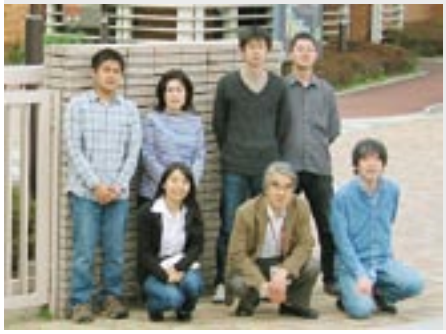
Elucidation of the Molecular Mechanisms of Protein Folding

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Kuwajima group is studying mechanisms of *in vitro* protein folding and mechanisms of molecular chaperone function. Our goal is to elucidate the physical principles by which a protein organizes its specific native structure from the amino acid sequence. In this year, we studied the equilibrium and kinetics of canine milk lysozyme folding/unfolding by peptide and aromatic circular dichroism and tryptophan fluorescence spectroscopy, and the unfolding pathways of goat α-lactalbumin by high-temperature molecular dynamics simulations.

1. Equilibrium and Kinetics of the Folding and Unfolding of Canine Milk Lysozyme[1)]

The equilibrium and kinetics of canine milk lysozyme folding/unfolding were studied by peptide and aromatic circular dichroism and tryptophan fluorescence spectroscopy. The Ca^{2+}-free apo form of the protein exhibited a three-state equilibrium unfolding, in which the molten globule state is well populated as an unfolding intermediate. A rigorous analysis of holo protein unfolding, including the data from the kinetic refolding experiments, revealed that the holo protein also underwent three-state unfolding with the same molten globule intermediate. Although the observed kinetic refolding curves of both forms were single-exponential, a burst-phase change in the peptide ellipticity was observed in both forms, and the burst-phase intermediates of both forms were identical to each other with respect to their stability, indicating that the intermediate does not bind Ca^{2+}. This intermediate was also shown to be identical to the molten globule state observed at equilibrium. The Φ-value analysis, based on the effect of Ca^{2+} on the folding and unfolding rate constants, showed that the Ca^{2+}-binding site was not yet organized in the transition state of folding. A comparison of the result with that previously reported for α-lactalbumin indicated that the folding initiation site is different between canine milk lysozyme and α-lactalbumin, and hence, the folding pathways must be different between the two proteins. These results thus provide an example of the phenomenon wherein proteins that are very homologous to each other take different folding pathways. It is also shown that the native state of the apo form is composed of at least two species that interconvert.

2. Unfolding Pathways of Goat α-Lactalbumin as Revealed in Multiple Alignment of Molecular Dynamics Trajectories[2)]

Molecular dynamics simulations of protein unfolding were performed at an elevated temperature for the authentic and recombinant forms of goat α-lactalbumin. Despite very similar three-dimensional structures, the two forms have significantly different unfolding rates due to an extra N-terminal methionine in the recombinant protein. To identify subtle differences between the two forms in the highly stochastic kinetics of unfolding, we 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 proteins, *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 in the Φ-value analysis.

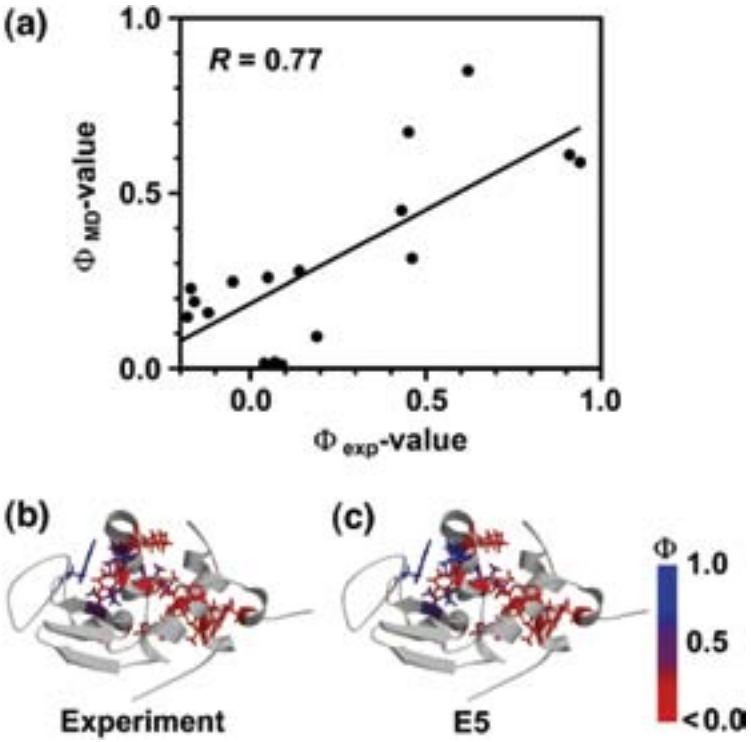

Figure 1. (a) Scatter plot showing the correlation between Φ_{exp} and Φ_{MD} of recombinant goat α-lactalbumin; Φ_{exp} contains small negative values, and Φ_{MD} was calculated for the structures around the center of cluster E5. (b) Φ_{exp} and (c) Φ_{MD} mapped onto the three-dimensional structure of goat α-lactalbumin.

References

1) H. Nakatani, K. Maki, K. Saeki, T. Aizawa, M. Demura, K. Kawano, S. Tomoda and K. Kuwajima, *Biochemistry* **46**, 5238–5251 (2007).

2) T. Oroguchi, M. Ikeguchi, M. Ota, K. Kuwajima and A. Kidera, *J. Mol. Biol.* **371**, 1354–1364 (2007).

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