

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 carried out comparative studies of folding pathways of highly homologous proteins, goat α -lactalbumin and canine milk lysozyme.

1. Different Folding Pathways Taken by Highly Homologous Proteins, Goat α -Lactalbumin and Canine Milk Lysozyme¹⁾

Is the folding pathway conserved in homologous proteins? To address this question, we compared the folding pathways of goat α -lactalbumin and canine milk lysozyme using equilibrium and kinetic circular dichroism spectroscopy. Both Ca^{2+} -binding proteins have 41% sequence identity and essentially identical backbone structures. The Φ -value analysis, based on the effect of Ca^{2+} on the folding kinetics, showed that the Ca^{2+} -binding site was well organized in the transition state in α -lactalbumin, although it was not yet organized in lysozyme. Equilibrium unfolding and hydrogen-exchange 2D NMR analysis of the molten globule intermediate also showed that different regions were stabilized in the two proteins. In α -lactalbumin, the Ca^{2+} -binding site and the C-helix were weakly organized, whereas the A- and B-helices, both distant from the Ca^{2+} -binding site, were well organized in lysozyme. The results thus provide an example of highly homologous proteins taking different folding pathways. To understand the molecular origin of this difference, we investigated the native three-dimensional structures of the proteins in terms of non-local

contact clusters, a parameter based on the residue-residue contact map and known to be well correlated with the folding rate of non-two-state proteins. There were remarkable differences between the proteins in the distribution of the non-local contact clusters, and these differences provided a reasonable explanation of the observed difference in the folding initiation sites. In conclusion, the protein folding pathway is determined not only by the backbone topology but also by the specific side-chain interactions of contacting residues.

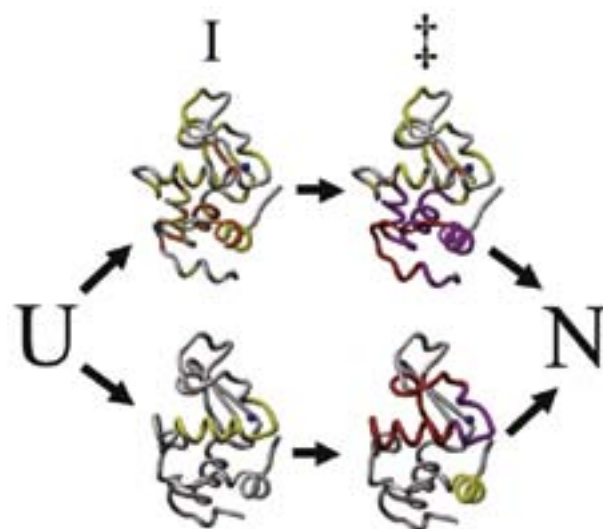


Figure 1. A folding model of α -lactalbumin/lysozyme family proteins. Two parallel pathways are available for the backbone topology of this family, and the lower and upper pathways are taken by goat α -lactalbumin and canine milk lysozyme, respectively, because of the distributions of the strong clusters in their native 3D structures.

2. Flexible Recognition of the tRNA G18 Methylation Target Site by TrmH Methyltransferase through First Binding and Induced Fit Processes²⁾

Transfer RNA (Gm18) methyltransferase (TrmH) catalyzes methyl transfer from S-adenosyl-L-methionine to a conserved G18 in tRNA. We investigated the recognition mechanism of *Thermus thermophilus* TrmH for its guanosine target. Thirteen yeast tRNA(Phe) mutant transcripts were prepared in which the modification site and/or other nucleotides in the D-loop were substituted by dG, inosine, or other nucleotides. We then conducted methyl transfer kinetic studies, gel shift assays, and inhibition experiments using these tRNA variants. Sites of methylation were confirmed with RNA sequencing or primer extension. Although the G18G19 sequence is not essential for methylation by TrmH, disruption of G18G19 severely reduces the efficiency of methyl transfer. There is strict recognition of guanosine by TrmH, in that methylation occurs at the adjacent G19 when the G18 is replaced by dG or adenosine. The fact that TrmH methylates guanosine in D-loops from 4 to 12 nucleotides in length suggests that selection of the position of guanosine within the D-loop is relatively flexible. Our studies also demonstrate that the oxygen 6 atom of the guanine base is a positive determinant for TrmH recognition. The recognition process of TrmH for substrate is inducible and product-inhibited, in that tRNAs containing Gm18 are excluded by TrmH. In contrast, substitution of G18 with dG18 results in the formation of a more stable TrmH-tRNA complex. To address the mechanism, we performed the stopped-flow pre-steady state kinetic analysis. The result clearly showed that the binding of TrmH to tRNA is composed of at least three steps, the first bi-molecular binding and the subsequent two uni-molecular induced-fit processes.

3. Adaptation of a Hyperthermophilic Group II Chaperonin to Relatively Moderate Temperatures³⁾

Group II chaperonins exist in archaea and the eukaryotic

cytosol, and mediate protein folding in an ATP-dependent manner. We have been studying the reaction mechanism of group II chaperonins using alpha chaperonin, the recombinant chaperonin alpha subunit homo-oligomer from a hyperthermophilic archaeon, *Thermococcus* sp. strain KS-1 (*T. KS-1*). Although the high stability and activity of *T. KS-1* alpha chaperonin provided advantages for our study, its high thermophilicity caused the difficulty in using various analytical methods. To resolve this problem, we tried to adapt *T. KS-1* alpha chaperonin to moderate temperatures by mutations. The comparison of amino acid sequences between 26 thermophilic and 17 mesophilic chaperonins showed that three amino acid replacements are likely responsible for the difference of their optimal temperatures. We introduced three single mutations and also their double combinations into *T. KS-1* alpha chaperonin. Among them, K323R single mutant exhibited the improvements of the folding activity and the ATP-dependent conformational change ability at lower temperatures, such as 50 °C and 40 °C. Since K323 may secure helix 12 in the closed conformation by interacting with D198, the replacement of Lys to Arg likely induced the higher mobility of the built-in lid, resulting in the higher activity at relatively low temperatures.

References

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