Molecular Structural Analysis of Actomyosin to Characterize the Motor Protein System*

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Muscle contraction results from the relative sliding motion between thick myosin and thin actin filaments. Actomyosin is a molecular machine that converts chemical energy produced by the hydrolysis of ATP into kinetic energy. The investigation of the contractile mechanism of muscles at the atomic/molecular level was motivated by the determination of the structures of actin and myosin head S1 monomers by X-ray diffraction analyses. In order to clarify microscopic kinetic function and material heterogeneity, the molecular structural analysis of actomyosin was carried out using the molecular mechanics simulation code “AMBER”. The 3-D molecular structures of actomyosin employ three models which consist of three kinds of myosin head S1 (with ATP, with ADP, and without nucleotide) and F-actin itself to reveal the fundamental micromechanism of activation in the motility assay. The minimum-energy conformations of actomyosin in the three models were determined from molecular mechanics analyses. The differences in atomic coordinates and potential energy distributions show the existence of local packing and microstructural heterogeneity. Then, molecular fluctuations were studied by molecular dynamics analysis. The fluctuations reveal the dynamic properties at the atomic level and the possibility of change in the mesoscale structure as well as the emergence of the sliding motion of the entire molecule.

**Key Words**: Muscle and Skeleton, Biomaterial, Biomechanics, Computer-Aided Analysis, Molecular Dynamics, Actomyosin, ATP, ADP, Minimum-Energy Conformation, Fluctuation

1. Introduction

The contraction of skeletal muscle occurs due to the relative sliding motion between thick myosin and thin actin filaments. Investigation of the contractile mechanism of muscles at the atomic/molecular level was initiated in the beginning of the 1990s, after the molecular structure of an actin monomer was determined experimentally by Holmes et al. in 1990(1), and the myosin head subfragment-1 (S1) by Raymond et al. in 1993(2). Using molecular structural data determined by X-ray crystallography, we elucidated the static and dynamic characteristics of molecular structure by molecular mechanics (MM) and molecular dynamics (MD) analyses(3)-(6). The characteristics of microstructural heterogeneity and atomic fluctuation were obtained using the MM simulation code “AMBER”(7)(8). First, the minimum-energy conformations of actomyosin in three models were determined by the MM analysis. The three models corresponded to myosin head S1 with adenosine triphosphate (ATP), that with adenosine diphosphate (ADP), and that without nucleotide. It was revealed that myosin head S1 with ATP has the highest fluctuation at a specified site, which might be related to the fundamental micromechanism of activation in the motility assay. In the MM analysis, the potential energy distributions showed the existence of local packing and heterogeneous characteristics. From the MD analysis, it was suggested that a specific atom is activated as r.m.s. fluctuation increases, and macrostructural vibration is induced, which generates the relative sliding motion.

2. Molecular Structural Analysis(9)

2.1 Molecular mechanics analysis

AMBER(7)(8), which Kollman and coworkers developed, is used in the MM analysis. Total conformation
energy $V$ is defined as the sum of each energy term as follows:

$$V = V_r + V_\theta + V_\phi + V_{et} + V_{dw} + V_{hos}.$$  (1)

Each of the terms on the right-hand side of Eq. (1) is expressed as follows:

**Bond Potential:**

$$V_r = \sum_i \frac{1}{2} k_r (r_i - r_{eq})^2$$  (2)

**Bond Angle Potential:**

$$V_\theta = \sum_i \frac{1}{2} k_\theta (\theta_i - \theta_{eq})^2$$  (3)

**Torsion Potential:**

$$V_\phi = \sum_i \frac{1}{2} k_\phi \{1 + \cos(n \phi_i + \gamma)\}$$  (4)

**Electrostatic Potential:**

$$V_{el} = \sum_i \frac{q_i q_j}{4\pi \varepsilon r_{ij}}$$  (5)

**Van der Waals Potential:**

$$V_{dw} = \sum_i \left( \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^6} \right)$$  (6)

**Hydrogen Bond Potential:**

$$V_{hos} = \sum_i \left( \frac{C_{ij}}{r_{ij}^{4}} - \frac{D_{ij}}{r_{ij}^{8}} \right)$$  (7)

where $r$, $\theta$, $\phi$, and $r_{eq}$ represent covalent interatomic distance, bond angle, dihedral angle and noncovalent interatomic distance, respectively. The coefficients $A_{ij}$, $B_{ij}$, $C_{ij}$ and $D_{ij}$ are obtained from X-ray diffraction analysis, and $k_r$ and $k_\theta$ are obtained from normal mode analysis. Those values are understood to be Kollman's empirical values\(^{(7,8)}\). Moreover, $q_i$ and $q_j$ represent the charge of each atom, and $\varepsilon$ represents the dielectric constant that depends on the distance.

We use a combination of the steepest descent method at the initial stage of calculation and the conjugate gradient method at the final stage to determine the minimum-energy conformations.

### 2.2 Molecular dynamics analysis

The equation of motion for a system of $N$ molecules, interacting via a potential energy function $V$, is derived as follows:

$$\dot{r}_i = -\frac{1}{m_i} F_i$$  (8)

$$F_i = -\frac{d}{dr_i} V(r_i, r_2, \ldots, r_N)$$  (9)

where $r_i$ is the relative position vector. The leapfrog method i.e., the modified Verlet method, is employed to solve Eq. (8) in “AMBER”. In this method, firstly, the velocities at time $t + h/2$ are calculated using

$$\nu_i(t+h/2) = \nu_i(t-h/2) + \frac{1}{m_i} F_i(t) h$$  (10)

and the equation for advancement in position is as follows:

$$r_i(t+h) = r_i(t) + \nu_i(t+h/2) h.$$  (11)

Using Eq. (10), the velocities at time $t$ are then computed as

$$\nu_i(t) = \frac{1}{2} [\nu_i(t+h/2) + \nu_i(t-h/2)].$$  (12)

### 3. Numerical Results

#### 3.1 Minimum-energy conformation

The 3-D molecular structures of the actomyosin system were determined by combining F-actin (fourteen actin monomers were helically assembled) and S1 with ATP, with ADP and without nucleotide (Fig. 1). The binding sites between actin and myosin head S1 were determined on the basis of the molecular structures of the actin monomer by Holmes \textit{et al.} in 1990\(^{(13,19)}\) and the myosin head S1 by Rayment \textit{et al.} in 1993\(^{(20,21)}\). For the analytical model, two light chains of S1 were removed. ATP (35 atoms) and ADP (31 atoms) binding sites with S1 were determined by employing the methods proposed by Yount \textit{et al.}\(^{(12)}\). Those methods were developed on the basis of the topological adaptation under ATPase activation in adenylate kinase. In order to reduce the degree of freedom and the number of atomic potential parameters, a united atom model was used, which dealt with atomic groups CHN as a single atom. The total number of actomyosin atoms became 58,521 (6,051 residues).

The model of the actomyosin system employed here and the determination of the minimum-energy conformation are discussed briefly. First, the

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**Fig. 1** Initial structure of ACM
minimum-energy conformations of actin and a single myosin were determined by employing the initial prediction of the molecular structure\cite{16,17} obtained from X-ray crystallography of the freeze-dried molecule\cite{18,19,20}. Next, three kinds of actomyosin models, actomyosin with ATP, that with ADP and that without nucleotide, were constructed. MM analyses revealed the minimum-energy conformations. It was considered that the minimum point converged on the global minimum point, because the initial prediction of the structure was determined by experimental observation. In this analysis, structural analyses were entirely executed in vacuum, regardless of the solvent used, such as water. Our simulation was aimed at investigating the structural characteristics peculiar to the actomyosin system. An elucidation of the emerging mechanism of kinetic function during muscle contraction could be found by analyzing the structural characteristics of three states (actomyosin) → (actomyosin + ATP) → (actomyosin + ADP, Pi) in the biochemical process of ATP hydrolysis. Here, actomyosin, actomyosin with ATP and actomyosin with ADP were denoted simply by ACM, ACM·ATP and ACM·ADP, respectively. The minimum-energy conformations are shown in Figs. 2 to 4. The ACM·ATP model shows that the cleft opened clearly as in Fig. 3. This structural change may correspond to biochemical activation, which is observed in the
experiment. On the other hand, ACM-ADP and ACM models show the cleft closing.

3.2 Potential energy distribution and structural characteristics

The potential energy distributions of amino acid residues in the three models were determined by MM and the results are shown in Figs. 5 to 10. Figures 5 to 7 show the cases of S1, and Figs. 8 to 10, the cases of F-actin. S1 and F-actin have several peaks with remarkably high potential energy. Energy localization and atomic packing may cause microstructural instability.

In the three models, common residues, such as Phe458, Phe459, or Phe470, show high-energy peaks which are similar to the case of the S1 monomer\(^{19}\): this means that these residues of S1 are not affected by the combination with F-actin. The number following

Fig. 5 Potential energy distributions of S1 in ACM

Fig. 8 Potential energy distributions of F-actin in ACM

Fig. 6 Potential energy distributions of S1 in ACM-ATP

Fig. 9 Potential energy distributions of F-actin in ACM-ATP

Fig. 7 Potential energy distributions of S1 in ACM-ADP

Fig. 10 Potential energy distributions of F-actin in ACM-ADP
the name of the residue, such as 458 of Phe458, shows the order of the primary arrangement of protein molecules. The binding of ATP with S1 generates new energy peaks at residues Val126 ($6.48 \times 10^{-18}$ J) and Pro677 ($4.20 \times 10^{-18}$ J) near the binding site, as shown in Fig. 6.

However, when ADP combines with S1, the energy peaks in these sites is canceled. It suggests the local increase in fluctuation and atomic activation. Ile341 of each G-actin, which has 375 residues, shows an energy peak ($6.7 \times 10^{-18}$ J) periodically, as shown in Fig. 8. The different values of the energy peaks for residues such as Pro32 ($5.2 \times 10^{-18}$ J), Asp56 ($5.5 \times 10^{-18}$ J), Phe223 ($10.5 \times 10^{-18}$ J), or Glu226 ($5.9 \times 10^{-18}$ J), are observed in the case of ACM. Phe233 in six of fourteen G-actins has the highest energy peak. This suggests that each G-actin plays a different role and has heterogeneous characteristics.

On the other hand, as shown in Fig. 9, when ATP combines with S1, there occurs a periodicity of energy peaks with the same values. These residues are Pro32 ($8.0 \times 10^{-18}$ J), Phe223 ($10.5 \times 10^{-18}$ J), Glu226 ($5.8 \times 10^{-18}$ J) and Ile341 ($6.7 \times 10^{-18}$ J). On the other hand, the potential energies of Phe223 and Glu226 of the 7th G-actin molecule that combined with S1 were low. This means that the atomic packing in the 7th G-actin molecule might be relaxed. ACM•ADP shows the recovery of the high-energy peaks for residues Phe223 and Glu226 of the 7th G-actin molecule, which are low in the case of ACM•ATP. The complete periodicity of the energy peak is obtained. It can be concluded that ATP generates a specific energy peak in S1 molecules and the periodic energy peak in F-actin filament, except for the peak drop at the particular G-actin molecule which combines with S1.

3.3 Numerical results of molecular dynamics analysis

Here the MD simulations for ACM, ACM•ATP and ACM•ADP were carried out to evaluate kinetic function, which is related to the motility assay of the actomyosin system. We adapted the minimum-energy conformations as the initial state, the incremental time step 1 fs, and the total time 100 ps. As the molecular models, a vacuum environment and the constraint–free condition were employed, similar to MM analyses. Figures 11 to 13 show G-actin and S1 molecules at the calculation step of 100 ps to investigate the change in molecular structure. ACM•ATP and ACM•ADP show clearly the selected binding domains, as indicated in Figs. 12 and 13. On the other hand, Fig. 11 shows that the selected domains of S1 for
combination with the actin filament disappear. It is also observed that almost all atoms of ACM vibrate randomly, which means uniform fluctuation in all domains. Because of the constraint-free condition, the tail part of S1 changes its structure markedly, by twisting or bending. It is confirmed that the influence of this tail part on the main body of S1 can be neglected. In the case of ACM·ATP, two selected domains approach the G-actin binding sites. These residues found in the selected domains are Arg95·Pro98 and Asp1·Glu4 in the 5th G-actin molecule of the F-actin filament, and Ile508·Trp510 and Val753·Gln757 of S1. The two binding sites obtained in our simulation were also reported by Holmes et al.\textsuperscript{[10]} and Rayment et al.\textsuperscript{[12]} It can be postulated that this molecular activation in the two specific interacting sites generates a large-scale structural fluctuation and motivates the relative sliding between S1 and F-actin filament. Figure 12 shows the cleft opening. MD results of ACM·ATP reveal a decrease in the atomic-scale fluctuation and an increase in the vibration of selected domains. It is concluded that this large-scale structural vibration in the selected domain of ACM·ATP, such as opening and closing of the cleft, is related to the partial charge and increase in long-range forces.

Figure 13 shows the MD results of ACM·ADP. Similar results concerning the two binding sites between S1 and G-actins to ACM·ATP were obtained, but the cleft was closed. Random atomic fluctuation in all domains increases as the macrostructural vibration of S1 becomes smaller than the case of ACM·ATP. This may mean that ADP can not induce the movement of an electric charge, due to a large vibration.

3.4 Investigation of the arrangement of S1

MM and MD analyses were executed by setting S1 in sequential positions along the longitudinal axis of F-actin, such as 1, 2, or 3 nm away from the binding position observed by experiment.\textsuperscript{[13]]}\textsuperscript{[14]}. We studied when and how S1 and F-actin molecules are activated to induce the relative sliding motion. When the S1 molecule moves away from the binding position, the energy packing at residues Val126 and Pro677 disappears and the opening action of the cleft is inactivated. It is confirmed that it is only when ACM·ATP locates at the binding site are macrostructural fluctuation and high kinetic function obtained.

4. Conclusion

In this study, MM and MD analyses were carried out to study the fundamental micromechanism of activation in the actomyosin motility assay. MM results showed that when ATP combines with S1, the potential energy peak at a specific residue of S1 occurs and the periodic energy peaks in the F-actin filament, except the 7th G-actin molecule, also appear. The energy peak corresponds to atomic packing and the possibility of the increase in atomic fluctuation. MD analyses revealed that ATP binding causes large vibrations in specific domains, such as two binding sites between S1 and two G-actins, or the cleft of S1. The atomic fluctuation in all domains of the actomyosin system decreases, but the macroscopic vibration increases when ATP combines with S1, which might induce the relative sliding motion.

In the future, we will investigate the entire sliding process of the actomyosin system by employing quantum biological simulations.

Acknowledgements

The authors would like to thank Mr. Hiroyuki Shimotsu of Bando Chemical Industries, Ltd., for his help. This research was supported by a Grant-in-Aid for Scientific Research given to “Development of Evaluation System by Analyzing Molecular Mechanical Adaptation Function of Biomimetics Material” (No. 08555029) from the Ministry of Education, Science, Sports and Culture of Japan.

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