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Molecular dynamics simulation for the reversed power stroke motion of a myosin subfragment-1

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ABSTRACT

Myosins are typical molecular motor proteins that convert the chemical energy from the ATP hydrolysis into mechanical work. The fundamental mechanism of this energy conversion is still unknown. To explain the experimental results already obtained, Masuda has proposed a hypothesis called the "Driven by Detachment" theory for the working principle of the myosins. This theory insists that the energy used during the power stroke of the myosins does not directly originate from the chemical energy of ATP, but is converted from the elastic energy within the molecule at the joint between the head and neck domains. One method for demonstrating the validity of this theory is a computational simulation using the molecular dynamics (MD) method. The MD software used was GROMACS. The target of the MD simulations was myosin subfragment-1 (S1), for which the initial structure was obtained from the Protein Data Bank entry 1M8Q. The AFM pull code of GROMACS was used to apply an external force of 17 pN at the end of the neck domain in the direction opposite to the power stroke to observe whether the myosin S1 takes the pre-power stroke conformation. The residues assumed to be engaged in the docking with an actin filament were fixed to the space. Starting from exactly the same initial position, 10 simulations were repeated by varying the random seeds for generating the initial velocities of the atoms. After 64 ns of calculations, the myosin S1 took the conformation of the pre-power stroke state in which the neck domain was bent around the joint between the head and the neck domains. This result agrees with the prediction expected by the DbD theory, the validity of which may be established by conducting similar simulations for the other steps of the myosin working processes.

1. Introduction

Myosins are typical molecular motor proteins that convert the chemical energy from the ATP hydrolysis into a variety of cellular mechanical work including muscle contraction (Geeves and Holmes, 1999; Berg et al., 2001). Myosins, in general, consist of head, neck and tail domains (Rayment et al., 1993; Geeves, 2002). The head domain contains both the actin-binding and the nucleotide-binding sites. The neck domain, to which up to six light chains bind, connects the head to the tail domain. The protein complex consisting of the head domain, the neck domain and the light chains is called myosin subfragment-1 (S1) and is sufficient for force production (Yanagida, 1985; Geeves and Holmes, 1999).

The fundamental mechanism of the chemical to mechanical energy conversion in molecular motors is still unknown in spite of the long history of research using various experimental methods. Currently, the most widely accepted theory is the "lever arm hypothesis" (Geeves and Holmes, 1999; Geeves, 2002), but the experimental results using the method of a single molecule observation and manipulation revealed serious problems with this theory (Yanagida et al., 1985). An alternative theory, mainly proposed by Yanagida and his coworkers, is the biased Brownian ratchet model (Ishijima et al., 1998; Nishiyama et al., 2003; Shimokawa et al., 2003; Buonocore et al., 2005; Esaki et al., 2007), which also has some fundamental problems (Masuda, 2013).

Under these circumstances, Masuda (2003, 2008, 2009, 2013) proposed a simple theory called the "Driven by Detachment (DbD)" theory to explain the experimental results so far obtained from various types of myosins. Based on this theory, the chemical energy from the ATP hydrolysis does not directly contribute to the mechanical work of the power stroke, but is used for the detachment of a myosin molecule from an actin filament. After returning to the detached state, a myosin molecule is again attracted to an actin filament and has potential energy originating from this attractive force. This potential energy is converted into the elastic energy at the joint between the head and the neck

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domains during the docking process, and the elastic energy is finally used for the power stroke.

Based on the DbD theory, every step in the myosin working processes, except for the ATP hydrolysis step, is explained by the intermolecular potential and the intramolecular elasticity, and, therefore, can be simulated using the molecular dynamics (MD) method. Masuda (2013) has already conducted MD simulations for the docking step of a myosin S1 against an actin filament, and successfully reproduced the rigor docking state of an actomyosin complex.

To convert the intermolecular potential energy into the intramolecular elastic energy, a myosin S1 should be docked to an actin filament by rotating the neck domain and generating an intramolecular distortion. However, a preliminary study showed that this simulation took a very long calculation time (Masuda 2013). Therefore, in the present study, instead of directly producing the pre-power stroke conformation of a myosin S1, the time course was reversed and, starting from the post-power stroke conformation, an external force was applied at the end of the neck domain to observe whether or not the myosin S1 took the pre-power stroke conformation.

2. Methods

A myosin S1 structure extracted from the Protein Data Bank (PDB) entry 1M8Q (Chen et al., 2002) was used as the initial atomic coordinates for the MD simulation. The chains used were D, E and F in the 1M8Q, which represented the myosin heavy chain, the regulatory light chain and the essential light chain, respectively.

GROMACS 4.6 (van der Spoel et al., 2005; Hess et al., 2008) was chosen as the MD simulation software, because the computation speed of this software is fast and the source code is freely available. The AFM pull code of GROMACS was used to apply a constant external force of 17 pN at the end of the neck domain in the direction opposite to that of the power stroke, that is, to the minus end direction of the actin filament. During the MD simulations, the myosin residues ILE^{520} – ASN^{560} assumed to be engaged in the docking with the actin filament (Milligan, 1996) were fixed in the space.

The parameters used for the MD simulations are summarized in Table 1. These parameters were the standard ones for proteins in general and not specific to the present study. The force field was GROMOS96 43a1, and the water model was SPC (simple point charge). The box for the MD simulations was 17 nm × 15 nm × 17 nm in size, which was sufficiently large for placing the myosin S1 with enough margins. After placing the myosin S1, this computation box was filled with water molecules, and then Na ions were added to make their concentration 140 mM. Finally, Cl ions were added to make the total electrical charge neutral. The resultant number of all the atoms for the myosin, water and ions became 426,578.

Table 1
List of parameters used for molecular dynamics simulations of the myosin subfragment-1.

Parameter name	Value
Force field	GROMOS96 43a1
Water model	SPC
Box size [nm]	17.0 × 15.0 × 17.0
Time step [fs]	2
nstlist	10
ns_type	Grid
Coulomb type	PME
Fourier spacing	0.15
pme_order	4
Rlist	0.9
Rcoulomb	0.9
Rvdw	1.0
Temperature coupling	V-rescale
Temperature [K]	300
Tau_t	0.1
Pressure coupling	No
Pull	Constant force
Pull_geometry	Direction
Pull_k1 [kJ/(mol × nm)]	10

The time step for the calculations was 2 fs, and the total calculation time was 64 ns. The atomic coordinates were recorded every 0.4 ns. The temperature was set at 300 K, and no pressure control was applied. Starting from exactly the same initial position, 10 simulations were repeated by varying the random seeds for generating the initial velocities of the atoms.

The calculations were carried out using a personal computer (MacPro Apple, Cupertino, CA, USA) equipped with dual Intel 6-core Xeon processors (2.66 GHz). The conformation of the myosin was displayed using Visual Molecular Dynamics (VMD 1.8.7) software (Humphrey et al., 1996). The screen images were captured to create the pictures shown in Fig. 2. To quantitatively analyze the structural changes in the myosin S1, the coordinates of the CA atom in the residue at the end of the neck domain were calculated. Furthermore, to investigate the contribution of the conformational changes within the neck domain to the displacement of the neck end, the bending angle of the long helix consisting of the neck domain of the myosin heavy chain was also calculated.

3. Results

The displacements of the CA atom at the end of the neck domain are shown in Fig. 1(A) for the 10 simulations. The displacement was set 0 nm for the initial position. Out of the 10 calculations, the result with the smallest displacement is shown by the thick gray line, while that with the greatest displacement is shown by the thick black line. The remaining trials are indicated by the thin black lines. At the end of the calculations for 64 ns, the smallest displacement was 8.5 nm, while the highest one was 13.8 nm, which was about 1.6 times greater than the smallest value.

Fig. 1(B) shows the average and standard deviation of the displacements for the 10 calculations. On average, the displacement of the neck end was 10.1 nm. The average displacement increased with time, but the rate of the increase became gradually lower. It is unclear whether the displacement had reached equilibrium after 64 ns. The standard deviation of the displacements among the 10 calculations was 1.2 nm at 36 ns and was relatively low compared with that beyond 36 ns. At 64 ns, the standard deviation became 2.3 nm, which was about 2 times greater than the value at 36 ns and was about 23% of the average displacement of 10.1 nm.

The initial structure of the myosin S1 is shown in Fig. 2(A), in which the neck domain was in the post-power stroke conformation and extended straight against the head domain. Two representative structures after the MD simulations for 64 ns are shown in Fig. 2(B) and (C). The structure in Fig. 2(B) is the result that demonstrated the smallest displacement of the neck end, while that in Fig. 2(C) showed the greatest displacement. The other 8 results showed intermediate structures between the ones shown in Fig. 2(B) and (C).

In Fig. 2(B), the neck domain is slightly bent around the joint between the neck and head domains and moved toward the pre-power stroke position. In Fig. 2(C), the neck domain is bent over 90 degrees around the joint and demonstrated the typical pre-power stroke conformation.

As shown in Fig. 2(B), the displacement of the neck end was mainly caused by the rotation around the joint between the neck and head domains, but it may also be a result of the bending within the neck domain (Fig. 2 (C)). Because the neck domain of the myosin heavy chain is a long helix of amino acids, three residues