Elastic properties of dynein motor domain obtained from all-atom molecular dynamics simulations

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Abstract

Dyneins are large microtubule motor proteins that convert ATP energy to mechanical power. High-resolution crystal structures of ADP-bound cytoplasmic dynein have revealed the organization of the motor domain, comprising the AAA+ ring, the linker, the stalk/strut and the C sequence. Recently, the ADP.vanadate-bound structure, which is similar to the ATP hydrolysis transition state, revealed how the structure of dynein changes upon ATP binding. Although both the ADP- and ATP-bound state structures have been resolved, the dynamic properties at the atomic level remain unclear. In this work, we built two models named ‘the ADP model’ and ‘the ATP model’, where ADP and ATP are bound to AAA1 in the AAA+ ring, respectively, to observe the initial procedure of the structural change from the unprimed to the primed state. We performed 200-ns molecular dynamics simulations for both models and compared their structures and dynamics. The motions of the stalk, consisting of a long coiled coil with a microtubule-binding domain, significantly differed between the two models. The elastic properties of the stalk were analyzed and compared with the experimental results.

Key words: ADP and ATP, dynein, elastic properties, molecular dynamics simulation

Introduction

Dyneins are large microtubule motor proteins that play important roles in various biological movements along microtubules (Gibbons and Rowe, 1965; Paschal and Vallee, 1987). Their motor activity is generated by the energy conversion gained from ATP hydrolysis (Neuwald et al., 1999). Cytoplasmic dynein is responsible for cell division, cell migration and other basic cellular functions (Karki and Holzbaur, 1999). Axonemal dynein plays a role in the motion of cilia and flagella (Gibbons, 1981). Dyneins exist as a complex of heavy, intermediate and light chains. The core part of the heavy chain that performs the major functions for motor activities is called the motor domain. The motor domain contains an N-terminal linker, central
ring-shaped AAA+ modules (AAA1–AAA6) (Neuwald et al., 1999) and a stalk extending from the AAA+ ring. The linker is considered to function as a mechanical element for generating the power stroke (Burgess et al., 2003). The three AAA+ modules, AAA1, AAA3 and AAA4, perform the ATP hydrolysis (Kon et al., 2012). The stalk consists of a long coiled coil with an ATP-dependent microtubule-binding domain (MTBD) at its tip (Gee et al., 1997).

Electron microscopy and FRET studies have clarified the existence of primed (pre-power stroke) and unprimed (post-power stroke) states, depending on the nucleotide state in the AAA1 module (Burgess et al., 2003; Kon et al., 2005; Roberts et al., 2009). The nucleotide state changes following the so-called mechanochemical cycle, from the apo-state to the ATP-bound state, ADP.Pi state, and ADP-bound state, and finally returns to the original apo-state. The apo- and ADP-bound states correspond to the unprimed state, while the ATP-bound and ADP.Pi states are the primed states. The linker moves during the mechanochemical cycle, and its tip is close to AAA4 and AAA2 in the unprimed and primed states, respectively.

X-ray crystallographic studies have revealed the structure of the stalk including the MTBD (Carter et al., 2008; Nishikawa et al., 2014) and the organization of the AAA+ ring, the linker, the stalk, the strut (or buttress) and the C sequence in the motor domain (Carter et al., 2011; Kon et al., 2011, 2012; Schmidt et al., 2012). The AAA+ ring is formed by the tandem linkage of the AAA1–AAA6 modules, from the N-terminal. The stalk and the strut, which both have a coiled-coil structural motif, extend from AAA4 and AAA5, respectively. The strut contacts the lower basis part of the stalk. The C sequence is linked to AAA6 at the C-terminal end. High-resolution structures of the motor domain of wild-type cytoplasmic dynein and its truncated MTBD mutant (∆MTBD), which are both in the unprimed states, revealed the interface between the AAA+ ring and the linker, and the junctional structures between the ring and the stalk (Kon et al., 2012). The nucleotide binding sites contain four ADP molecules in AAA1–AAA4, while no nucleotides are bound to AAA5 and AAA6. A mutational analysis demonstrated that the nucleotide binding site of AAA1 is essential for the dynein motility (Kon et al., 2004). Recently, a high-resolution structure of dynein in the primed state, with ADP.vanadate being bound to AAA1 to mimic the ATP hydrolysis transition state, was reported (Schmidt et al., 2015). The structures of the linker and the stalk are quite different between the primed and unprimed states. In the primed state, one of the α-helices of the stalk contains a kink, resulting in the sliding of the kinked helix relative to the other helix. Thus, while the linker forms a straight structure in the unprimed state, it is bent in the primed state. Although the structures of the two states have been resolved, the dynamic properties of the states at the atomic level remain unclear.

We previously introduced the zero-dipole summation (ZD) method (Fukuda et al., 2011) to treat electrostatic interactions for molecular dynamics (MD) simulation. The ZD method takes into account the neutrality of charges (zero-monopole) and dipoles (zero-dipole) in a truncated subset. Recently, the ZD method has been expanded to the zero-multipole method (Fukuda, 2013; Fukuda et al., 2014; Wang et al., 2016). The ZD method has reproduced structures and dynamics properties similar to those obtained by the particle mesh Ewald method (Essmann et al., 1995), which is one of the most accurate methods, in a pure TIP3P water system (Fukuda et al., 2012), a membrane protein system (Kamiya et al., 2013) and a DNA-ion–water system (Arakawa et al., 2013). We have performed MD simulations on the stalk fragment of the dynein motor domain using the ZD method, which revealed the function-related motion of the stalk (Nishikawa et al., 2014). Since the ZD method is free from the evaluation of the reciprocal Fourier part required in the particle mesh Ewald method, an order N scheme is implied for a large system by computing only local interactions. In fact, by using the ZD method, effective scalability has been attained from the EGFR system consisting of ∼40 000 atoms to the dynein motor domain system consisting of one million atoms (Mashimo et al., 2013).

In this work, we built two models, named the ADP and ATP models, where ADP and ATP ligands are bound to AAA1, respectively. As the ADP model, the X-ray crystal structure of the dynein from Dictyostelium discoideum was used, in which four ADP molecules are bound to AAA1–AAA4. In the ATP model, the ADP molecule in AAA1, which is essential for dynein function, was substituted with ATP. We executed an MD simulation starting from the dynein structure of the unprimed state, to investigate the dynamic properties of both models. The computational system consists of the dynein motor domain, nucleotides, explicit water molecules and ions (size of the systems: approximately one million atoms). To execute the MD simulation of such a large system, we used our program, psygene-G (Mashimo et al., 2013), which utilizes GPGPUs for acceleration of the non-bonded interactions, including the electrostatic computation by the ZD method. We performed 200-ns MD simulations for both the ADP and ATP models, and compared their structures and dynamics. The motions of the stalks in the two models were quite different. The elastic properties of the stalks were analyzed and compared with the experimental results.

Materials and methods
Computational system
Our target, cytoplasmic dynein, exists as a homodimer in vivo, but the monomer is the minimal functional unit (Roberts et al., 2013; Schiavo et al., 2013). We built a system consisting of a dynein monomer to reduce the computational costs. Two different models were prepared for MD simulations.

The ADP model, in which all four AAA+ modules, AAA1–AAA4, bind ADPs, was built from the high-resolution structures of D. discoideum dynein (Kon et al., 2012). The wild-type and ∆MTBD mutant structures (PDB IDs: 3 vkh and 3 vkg, respectively) were obtained from the Protein Data Bank (berman et al., 2007). Since the resolution of the ∆MTBD mutant structure (2.8 Å) is higher than that of the wild-type structure (3.8 Å) and the ∆MTBD mutant retains the ATPase activity, the structure of the ∆MTBD mutant including nucleotides was chosen as the initial model for the current MD simulations. The missing MTBD domain, residues Ala3372-Lys3495, was modeled from the wild-type structure. In order to include the effects of the missing charged residues, we built the atomic models for the other residues with electron densities that were not observed in the crystal structures, listed in Table I, by the spanner modeling server (Lis et al., 2011). The N- and C-termini were capped by acetyl and N-methyl groups, respectively. After the modeling, the system consists of 3320 residues (Glu1410–Asp4729), four ADP molecules and one magnesium ion (Fig. 1A). A rectangular water box with dimensions of 167 × 333 × 180 Å3 was placed around dynein. Sodium and chloride ions were added to neutralize the system. The sodium and chloride ions were added to the system, and to achieve a physiological concentration (100 mM). The computational system consists of 980 265 atoms (53 443 dynein atoms, 4 ADP molecules, 1 magnesium ion, 574 sodium ions, 528 chloride ions and 308 521 water molecules).

The ATP model, in which ATP is bound to AAA1 and the structure of dynein is exactly the same as the ADP model, was built from the
Table I. Domain names and residue numbers of *D. discoideum* dynein

| Domain name          | Residue number (initial) | Residue number (final) | Missing residues* |
|----------------------|--------------------------|------------------------|-------------------|
| Linker               | 1410                     | 1935                   | 1425–1449; 1620–1624 |
| AAA1                 | 1936                     | 2229                   | 2061–2063; 2170–2175 |
| AAA2                 | 2230                     | 2631                   | 2454–2487; 2631    |
| AAA3                 | 2632                     | 2948                   | 2632–2633          |
| AAA4 (N-terminal end)| 2949                     | 3262                   | 2958–2961; 2981–2986; 3042–3045; 3157–3160; 3212–3215 |
| Stalk (N-terminal helix) | 3263                   | 3371                   |                   |
| Stalk (MTBD)         | 3372                     | 3486                   |                   |
| Stalk (C-terminal helix) | 3487                   | 3596                   | 3492–3495         |
| AAA4 (C-terminal end) | 3597                     | 3638                   |                   |
| AAA5 (N-terminal end) | 3639                     | 3806                   | 3699–3703; 3725–3758 |
| Strut                | 3807                     | 3885                   |                   |
| AAA5 (C-terminal end) | 3886                     | 4114                   | 4114              |
| AAA6                 | 4115                     | 4411                   | 4115              |
| C-sequence           | 4412                     | 4729                   | 4440–4493; 4522–4530; 4582–4588; 4614–4634; 4674–4676 |

*Missing residues of each domain in the crystal structure are shown.

ADP model by adding the PO$_3$ group to the terminal oxygen atom of the ADP molecule. Then, the chloride ion and water molecule nearest to the PO$_3$ group of the ATP were removed. In total, 980 263 atoms (53 443 dynein atoms, 1 ATP molecule, 3 ADP molecules, 1 magnesium ion, 574 sodium ions, 527 chloride ions and 308 520 water molecules) were in the rectangular box.

The force field parameters for dynein, the other ions and water molecules were derived from our AMBER-based hybrid force field (Kamiya *et al.*, 2005), the halide monovalent ion parameters (Joung and Cheatham, 2008) and the flexible TIP3P water model (Jorgensen *et al.*, 1983), respectively, and the parameters for the nucleotides and the magnesium ion originated from the General AMBER Force Field (Wang *et al.*, 2004). The partial atomic charges of ADP and ATP were taken from the AMBER parameter database (http://www.pharmacy.manchester.ac.uk/bryce/amber).

**MD simulations**

All of the energy minimization calculations and MD simulations were performed by the psygene-G program (Mashimo *et al.*, 2013). The simulation protocols for the ADP and ATP models were the same. Conjugated gradient energy minimization was executed with position-al restraints on the heavy atoms of dynein, nucleotides and the magnesium ion, using a force constant of 10 kcal/mol Å$^2$, followed by minimization without these restraints. The system was equilibrated for 100 ps by adopting Berendsen’s NPT algorithm (Berendsen *et al.*, 1984), with the temperature set at 300 K, pressure under 1 bar and a time step of 0.5 fs. Here, the positions of the heavy atoms of dynein, nucleotides and the magnesium ion were restrained with a force constant of 10 kcal/mol Å$^2$. Electrostatic interactions were treated by the ZD method, with a cutoff distance of 12 Å and a damping factor α of 0.00 Å$^{-1}$. The cutoff distance of the van der Waals interactions was set to 12 Å. After the NPT simulations, the cell sizes of the ADP and ATP models were 166.2 × 329.4 × 176.1 and 165.9 × 333.7 × 175.0 Å$^3$, respectively. An NVT heating run from 0 to 300 K for 1 ns was performed with a time step of 1.0 fs. The SHAKE algorithm (Ryckaert *et al.*, 1977) was applied to constrain the covalent bonds between heavy and hydrogen atoms. Finally, the NVT production run at 300 K was performed for 200 ns with SHAKE and a time step of 1.0 fs for each model. The electrostatic and van der Waals treatments were the same as those of the NPT simulation. The structure trajectory was stored every 10 000 MD steps (10 ps). All of the productive runs were performed using the HA-PACS supercomputer system at Tsukuba University (http://www.ccs.tsukuba.ac.jp/eng/research-activities/projects/ha-pacs/). It took ~12 h to execute a 1-ns MD simulation in parallel with 64 CPUs and 64 GPGPUs.

**Analysis of elastic properties**

The stalk of dynein consists of a long coiled coil (~150 Å) and the MTBD. In the coiled coil, two α-helices are aligned with anti-parallel geometry (see Fig. 1B). The elastic properties of the coiled coil were analyzed by the same method used for the analysis of myosin II (Adamovic *et al.*, 2008). Here, the method is briefly summarized...
below. The elastic properties of the coiled coil are divided into the stretching stiffness (i.e. spring constant) \( (k_s) \) and the bending stiffness \( (k_b) \), which are given by the following equations:

\[
k_s = \frac{kT}{\langle \Delta L^2 \rangle} \quad (1)
\]

\[
k_b = \frac{kT}{\langle \Delta r^2 \rangle} \quad (2)
\]

Here, \( k, T, \Delta L, \Delta r \) and \( \langle \rangle \) are the Boltzmann constant, temperature \((= 300 \text{ K})\), fluctuations of the stalk in the parallel (stretching) direction, those in the perpendicular (bending) direction and the time average over the MD trajectory, respectively.

In the current study, the length of \( L \) was calculated as the average distance between selected pairs of \( \text{C}^\alpha \) atoms (Val3267–Gln3358, Ile3271–Glu3354, Leu3504–Val3593 and Ala3508–Val3589 in Fig. 1B), which are close to the N- and C-termini of the coiled coil.

The first principal axis of the moment of inertia for the stalk (solid line with arrow in Fig. 1B) was calculated from the X-ray structure (Kon et al., 2012). The atomic structures obtained during the MD trajectory were superimposed on those of the X-ray structure, and all of the heavy atoms other than the stalk were used for the superimposition. The value \( r \) was then calculated as the minimum distance from selected \( \text{C}^\alpha \) atoms to the principal axis, using the \( \text{C}^\alpha \) atoms of Glu3354, Gln3358, Leu3504 and Ala3508 close to the MTBD (Fig. 1B).

Since there is currently no experimental data available on the stretching stiffness of the stalk, we calculated the apparent stretching modulus \( (E) \), for comparison with that of another protein (i.e. myosin II), according to the following equation:

\[
E = \frac{<L>/kT}{\langle\langle\Delta L^2\rangle\rangle} \quad (3)
\]

Here, \( <L> \) is the average distance over the MD trajectory.

Fortunately, the bending stiffness of the stalk has been obtained from the analysis of the electron micrograph data (Burgess et al., 2003) and is 0.14 pN/nm in the ADP-vanadate configuration, and 0.47 pN/nm in the apo-state (Lindemann and Hunt, 2003). Here, the ADP-vanadate state is a model for the ADP.Pi state, which corresponds to the primed state, while the apo-state corresponds to the unprimed state. Therefore, it is possible for us to compare the bending stiffness of the ATP and ADP models from our simulation data with those of the ADP-vanadate and apo-states from the experimental data, respectively.

Results and discussion

To compare the thermodynamic stability between the ADP and ATP models, we analyzed the root-mean-square deviations (rmsds) of the backbone atoms along the 200-ns MD trajectory (Fig. 2). The rmsds of all backbone atoms for both models are shown in Fig. 2A. The rmsd of the ATP model fluctuated much more broadly between 4 and 20 Å than that of the ADP model. In contrast, the trajectories of the rmsds of the backbone atoms other than the stalk were similar in both models, where each of them reached a plateau within 40 ns, followed by fluctuations between 4.0 and 5.0 Å in the ADP model and 4.5 and 5.5 Å in the

![Fig. 2 The rmsds of (A) all backbone atoms and (B) backbone atoms other than the stalk, and (C) backbone atoms in AAA1 along the MD trajectory. (D) The distance between Pβ of ADP or ATP in AAA1 and Cζ of Arg2410 in AAA2 along the MD trajectory. Black and red lines represent the rmsd or the distance of the ADP and ATP models, respectively.](image-url)
ATP model (Fig. 2B). The result suggests that the stalk in the ATP model fluctuated broadly, even after the system was equilibrated. The dynamics of the AAA1 module itself were similar in both ADP and ATP models from Fig. 2C. However, the distances between the phosphate group of ATP bound to the AAA1 module and Arg2410 in the AAA2 module were much shorter in the ATP model than the corresponding distances in the unprimed ADP-bound model along the MD trajectories (Fig. 2D).

Next, to determine which residues moved during the initial stage of the trajectory (i.e. <40 ns), the rmsd of each residue with respect to the initial structure was calculated. In the AAA2 module, the modeled residues, which form the loop structure as shown in the missing residues column in Table I (i.e. 2454–2487), were flexible in both models (Fig. 3). The stalk of the ATP model moved in a broader conformational space than that of the ADP model at the beginning (<10 ns). The linker, strut and C-sequence of the ATP model began to deviate from the X-ray structure at 2, 27 and 8 ns, respectively (Fig. 3B), while those of the ADP model remained stable with similar structures to the initial ones (Fig. 3A). The magnitudes of these movements in the ATP model were, however, limited to the local area in comparison with those of the stalk.

The snapshot structures during the MD simulations, taken every 40 ns, are depicted in Fig. 4. While the stalk and the MTBD underwent significantly large motions in random directions in the ATP model, they slightly fluctuated around the initial structure in the ADP model. In the ATP model, the boundary region between the stalk and the MTBD, which formed a helical structure before the MD, adopted disordered conformations during the MD. Therefore, the ATP in AAA1 appeared to contribute to the large movement of the stalk in the ATP model. No other significant structural changes occurred in either model, and no dissociation of the nucleotides was observed. In order to confirm these characteristic dynamic properties, we performed further MD simulations, in which the ADP model was switched to the ATP model and vice versa, at the middle of the MD trajectory. As shown in Supplementary Fig. S1A and B, the dynamic properties changed from the ATP to the ADP model, and the ADP to the ATP model, respectively. It is evident that although the dynamic feature did not change after 44 ns switching from the ATP to the ADP model in Supplementary Fig. S1A, it dramatically changed in Supplementary Fig. S1B after switching from the ADP to the ATP model at 40 ns. Namely, the current study could simulate the initial procedure from the unprimed to the primed state in a reproducible manner.

In Fig. 5, the cross-correlation matrix elements are shown in the fluctuations of the unprimed ADP model (upper triangle) and those in the ATP model (lower triangle). Because the amplitudes of the structural fluctuation of the ATP model are much larger than those of the ADP model as shown in Figs 2–4, the absolute values of the cross-correlation matrix elements of the ATP model are also significantly larger than those of the ADP model. In addition, when the matrix elements between the two models are precisely compared, the following strong correlations are found:

(i) Strong negative correlations between N-terminal linker region and AAA1, and between the linker and C-sequence plus strut, as indicated by dotted circles a and b, respectively.
(ii) Positive correlation between AAA1 and AAA2 by c.
(iii) Strong negative and positive correlations between stalk (CC1, CC2 and MTBD) and both AAA2 and AAA3 by d.
(iv) Strong negative correlation between strut and both AAA2 and AAA3 by e.
(v) Negative correlation between strut and stalk by f.
(vi) Positive correlations between C-sequence and AAA1 plus AAA2 by g.
(vii) Strong positive correlation between C-sequence and strut by h.

These correlations seem to well support the mechanism underlying the motor actions of dynein proposed by Kon et al. (2012).

In fact, the correlation identified by a dotted circle c in Fig. 5 could correspond to the interaction between the bound ATP to AAA1 and the Arg2410 in AAA2 (see Fig. 2D). In order to dissect the interaction between the bound nucleotide, ADP or ATP, and the side chain of Arg2410 in AAA2, the shortest distances between the atom pairs of a negatively charged phosphate oxygen atom in ADP or ATP and a positively charged hydrogen atom in the guanidinium group of Arg2410 side chain are plotted during the MD simulations in Supplementary Fig. S2A. In the ADP model, the shortest distances between an ADP phosphate oxygen and the side chain of Arg2410 along the MD trajectory were large, separating the pairs far around 8–10 Å as shown in a snapshot at 200 ns (Supplementary Fig. S2B). On the contrary, in the ATP model, the shortest distances became very close, sometimes form-
As shown in the Materials and Methods section, the bending stiﬀnesses of the stalk corresponding to the ADP and ATP models have been experimentally obtained (Lindemann and Hunt, 2003). We estimated the bending stiﬀness from the MD data, for comparison with the experimental value. The distance from the principal axis of the moment of inertia (r) to each of the four atoms is shown in Fig. 7. The changes in the distance from the principal axis to the atoms in the CC1 helix (Glu3354 and Gln3358) and the CC2 helix (Leu3504 and Ala3508) clearly correlated in both models, indicating that these helices moved collectively in the direction perpendicular to the principal axis. Although the magnitude of the distance (∼50 Å) is similar in both models, the distance in the case of the ATP model (Fig. 7B)

### Table II. Stretching property of the stalk obtained from MD simulation

| Model   | Residue pair     | \(L_{\text{ini}}\) (Å) | \(L_{\text{ave}}\) (Å) | \(<(L - L_{\text{ave}})^2>\) (Å²) | Stiffness (pN/nm) | Apparent stretching modulus (pN) |
|---------|------------------|--------------------------|--------------------------|----------------------------------|-------------------|----------------------------------|
| ADP     | Val3267–Gln3358  | 134.0                    | 132.9                    | 3.0                              | 138.5             | 1855.9                           |
|         | Ile3271–Glu3354  | 121.8                    | 121.5                    | 2.6                              | 162.4             | 1978.0                           |
|         | Leu3504–Val3593  | 131.6                    | 129.7                    | 3.4                              | 120.9             | 1591.0                           |
|         | Ala3508–Val3589  | 120.6                    | 119.6                    | 1.9                              | 221.6             | 2672.5                           |
| ATP     | Val3267–Gln3358  | 134.0                    | 130.1                    | 12.8                             | 32.3              | 432.8                            |
|         | Ile3271–Glu3354  | 121.8                    | 118.8                    | 9.9                              | 41.7              | 507.9                            |
|         | Leu3504–Val3593  | 131.6                    | 124.8                    | 14.0                             | 29.7              | 390.9                            |
|         | Ala3508–Val3589  | 120.6                    | 115.8                    | 10.9                             | 37.9              | 457.1                            |

\(L_{\text{ini}}\) is the length of the stalk of the initial structure.
\(L_{\text{ave}}\) is the average length over from 40 to 200 ns trajectories.
\(<(L - L_{\text{ave}})^2>\) is the average deviation of the length over from 40 to 200 ns.

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**Fig. 6** The length of the stalk (L) calculated by the distances between four pairs of Cα atoms in (A) the ADP model and (B) the ATP model. The lengths of Val3267–Gln3358, Ile3271–Glu3354, Leu3504–Val3593 and Ala3508–Val3589 are shown by red, green, blue and purple lines, respectively.

**Fig. 7** The distance from the principal axis of the moment of inertia (r) to each of four Cα atoms in (A) the ADP model and (B) the ATP model. The distances to Gln3358, Glu3354, Leu3504 and Ala3508 are shown by red, green, blue and purple lines, respectively.
The similar coiled-coil structure in the myosin II s2 subdomain. The
lus derived from those values has a more
been previously reported, was estimated to be 160.9 pN/nm in the
analyzed using the trajectory. The stretching stiffness, which has not
stiffness of the stalk were
of the ADP model, based on analyses of the rmsds and the backbone
primed state. The stalk of the ATP model was more
for both models were executed, starting from the structure of the un-
bound in AAA1, 200-ns MD simulations of the dynein motor domain
with ADP bound in AAA1 to the initial transition state with ATP

Conclusion
To clarify the structural changes in dynein from the unprimed state
with ADP bound in AAA1 to the initial transition state with ATP
bound in AAA1, 200-ns MD simulations of the dynein motor domain
for both models were executed, starting from the structure of the un-
primed state. The stalk of the ATP model was more flexible than that
of the ADP model, based on analyses of the rmsds and the backbone
torsion angles. The stretching and bending stiffness of the stalk were
analyzed using the trajectory. The stretching stiffness, which has not
been previously reported, was estimated to be 160.9 pN/nm in the
ADP model and 35.4 pN/nm in the ATP model. The stretching modu-
lus derived from those values has a more flexible nature than that of
the similar coiled-coil structure in the myosin II s2 subdomain. The
bending stiffness was estimated to be 7.6 pN/nm in the ADP model
and 3.2 pN/nm in the ATP model, and these values qualitatively
agree with the experimental values. The simulation and analysis of
the stiffness in the present work should help to clarify the structure-
function relationships during the mechaanochemical cycle of dynein.

Supplementary data
Supplementary data are available at PEDS online

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| Model       | Residue       | $r_{ini}$ (Å) | $r_{av}$ (Å) | $<(r-r_{ini})^2>$ (Å²) | Stiffness (pN/nm) |
|------------|--------------|---------------|--------------|------------------------|-------------------|
| ADP model  | Glu3358      | 6.9           | 16.4         | 70.6                   | 5.9               |
|            | Glu3354      | 9.2           | 16.3         | 60.6                   | 6.8               |
|            | Leu3504      | 0.7           | 12.9         | 54.0                   | 7.7               |
|            | Ala3508      | 2.5           | 10.8         | 40.5                   | 10.2              |
| ATP model  | Glu3358      | 6.9           | 29.4         | 134.3                  | 3.1               |
|            | Glu3354      | 9.2           | 26.9         | 119.6                  | 3.5               |
|            | Leu3504      | 0.7           | 28.7         | 141.5                  | 2.9               |
|            | Ala3508      | 2.5           | 24.3         | 117.9                  | 3.5               |

$^{a}r_{ini}$ is the distance from the principal axis (see Fig. 1B) of the initial structure.
$^{b}r_{av}$ is the average distance over from 40 to 200 ns trajectories.
$^{c}<|r-r_{ini}|^2>$ is the average deviation of the distance over from 40 to 200 ns.

The distances from the principal axis of moment of inertia, average
distances, average deviations and bending stiffnesses are listed in
Table III. The average distance in the ATP model was approximately
twice that in the ADP model, and the deviation was also significantly
different.

The bending stiffnesses estimated from the average over the stiffness of
the four pairs were 7.6 pN/nm in the ADP model and 3.2 pN/nm in the
ATP model, indicating that the stalk of the ADP model was 2.4 times
stiffer than that of the ATP model. The experimental bending stiffness
was 0.14 pN/nm in the ADP–vanadate configuration and 0.47 pN/nm
in the apo-state (Lindemann and Hunt, 2003), which correspond to the
ATP and ADP models, respectively. The ratios of the stiffnesses between
the ADP and ATP models were 2.4 in our simulation and 3.4 in the ex-
periment, and these values agree well with each other. Thus, the ten-
dency of the stalk to become more flexible in the presence of ATP at
AAA1 seemed to be reproduced by our simulation.

The absolute value of the bending stiffness in our simulation was,
however, ~10 times higher than the experimental value. The reason
may be due to the fact that the current simulation time was too
short to capture any large-scale structural changes. Since the computa-
tional system consisting of about one million atoms is very big, a
simulation with a duration longer than 1 ms should be necessary to
see large-scale motion. In fact, the sliding of the helix of the stalk
and the structural change of the linker from the straight to bent con-
formation (Schmidt et al., 2015), upon binding the ATP molecule in
AAA1, were not observed during our simulation. Further simulation
studies should be required. However, the simulation and analysis of
the stiffness in the present work will surely help us to understand
the initial procedure of the structural change from the unprimed to
primed state during the mechaanochemical cycle of dynein.
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