論文の内容の要旨

論文題目

Actin Stability and Dynamics Investigated by Molecular Dynamics Simulations

（アクチン安定性及び動態解析の分子動力学シミュレーション）

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In this study, I investigated the protein stability and dynamics of globular (G-) and filamentous (F-) actins. Actin is a protein responsible for numerous cellular functions. One of the main components of muscle fiber is actin, which plays many important roles in both muscle and non-muscle cells. I performed molecular dynamics simulations to analyze actin properties and mechanisms.

In the study of protein stability, the pressure tolerance of monomeric actin from the deep-sea fish *Coryphaenoides armatus* and *C. yaquinae* was compared to that of non-deep-sea fish *C. acrolepis*, carp, and rabbit/human/chicken actins using the simulations at 0.1 and 60 MPa. The amino acid sequences of actins are highly conserved across a variety of species. The actins from *C. armatus* and *C. yaquinae* have the specific substitutions Q137K/V54A and Q137K/L67P, respectively, relative to *C. acrolepis*, and are pressure tolerant to depths of at least 6000 m. At high pressure, I observed significant changes in the salt bridge patterns in deep-sea fish G-actins, and these changes are expected to stabilize ATP binding and subdomain arrangement. Salt bridges between ATP and K137, formed in deep-sea fish actins, are expected to stabilize ATP binding even at high pressure. At high pressure, deep-sea fish actins also formed a greater total number of salt bridges than non-deep-sea fish actins owing to the formation of inter-helix/strand and inter-subdomain salt bridges. Free energy analysis suggests that deep-sea fish actins are stabilized to a greater degree by the conformational energy decrease associated with pressure effect (Table 1).

Table 1. Energy differences between 60 and 0.1 MPa.

Label	ΔE_{conf}	$\Delta\Delta\mu$	$T\Delta S$	ΔG	$\Delta\Delta G$
Rab	−59 ± 53	520 ± 32	18 ± 18	444 ± 57	−135
Ac1W	11 ± 103	532 ± 14	16 ± 26	527 ± 122	−51
Ac1Q	16 ± 85	575 ± 43	13 ± 15	579 ± 65	0
Ac2	−52 ± 127	512 ± 29	19 ± 24	441 ± 112	−138
Arm	**−147 ± 67**	**510 ± 22**	**29 ± 20**	**334 ± 69**	**−244**
Yaq	**−153 ± 92**	**535 ± 25**	**30 ± 11**	**352 ± 77**	**−226**

Unit: kcal/mol. Rabbit actin is labelled as Rab. Actin 1 and 2a of the non-deep-sea fish species *C. acrolepis* are labelled as Ac1 and Ac2, respectively. I obtained two distinct Mg^{2+} coordination patterns in Ac1: 1) coordination by four water molecules and two γ-oxygen atoms of ATP (Ac1W), and 2) coordination by three water molecules, two γ-oxygen atoms of ATP, and a Q137 side-chain oxygen atom (Ac1Q). Actin 2b of the deep-sea fish species *C. armatus* and *C. yaquinae* are referred to as Arm and Yaq, respectively. E_{conf}, $\Delta\mu$, and S represent the solute conformational energy, solvation free energy, and solute entropy, respectively. T represents the absolute temperature. $\Delta X = X_{60MPa} - X_{0.1MPa}$ where $X = E_{conf}$, $\Delta\mu$, TS, or G. $\Delta G = \Delta E_{conf} + \Delta\Delta\mu - T\Delta S$. $\Delta\Delta G$ is the difference from ΔG of Ac1Q. The value after "±" indicates standard deviation.

In the study of protein dynamics, I investigated the conformational transition of actin which is related to the elongation mechanism of F-actin. I proposed the method which combines the parallel cascade selection molecular dynamics (PaCS-MD) and Markov state model (MSM). With these methods, free energy changes upon conformational change were calculated without any bias during MD simulations. The dihedral angle defined by four subdomains in actin (propeller angle) is known to be different between ATP-bound G-actin (twisted-ATP) and ADP-bound F-actin (flat-ADP). PaCS-MD enhanced sampling and successfully generated wide range of propeller angles compared to the conventional molecular dynamics (CMD) simulations. By the free energy analysis along the propeller angle, ATP-bound actin was confirmed to be stable in a twisted structure whereas ADP-bound actin was stable in a flat structure (Figure 1). Comparison among G-actin and actin pentamer was conducted to investigate the relation of the propeller angle difference to the ATP hydrolysis and filament elongation. The free energy difference in flat and twisted conformation for ATP-bound actin is larger than that for ADP-bound actin. This observation might imply that conformational transition between flat- and twisted-form could occur in ADP-bound actin more easily than in ATP-bound actin because the conformational transition from twisted- to flat-form would be allowed only when ATP is hydrolyzed into ADP.

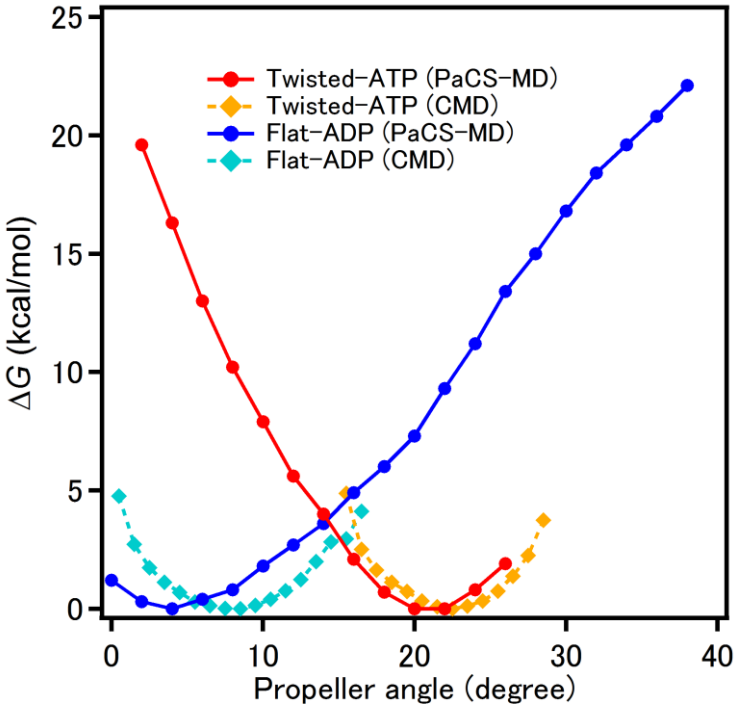

Figure 1. Free energy profiles along the propeller angle in twisted-ATP and flat-ADP. Red and blue solid lines denote the free energies in twisted-ATP and flat-ADP using PaCS-MD, respectively. Orange and cyan broken lines denote the free energies in twisted-ATP and flat-ADP using CMD simulations, respectively.