Elucidation of the Molecular Mechanisms of Protein Folding

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Kuwajima group is studying mechanisms of *in vitro* protein folding and mechanisms of molecular chaperone function. Our goals are 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 folding/unfolding of goat α-lactalbumin, the single-molecule unfolding of staphylococcal nuclease, and the comparative analysis of folding reactions of tear lipocalin and β-lactoglobulin.

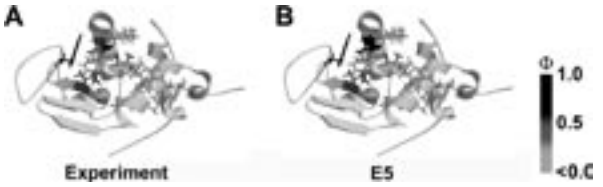

Figure 1. The Φ-values ((a) experimental Φ-values, and (b) Φ_{MD} obtained from molecular dynamics trajectories) mapped onto the three-dimensional structure of goat α-lactalbumin.

1. Experimental and Simulation Studies of the Folding/Unfolding of Goat α-Lactalbumin[1)]

We studied (1) the unfolding behavior of the authentic and recombinant forms of goat α-lactalbumin and (2) the structure of the transition state of folding/unfolding of the protein, both experimentally and by simulation of the molecular dynamics. Experimentally, the recombinant protein exhibited remarkable destabilization and unfolding-rate acceleration as compared to those of the authentic protein; these differences were caused by the presence of an extra N-terminal methionine residue in the recombinant form. We also characterized the transitionstate structure by mutational Φ-value analysis, based on which the structure was localized in a region containing the C-helix and the Ca^{2+}-binding site of the protein. Simulation of the molecular dynamics of unfolding at high temperatures (398 and 498 K) yielded good reproduction of the experimental observations and gave atomically detailed descriptions of the unfolding behavior and the transition-state structure of folding/unfolding. The present series thus demonstrated the power of combination of experiments and simulations for studying the problems of protein folding.

2. Probing Force-Induced Unfolding Intermediates of a Single Staphylococcal Nuclease Molecule and the Effect of Ligand Binding[2)]

Single-molecule manipulation techniques have given experimental access to unfolding intermediates of proteins that are inaccessible in conventional experiments. A detailed characterization of the intermediates is a challenging problem that provides new possibilities for directly probing the energy landscape of proteins. We investigated single-molecule mechanical unfolding of a small globular protein, staphylococcal nuclease (SNase), using atomic force microscopy. The unfolding trajectories of the protein displayed sub-molecular and stochastic behavior with typical lengths corresponding to the size of the unfolded substructures. Our results support the view that the single protein unfolds along multiple pathways as suggested in recent theoretical studies. Moreover, we found the drastic change, caused by the ligand and inhibitor bindings, in the mechanical unfolding dynamics.

3. Non-Native α-Helix Formation Is Not Necessary for Folding of Lipocalin: Comparison of Burst-Phase Folding between Tear Lipocalin and β-Lactoglobulin[3)]

Tear lipocalin and β-lactoglobulin are members of the lipocalin superfamily. They have similar tertiary structures but unusually low overall sequence similarity. Non-native helical structures are formed during the early stage of β-lactoglobulin folding. To address whether the non-native helix formation is found in the folding of other lipocalin superfamily proteins, the folding kinetics of a tear lipocalin variant were investi gated by stopped-flow methods measuring the time-dependent changes in circular dichroism (CD) spectrum and small-angle X-ray scattering (SAXS). CD spectrum showed that extensive secondary structures are not formed during a burst-phase (within a measurement dead time). The SAXS data showed that the radius of gyration becomes much smaller than in the unfolded state during the burst-phase, indicating that the molecule is collapsed during an early stage of folding. Therefore, non-native helix formation is not general for folding of all lipocalin family members. The non-native helix content in the burst-phase folding appears to depend on helical propensities of the amino acid sequence.

References

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Award

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