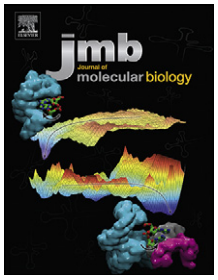

Structure of the Entire Stalk Region of the Dynein Motor Domain

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Abstract

Dyneins are large microtubule-based motor complexes that power a range of cellular processes including the transport of organelles, as well as the beating of cilia and flagella. The motor domain is located within the dynein heavy chain and comprises an N-terminal mechanical linker element, a central ring of six AAA+ modules of which four bind or hydrolyze ATP, and a long stalk extending from the AAA+ ring with a microtubule-binding domain (MTBD) at its tip. A crucial mechanism underlying the motile activity of cytoskeletal motor proteins is precise coupling between the ATPase and track-binding activities. In dynein, a stalk region consisting of a long (~15 nm) antiparallel coiled coil separates these two activities, which must facilitate communication between them. This communication is mediated by a small degree of helix sliding in the coiled coil. However, no high-resolution structure is available of the entire stalk region including the MTBD. Here, we have reported the structure of the entire stalk region of mouse cytoplasmic dynein in a weak microtubule-binding state, which was determined using X-ray crystallography, and have compared it with the dynein motor domain from *Dictyostelium discoideum* in a strong microtubule-binding state and with a mouse MTBD with its distal portion of the coiled coil fused to seryl-tRNA synthetase from *Thermus thermophilus*. Our results strongly support the helix-sliding model based on the complete structure of the dynein stalk with a different form of coiled-coil packing. We also propose a plausible mechanism of helix sliding together with further analysis using molecular dynamics simulations. Our results present the importance of conserved proline residues for an elastic motion of stalk coiled coil and imply the manner of change between high-affinity state and low-affinity state of MTBD.

Introduction

Dyneins are large microtubule-based motor complexes (over 1000 kDa) that power a wide variety of cellular processes through the coordinated action of their subunits [1,2]. There are two major classes of dynein: (i) cytoplasmic dynein that is involved in nuclear segregation, cell mitosis, and translocation of various cargo and intracellular organelles and (ii) axonemal dynein that drives the motions of flagella and the beating of cilia [3–5]. In each class of dyneins, the complex is made up of heavy, intermediate, and light chains. The heavy chain is required for basic motor activities, and its functional core is called the motor domain. This domain comprises an N-terminal linker (i.e., the mechanical element), a central ring of six AAA+ modules (four of which bind or hydrolyze ATP), and a long stalk connected to a microtubule-binding domain (MTBD) at its tip extending from the fourth module of the AAA+ ring [6–8]. The domain organization of dynein was originally determined using electron microscopy (EM) and more recently by X-ray crystallographic analysis of dynein motor domains [7,9–14]. These analyses show that the unique domain organization of dynein comprises several functional units embedded within the common AAA+ ring [15].

The motile activity of cytoskeletal motor proteins requires precise coupling between the ATPase and

track-binding activities [16,17]. In dynein, these two activities are spatially separated by the ~15-nm coiled-coil stalk, and thus, they must communicate with each other through this long coiled-coil structure. This communication may be mediated by a half-heptad of helix sliding in the stalk region [18,19]. This unique communication mechanism was first proposed according to the results of a microtubule-binding assay of MTBD with the distal portion of the coiled-coil stalk fused to seryl-tRNA synthetase (SRS) from *Thermus thermophilus* [18]. Mouse cytoplasmic dynein MTBDs fused to the coiled-coil region in the two alternate registries (denoted as "α" or "+β") have displayed high and low microtubule-binding affinity, respectively, although the coiled-coil portion of dynein was truncated and did not include the entirety of the coiled-coil helices [10]. That is, the register change of the stalk coiled coil can alter the affinity for microtubules.

The first X-ray structure of dynein was acquired by analysis of the MTBD fused with SRS in a weak microtubule-binding form with the heptad repeat fixed in the +β registry. The microtubule-binding surface of dynein and the exact heptad registry were determined at 2.3 Å resolution [10]. However, the SRS-MTBD fusion protein in a strong microtubule-binding form has been not yet crystallized. Quite recently, a pseudoatomic-resolution structure of the SRS-MTBD bound to the microtubule was determined using cryo-transmission EM reconstruction at 9.7 Å resolution and molecular dynamics (MD) simulations [20]. The initial model, which was revealed by the SRS-MTBD structure in a weak microtubule-binding form, agreed well within the experimental density, although the coiled coil remained in the low-affinity registry of +β. The final model with the half-heptad registry shift from +β to α was obtained by applying targeted MD. Based on these initial and fitted structures, a large displacement of the H1 helix within the MTBD and an opening of CC1 helix at the base of the stalk are proposed as required for the structural reorganization that determines the registry change from a weak (+β) to a strong (α) microtubule-binding mode.

Analysis of the recombinant 380-kDa motor domain of cytoplasmic dynein from *Dictyostelium discoideum* revealed the relationship between stalk conformation, ATPase, and microtubule-binding activities [19]. Introduction of disulfide bonds between engineered cysteine residues locks the registry of the two helices in the stalk coiled coil, which traps ATPase activity in either a high state or a low state, and microtubule-binding activity is fixed in either a strong state or a weak state depending on the locked registry (α or ±β). Together with this functional analysis, X-ray structures are available for the ADP-bound form of the wild-type and the ΔMTBD dynein motor domain from *D. discoideum* at 3.8 Å and 2.8 Å resolutions, respectively [13]. These structures show that the entire portion of the stalk region is in the strong microtubule-binding form; that is, the heptad repeat is in the α registry.

Despite these unambiguous results from functional studies, the structural basis for the helix-sliding mechanism involving the entire stalk region is poorly understood, and two fundamental issues remain to be resolved. First, a high-resolution structure of the SRS-MTBD fusion protein includes only a small portion of the stalk coiled coil [10]. Therefore, it cannot be determined whether the remainder of the coiled coil forms a canonical coiled coil with a registry of +β through the entire stalk. Second, although the 3.8-Å structure of the ADP-bound form of the dynein motor domain includes the intact stalk region, the electron density of the distal portion of the CC1 coiled-coil helix is not sufficiently clear to model the coordinates. The complete structure of the dynein stalk is required to fully understand the structural basis for the helix-sliding mechanism through the entire stalk region. Indeed, the structure of the stalk region provides a good starting model for performing MD simulations that might provide a structural basis to explain how the dynein stalk coordinates the action of the AAA+ ring and the MTBD.

Here, we have performed such a study by means of biophysical analysis of the entire portion of the microtubule-binding stalk. We have determined the crystal structure of the mouse cytoplasmic dynein stalk at 3.5 Å resolution using the single-wavelength anomalous dispersion (SAD) method. Helical content of the entire stalk region with two different registries (α and +β) was estimated by circular dichroism (CD) spectra analysis. We also show completely different motions between the wild-type stalk and its alanine double mutant (whose conserved proline residues were replaced to alanine) obtained from MD simulations followed by principal component analysis, which may be relevant to the stalk-mediated communication.

Results

The mouse dynein stalk region comprises 277 amino acid residues (Stalk277) that extend from Lys3207 to Glu3483. The recombinant protein comprising the stalk coiled coil, the MTBD, and the C-terminal 6× His tag was overexpressed in *Escherichia coli*. The recombinant portion is named Stalk277 and comprises the MTBD and a long coiled-coil structure interacting with the strut (buttress) coiled coil that was recently identified in the X-ray structure of the dynein motor domain [11]. Interactions between the stalk and strut coiled coils are so extensive in width that we excluded the strut-interacting region for structural analysis. Microtubule-binding activity was assayed using the cosedimentation method. Stalk277 showed a very