

Theoretical Analysis of α -Actin Stability at High Pressure

若井信彦

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1 Introduction

The main component of muscle fiber is α -actin which is one of the most abundant proteins. Monomeric actin (G-actin) can polymerize into fiber (F-actin) with ATP hydrolysis. Actin plays many important roles in cell functions. High pressure has been shown to induce significant effects on the actins purified from land or shallow water species as decrease of DNase I inhibiting, decrease of volume change at polymerization, and increase of the dissociating rate for ligands¹⁻⁴. The amino acid sequences of actins are known to be highly conserved in many species. Compared to non-abysal fish

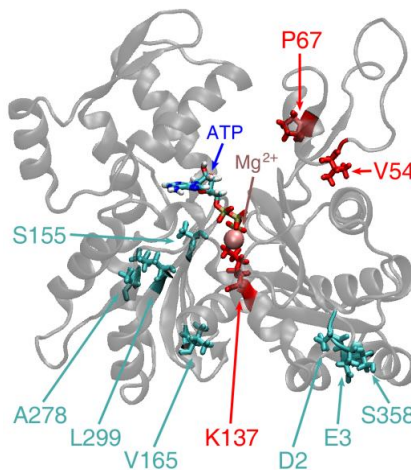


Figure 1. Structure of *yaquinae* actin.

Coryphaenoides acrolepis actin 1 (*acrolepis*) and deep-sea fish actins, deep-sea fish *C. armatus* actin 2b (*armatus*) and *C. yaquinae* actin 2b (*yaquinae*) were found to have only three substitution sites: Q137K/V54A (*armatus*) or Q137K/L67P (*yaquinae*) are the specific substitutions in deep-sea fish actins. Among these substitutions, Q137K near the active site of hydrolysis is expected to play essential role on the pressure tolerance of ligand binding. Although the effects of these substitutions were observed in some experiments, the detailed mechanisms are still unclear in molecular level. The purpose of this study is to elucidate the mechanism of the pressure tolerance induced by substitutions.

2 Methods

I performed molecular dynamics (MD) simulations to investigate pressure effects on actin structure and dynamics. First, I carried out structure modeling of *acrolepis*, rabbit, *armatus*, and *yaquinae* actins. Then each actin was solvated in KCl solution (50 mM) with explicit solvent molecules. The box size of simulated systems is $\sim 80 \times 80 \times 90 \text{ \AA}^3$. In *acrolepis*, two distinct states of Mg^{2+} coordination are considered; Coordination by three water molecules, ATP, and a Gln-137 side-chain atom (*acrolepis*_{Gln}) and coordination by four water molecules and ATP (*acrolepis*_{Wat}). After energy minimization, MD simulations of *acrolepis*_{Gln}, *acrolepis*_{Wat}, rabbit, *armatus*, and *yaquinae* actins were carried out for 40 ns equilibration and 30 ns samplings using at 1 or 600 bar. These simulations were performed using NAMD program package and CHARMM22 parameters.

3 Results and Discussion

I examined the changes of volume, solvent-accessible surface area (SASA), and principal moment of inertia (PMOI) induced by high pressure. Although there were slight tendencies of volume decrease and SASA increase in both deep-sea fish and other species at high pressure, the most notable difference was seen in PMOI of deep-sea fish actins that are less affected by high pressure. The number of total salt bridges (NSB) was also examined to analyze the effect of substitutions. Deep-sea fish actins maintain 25 salt bridges at 600 bar whereas other species have 20-24 salt bridges. Therefore, deep-sea fish actins are suggested to be more stabilized by the salt bridges.

Table 1. Comparison of the number of total salt bridges

Actin	NSB _{1bar}	NSB _{600bar}	Δ NSB
<i>acrolepis</i> _{Wat}	21.6 \pm 1.9	20.0 \pm 1.5	-1.6
<i>acrolepis</i> _{Gln}	19.5 \pm 1.9	22.1 \pm 1.5	2.6
Rabbit	24.4 \pm 1.6	24.2 \pm 1.7	-0.2
<i>armatus</i>	25.1 \pm 1.4	25.7 \pm 1.9	0.6
<i>yaquinae</i>	23.2 \pm 1.5	25.1 \pm 1.4	1.9

$$\Delta\text{NSB} = \text{NSB}_{600\text{bar}} - \text{NSB}_{1\text{bar}}$$

Only deep-sea fish actins have Lys-137 that forms a salt bridge with γ -oxygen atom in ATP. The salt bridge may prevent ATP dissociation at high pressure. In addition, there were nine salt bridges which both *armatus* and *yaquinae* have in common at high pressure. These salt bridges mostly connect pairs of secondary structures as well as different subdomains. These salt bridges are expected to contribute to the pressure tolerance of deep-sea fish actins.

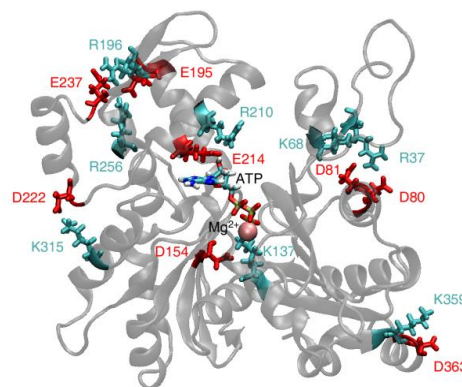


Figure 2. Characteristic salt bridges which deep-sea fish have.

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