

Fleximers of the dynein motor protein

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Introduction

Flexible macromolecules pose special difficulties for structure determination by crystallography or NMR. Progress can be made by electron microscopy, because the shapes of individual molecules are recorded, but electron cryo-microscopy of unstained, hydrated specimens is limited to larger macromolecules because of the inherently low signal-to-noise ratio. For 3-dimensional structure determination, the single particles must be invariant in structure. We have used negative staining and single-particle image processing techniques to overcome some of these limitations and to explore the structure and flexibility of single molecules of the microtubule motor protein dynein.

The approach we have used is to align the images based on features of one part of the molecule (e.g. the head) and then group the images into classes based on features of another part (e.g. the position of the stalk or tail). The averaged image of each group then shows consistent features of the class, and the whole series of class averages shows the range of shapes (the ‘fleximers’) the molecule adopts.

We show two-dimensional projection images of negatively-stained dynein-c, a flagellar inner arm dynein from *Chlamydomonas reinhardtii*, revealing new details of its structure. The dynein heavy chain (~500 kDa) comprises three domains: tail, head, and stalk. The head contains six AAA+ modules and forms a ring-like globular domain ~13 nm in diameter. The stalk, which emanates from the head, is a 15nm-long, intramolecular, anti-parallel, coiled coil, which has at its distal end the microtubule-binding domain. The ~25nm-long tail domain contains dimeric p28 and monomeric actin light chains, responsible for attachment of this motor to its cargo (a doublet microtubule within the 9+2 axoneme). Both the proximal tail and the stalk are flexible, suggesting that they act as compliant elements within this motor. This is shown in three different views of the molecule (Fig. 1).

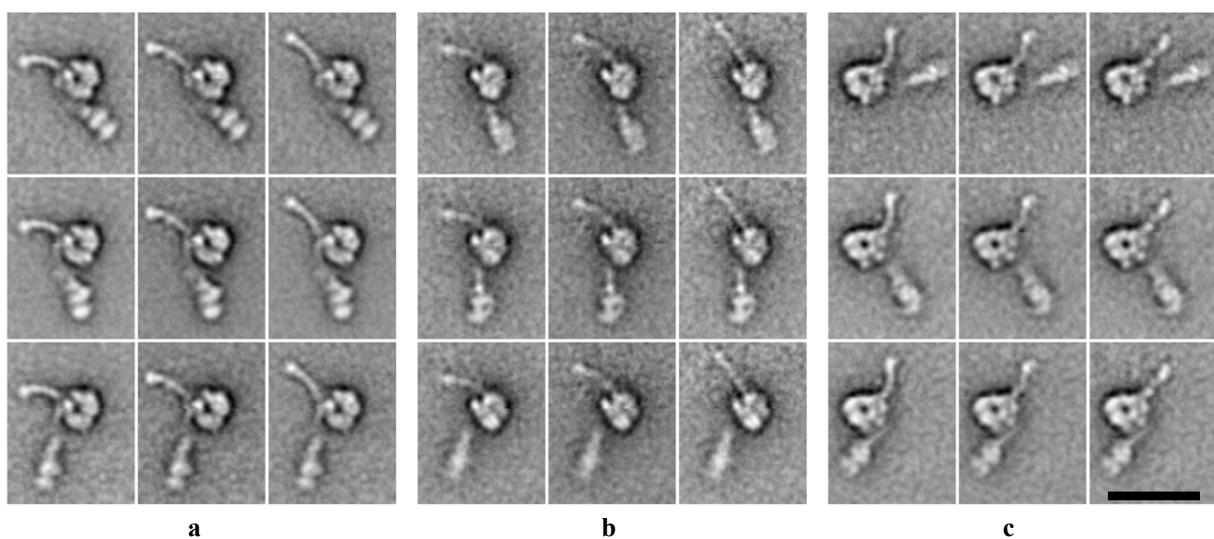


Fig. 1 Fleximers of dynein-c.

(a) Left, (b) side and (c) right views of apo-dynein-c, each showing 3 different stalk (columns) and tail (rows) conformations as illustrating the range of positions these domains adopt in relation to the head domain.

Scale bar 30nm.

Analysis of left views of ADP.Vi-dynein and apo-dynein (representing pre- and post-power stroke conformations of the motor) shows the combined effect of tail and stalk flexibility on the spatial distribution of the microtubule-binding domain relative to a fixed distal tail (Fig. 2).

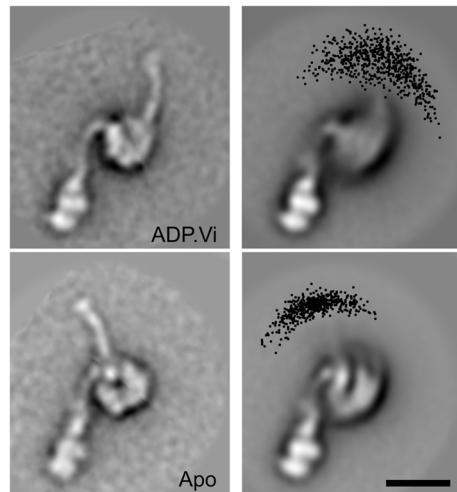


Fig. 2 Powerstroke of dynein-c.

(Left) Mean conformations of ADP.Vi (upper) and apo- (lower) molecules after alignment of their tail domains. (Right) Distribution of microtubule-binding domain positions from all fleximers. Scatter plots are superposed on the global averages which shows, as expected, smearing of the head and stalk. Scale bar: 15 nm.

The mean conformational change produces a ~15nm displacement of the microtubule-binding domain. However, the analysis of fleximers indicates the potential range of power stroke sizes about this mean which might underlie the inverse dependence of the step size on load seen by others in optical trap experiments.

Collaborators

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Publications

Burgess, S.A., Walker, M.L., Sakakibara, H., Oiwa, K. and Knight, P.J. (2004) The structure of dynein-c by negative stain electron microscopy. *J. Struct. Biol.* **146**, 205-216.

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