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Dynamic conformational changes due to the ATP hydrolysis in the motor domain of myosin: 10-ns molecular dynamics simulations

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ABSTRACT

Muscle contraction is caused by directed movement of myosin heads along actin filaments. This movement is triggered by ATP hydrolysis, which occurs within the motor domain of myosin. The mechanism for this intramolecular process remains unknown owing to a lack of ways to observe the detailed motions of each atom in the myosin molecule. We carried out 10-ns all-atom molecular dynamics simulations to investigate the types of dynamic conformational changes produced in the motor domain by the energy released from ATP hydrolysis. The results revealed that the thermal fluctuations modulated by perturbation of ATP hydrolysis are biased in one direction that is relevant to directed movement of the myosin head along the actin filament.

1. Introduction

Muscle contraction is caused by movement of myosin heads along actin filaments. The movement is driven by cyclic interactions between myosin and actin molecules caused by ATP hydrolysis, which occurs in the nucleotide active pocket within the myosin motor domain. For the process of cyclic myosin–actin interactions, several intermediate states and conformational changes have been presented on the basis of X-ray structure analyses [1–6], cryo-electron-microscopy [7], electron paramagnetic resonance (EPR) spectra [8], and mutational studies [9] of myosin heads complexed with nucleotide analogues. The following model for the cyclic process has been argued: association of ATP molecule with the myosin head, weakening of the myosin–actin binding, hydrolysis of ATP, release of ATP hydrolysis products through the back door of the myosin head, conformational changes in the motor domain including the junction between the lever arm and the myosin head, creation of the power stroke and a return to strong myosin–actin binding. Of these steps, bending of the junction between the lever arm and the head induced by ATP hydrolysis [2,3,5] is crucial for global movement of myosin along actin filament, but its mechanism still remains unknown. Furthermore, a kind of reversely-directed myosin motor, called myosin VI, is reported [10] and what determines the direction of movement of the myosin motor is of great importance.

On the other hand, single molecule observations by means of fluorescence microscopy revealed that the movement of the myosin head relative to the actin filament following hydrolysis of a single ATP consists of multiple random steps that are not always in the forward direction [11,12]. Furthermore, simultaneous measurements of the nucleotide release and the mechanical reaction of the myosin head showed that the reaction was not directly coupled with the nucleotide release [13].

To fully understand how a small chemical trigger at the nucleotide active site induces the mechanical reaction, it is desirable to examine the intramolecular processes following ATP hydrolysis in the myosin head. At present, however, there are no experimental means for observing the dynamic behaviors of each atom in a molecule. Molecular dynamics (MD) simulations provide us with information regarding both the static stability and the dynamic flexibility of subdomain conformations of a protein. In recent years, several MD simulations of myosin heads have been performed and are becoming complementary to experimental studies. Higo et al. [14] derived collective modes for subfragment-1 S1 of chicken skeletal myosin from MD trajectories of a backbone model and found large flipping motions of the α-helical C-terminal tail of S1 as the first and second largest amplitude collective modes, which are perpendicular to each other. Minehardt et al. [15] examined the molecular mechanism of ATP hydrolysis in the nucleotide-binding pocket by means of classical molecular mechanics (MM) simulations and quantum mechanical (QM) structural relaxations, and investigated the extent to which the P-loop, Switch I, and Switch II are involved in hydrolysis. MD simulations performed by Lawson et al. [16] revealed that relaxation

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of ADP and free Pi from their initial position reduced the diameter of the back door via motion of Switch I and Switch II located in the upper and lower 50-kDa subdomains, respectively. Li and Cui [17] analyzed the functional motions of myosin using the block normal approach method and suggested that the collective displacement mainly concerns the converter region, whereas the local rearrangement involves the salt-bridge region between Switch I and Switch II. Zheng and Doniach [18] carried out normal mode analyses of the deviations of all Cα atomic coordinates induced by a local displacement of atoms in the nucleotide-binding pocket using an elastic network model and revealed that the displacements of atoms for myosin and F1-ATPase could be expressed in terms of one or two dominant lowest-frequency modes and their amplitudes were in good agreement with the measured ones. Navizet et al. [19] calculated B-factors as functions of the residue number for three conformations (near-rigor, detached and transition states) of the myosin head, containing the motor domain and the lever arm, with a coarse-grained elastic network model; and their results identified rigid and flexible domains within the myosin structure and highlighted the respective roles of the light chains and nucleotide binding. Takagi and Kikuchi [20] investigated the structural relaxation of the myosin motor domain from the pre-power stroke state to the near-rigor state in connection with the nucleotide release using MD simulations of a coarse-grained Go-like model and found that the myosin motor does not relax to the near-rigor conformation before the nucleotide dissociates. Koppole et al. [21] carried out MD simulations of *Dictyostelium discoideum* Myosin II motor crystallized with various ATP analogues and revealed that residue Asn475 in the Relay helix was pushed away from Switch II upon ATP hydrolysis and this sensing allowed the power stroke to start upon initial binding to actin. Liu et al. [22] performed an MD study of detailed residue-wise interactions involved in the binding of myosin to actin in the absence of nucleotide using the crystal structure of chicken skeletal myosin. They found that the 50-kDa/20-kDa loop (Loop 2) is in a conformation stabilized with internal salt bridges, and further revealed that the Cardiomyopathy loop forms interprotein salt bridges with actin monomers, whereas its Arg 405 residue, the mutation site associated with the hypertrophic Cardiomyopathy, forms an internal strong double salt bridges with Glu 605 of S1. In a previous paper [23], we examined how the energy released by ATP hydrolysis at the nucleotide-binding site expands throughout the motor domain, using 1-ns all-atom MD simulations of the myosin head at 300 K and found that intramolecular collective modes were stimulated at the actin-binding site and the junction with the neck. However, the run time of 1-ns was too short compared with the experimental time resolution. In the present study, we extended the run time of MD simulations to 10 ns and examined how ATP hydrolysis gives rise to dynamic conformational changes including fluctuations in the overall myosin motor domain.

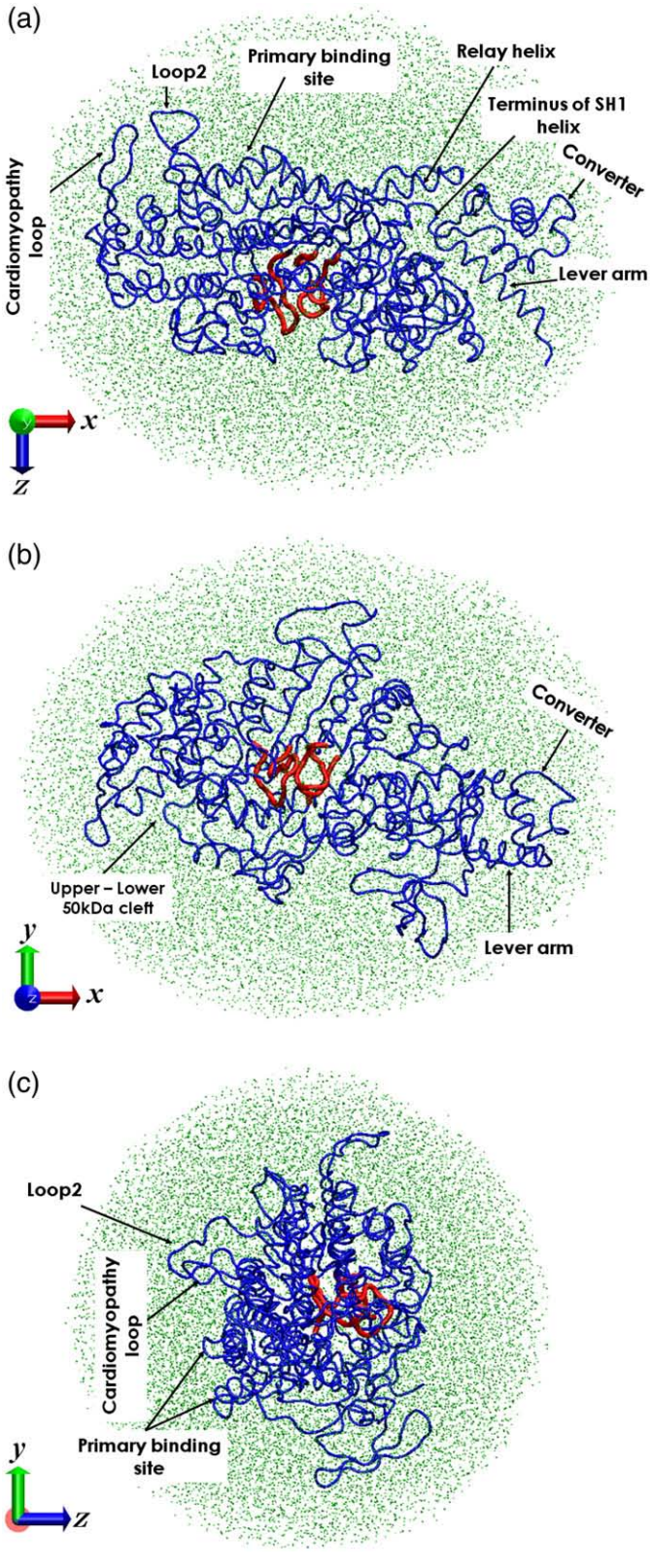

Fig. 1. Top (a), side (b) and front (from the actin side) (c) views of the backbone structure of the motor domain of myosin solvated with a spheroidal water droplet speckled in green. The ATP hydrolysis energy is distributed to the nucleotide-binding site shown in red. The conventional names of several parts of the myosin motor are included for convenience. The Cardiomyopathy loop, Loop 2 and Primary binding site are regions that directly interact with actin molecules. The converter and the lever arm constitute a tail, which links to the myosin filament. The upper–lower 50-kDa cleft is the exit for ATP hydrolysis products. The Relay helix is a signal transmission path from the nucleotide active site to the Primary binding site on one side and to the converter through the SH1 helix on the other side. It is noticeable that a terminus of the SH1 helix, which appears as a curved loop in the top view, is located between the head and the tail of the motor domain and seems to be a hinge joint connecting them.

2. Methods

The initial process in the MD simulations was the same as that in our previous study using a run time of 1 ns [23]. We truncated the motor domain (N-terminal 785 residues) from the structural data for nucleotide-free subfragment-1 S1 of scallop myosin (PDB; 1kk7, M. Himmel et al.) and added 34 missing residues (1–4, 23–25, 204, 211, 212, 365, 366, 406, 407, 451, 628–642 and 730–733) in the original data using the similar method explained by Lawson et al. [16] as follows. We built the missing parts by linking one resolved residue to the other resolved one with the missing residues using the Monte Carlo method. In this process the position of the alpha carbon atoms of the missing residues