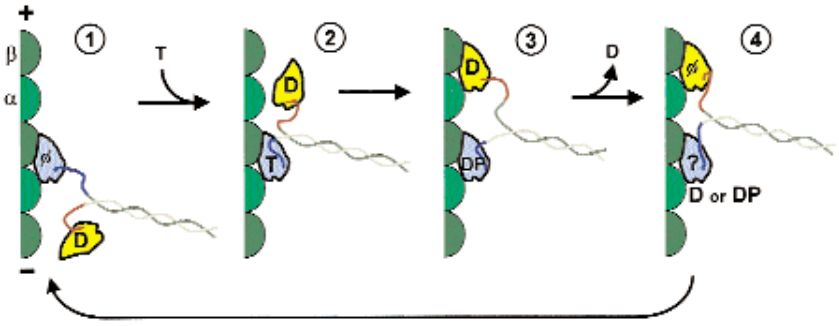
To overcome this difficulty, Sosa *et al.*[5] measured fluorescence polarization of individual kinesin heads docked onto microtubules, eliminating the ensemble distribution that obscured the interpretation. In this case, any depolarization measured must be generated by motion of the fluorophore[13,15]. Its application in the current paper highlights the power of single molecule spectroscopy to obtain structural information about functioning proteins that is not readily accessible with classical structural biological methods.

The single molecule experiments indicated extensive probe motions on the μs–ms timescale in the microtubule–kinesin–ADP complex, but not in the other states. The authors argue that this motion is not restricted to a local region but reflects axial rotations of the whole motor domain because their probe is fixed by its double attachment and the strand containing the probe (termed β5) is rigid in currently available crystal structures of kinesin–ADP. Another surprise to add to kinesin's tally: the protein wobbles through a large angle and yet remains attached to the microtubule. How does it do that? Perhaps one of the binding sites shown in Fig. 1 is disrupted and/or flexible loops allow the large rotational motions. The floppy kinesin–ADP state is probably an intermediate between a more tightly bound complex and a detached head. In the mechanochemical cycle shown in Box 1, candidates for weakly bound ADP intermediates are the leading head in state 3, which becomes more strongly bound upon ADP release and the trailing heads in states 3 and 4, which will detach to form state 1. Several of these states may exhibit disorder.

The extent of mobility in kinesin–ADP is controversial. A segment extending

Box 1 Energy transduction by kinesin and microtubules

When an individual kinesin molecule encounters a microtubule, it takes ~100 steps of 8 nm each toward the plus end (the faster growing end) before dissociating. This high degree of processivity is accomplished by repeated hand-over-hand interactions with successive microtubule binding sites and requires the heads to act reciprocally to prevent both from detaching simultaneously. Coupling between movement and ATP hydrolysis is tight; each step uses one ATP molecule.



In a simplified enzymatic cycle, an attached head (state 1; light blue) is free of nucleotide and its detached partner (yellow) has ADP (D) bound to the active site. On binding of ATP (T), a conformational change (step 1 → 2) positions the detached head forward. The structural change may be zipping of a 'neck linker' (blue peptide segment) down onto the attached head (ref. 16, but see also refs. 17 and 18). Repositioning of the unattached head allows it to bind to the next microtubule site (step 2 → 3). ATP is hydrolyzed to ADP and orthophosphate (P) and dimerization helices adjacent to the heads (gray peptides) may partly unwind. ADP is released from the newly attached leading head (step 3 → 4). It remains to be determined when orthophosphate is released from the trailing head. The question mark thus indicates either D or DP. Finally, the trailing head detaches from the microtubule (step 4 → 1) and another cycle begins. This step is possibly accelerated by stress in the double-headed attachment. Mechanical and biochemical steps alternate to synchronize the events in the two heads.

Many hypotheses have been proposed for the structural changes that induce motion and determine directionality. Among these possibilities are rearrangement of the neck[21,22], a disorder-to-order transition of the 'neck linker'[16] and recoiling of the dimerization domain[23,24]. Given the new results of Sosa *et al.*[5], it is also possible that a disorder-to-order transition of the entire head could play a role in generating directed motion.

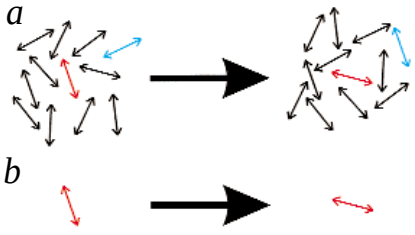


Fig. 2 Sensitivity to orientational motions in ensemble and single molecule measurements. ***a***, When a particular molecule in the ensemble (for example, the red molecule) rotates, on average some other molecule (for example, the blue molecule) will rotate to the initial orientation of the first molecule, thereby retaining the original distribution. Measurements of the ensemble distribution cannot differentiate between a steady state of tumbling molecules and a broad distribution of stationary ones. ***b***, Motion of a single molecule is unambiguous and can be observed directly.

through strands β9 and β10, that connects the motor domain to the coiled coil stalk, is called the 'neck linker'. Spectroscopic and EM evidence suggested that ATP binding causes docking of this segment onto the head[16] (Box 1). In these experiments, the neck linker appeared to be mobile in kinesin–ADP. Dissenters from this view have cited the stability of the neck linker in X-ray crystal structures of kinesin–ADP and the low apparent mass of the unattached head in cryo-EM reconstructions of two-headed microtubule-kinesin–AMP-PNP complexes[17,18] The low apparent mass suggests mobility in this complex.

The present results indicate a new and different type of mobility for kinesin-ADP: the whole head seems to wobble. This is consistent with the weaker microtubule affinity of kinesin–ADP compared to that of the other states[6,19] and with an earlier cryo-EM study showing low apparent mass of single-headed microtubule–kinesin–ADP complexes[20].

The present results also raise a number of new questions. As Sosa *et al.*[5] mention, confirmation that the probe wobble is really indicative of motions of the entire head, rather than of a subdomain, will require similar experiments with probes placed in different locations. Further puzzles need to be solved. Where in the cycle does the floppy ADP state fit? What internal structural changes mediate the transition from stable to wobbly state? How are these structural changes tied to the enzymatic cycle? Is there more than one microtubule–kinesin–ADP state? Sosa *et al.*[5] found that the mobility parameter characterizing individual kinesin–ADP heads varied widely. This observation may suggest a lack of homogeniety. Undoubtedly, measurements of dynamic structural changes at the single molecule level will figure importantly in answering these questions.

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Helicobacter pylori springs another surprise

Bruce E. Dunn and Markus G. Grütter

The crystal structure of *Helicobacter pylori* urease helps to explain how this enzyme contributes to bacterial survival in the acid environment of the human stomach.

Helicobacter pylori, perhaps the most common bacterial infection in humans, causes chronic gastritis and duodenal and gastric ulcers and is associated with gastric carcinoma and lymphoma. *H. pylori* is able to colonize the acidic gastric environment by producing abundant amounts of the enzyme urease, which hydrolyzes urea present in gastric juice. The ammonia produced from this reaction is thought to provide a neutral microenvironment within and surrounding the bacteria, thereby protecting the microorganism against the harsh acidic conditions in the stomach. Therefore, urease is a valid target for development of new therapies to eradicate *H. pylori* infection.

In order to better understand the mechanism(s) by which *H. pylori* urease contributes to acid resistance, Ha *et al.*[1], on page 505 of this issue of *Nature Structural Biology*, have determined the crystal structure of the enzyme. In contrast to known structures of other ureases[2,3], the *H. pylori* enzyme has a unique supramolecular assembly that may be important for protecting the microorganism against acid. To test this hypothesis, the authors have convincingly demonstrated that the free *H. pylori* urease *in vitro* is indeed active at low concentrations of urea in unbuffered acid, a condition that most likely mimics the environment *in vivo*.

A controversy

In contrast to other bacteria in which urease is present only within the cytoplasm, the urease of *H. pylori* is also located — and is enzymatically active — on the cell surface[4,5]. Urease becomes associated with the surface of *H. pylori* by a unique mechanism: cytoplasmic urease is released upon spontaneous autolysis of a subpopulation of bacteria; subsequently, the released urease adsorbs onto the surface of intact bacteria. This mechanism may ensure survival of the population at the expense of individual cells.

Although results from *in vitro* experiments suggest that both external and cytoplasmic urease are essential to protect *H. pylori* from the deleterious effects of acid[6], the protective role of external urease has been challenged on two grounds[7]. First, since the purified urease of *H. pylori* has a pH optimum of ~8.0 and is rapidly inactivated by short term exposure to