論文内容の要旨

論文題目　Experimental and Simulation Studies on the Folding/Unfolding of Goat α-Lactalbumin
（実験とシミュレーションによるヤギαラクトアルブミンのフォールディング/アンフォールディングの解析）

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Elucidation of the molecular mechanisms of protein folding remains a challenge for molecular biophysics. The combination of molecular simulations and experimental observations has become increasingly important in this regard. Molecular dynamics simulation is now widely used to scrutinize the molecular processes of folding/unfolding transitions.

In this study, I investigated the folding/unfolding process of the authentic and recombinant forms of goat α-lactalbumin by the combined use of molecular dynamics simulations and kinetic experiments. The recombinant form of wild-type goat α-lactalbumin expressed in *Escherichia coli* has an extra methionine residue at the N-terminus, Met0. The presence of Met0 destabilizes the native state, despite no appreciable change in the three-dimensional structure. This destabilization is due primarily to an increase in unfolding rate, which is eight times faster than that of the

authentic form. In addition to these experimental facts, the transition state structure of the recombinant form has also been characterized by ϕ-value analysis, which was performed in the previous study in our laboratory. The wealth of experimental data about the two proteins makes them ideal models to study the folding/unfolding process by molecular dynamics simulations.

There are two goals in this study: (1) the first is to obtain an atomically detailed description of the role of Met0 at the very early stage of unfolding; (2) the second is to investigate the unfolding transitions of goat α-lactalbumin from the initial stage to beyond the transition state.

To investigate the initial stage of unfolding, I performed unfolding simulations for the authentic and recombinant forms of goat α-lactalbumin at 398 K (ten trajectories of 5 ns for each form, 100 ns total). The results of these simulations precisely reproduced the experimentally observed differences in unfolding behavior between the authentic and recombinant forms of goat α-lactalbumin. In addition, experiments reproduced the destabilization of a mutant protein, T38A, faithfully as predicted by the simulations. This bi-directional verification between experiments and simulations enabled the atomically detailed description of the role of the extra methionine residue at the early stage of unfolding process.

To investigate the unfolding transitions from the initial stage to beyond the transition state, I performed unfolding simulations for the authentic and recombinant forms at 498 K (ten trajectories of 5 ns for each form, 100 ns total). At this high temperature, the two proteins were found to undergo an unfolding transition, losing most of the native tertiary contacts at the early stage. To identify subtle differences between the two forms in the highly stochastic kinetics of unfolding, I classified the unfolding trajectories using the

multiple alignment method based on the analogy between the biological sequences and the molecular dynamics trajectories. A dendrogram derived from the multiple trajectory alignment revealed a clear difference in the unfolding pathways of the authentic and recombinant forms, i.e., the former reached the transition state in an all-or-none manner while the latter unfolded less cooperatively. It was also found in the classification that the two forms of the protein shared a common transition state structure, which was in excellent agreement with the transition state structure observed experimentally by the ϕ-value analysis.