論文内容の要旨

論文題目　　Folding Mechanism of Green Fluorescent Protein

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The green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is a soluble globular protein consisting of 238 residues. It has a chromophore that emits green fluorescence with a maximum intensity at 508 nm and is surrounded by an 11-stranded β-barrel. GFP forms the chromophore by the autocatalytic cyclization of a polypeptide backbone of Ser65, Tyr66, and Gly67 without the use of any substrate.

It is important to investigate the folding mechanism of GFP for the following reasons. (1) GFP has symmetric and highly complicated topology mainly composed of β-sheet, and it is important to investigate the folding mechanism of a protein with such structural characteristics. (2) GFP is important as a model protein to understand the folding mechanism in a complex environment with molecular chaperons and other molecules such as those in biological cells because of its green fluorescence in the native state. (3) It is useful to investigate physicochemical properties of GFP because GFP is used widely as a marker of the protein expression or localization in the field of

cell biology.

I investigated the kinetic refolding of a mutant (F99S/M153T/V163A) of green fluorescent protein, which is known to mature more efficiently than the wild-type protein, from the acid-denatured state. The refolding was observed by chromophore fluorescence, tryptophan fluorescence, and the far-UV CD, using a stopped-flow technique. In this study, I show that the kinetics of the refolding of the mutant have at least six kinetic phases, which involve nonspecific collapse within the dead time of a stopped-flow apparatus (5 ms) and the subsequent formation of the two (first and second) intermediates with the characteristics of the molten globule state. When refolding was monitored by GFP chromophore, the second intermediate was formed during a lag phase that was evidence that the intermediate was an on-pathway intermediate. I also show that the slowest phase and the second slowest phase were rate-limited by slow prolyl isomerization in the intermediate states, as evidenced by the native-to-denatured-to-native double jump measurement and the refolding measurements in the presence of *human* Cyclophilin A, which has peptidyl-prolyl isomerase activity.

Furthermore, I investigated the characteristics of the intermediates of GFP in detail. I studied the acid denaturation of GFP by chromophore fluorescence, tryptophan fluorescence, and small-angle X-ray scattering. Noncoincidence of transition curves measured by the different probes has indicated that there are at least one equilibrium intermediates populated at pH 4.0. The intermediate was shown to have all the characteristics of the molten globule state. In order to investigate the relationship between the equilibrium intermediates and kinetic folding intermediates, I measured the

refolding reactions induced by pH jumps from pH 2.0 (denatured) to 7.5 (native), from pH 4.0 (intermediate) to 7.5 (native), and from pH 2.0 (denatured) to 4.0 (intermediate) monitored by chromophore and tryptophan fluorescence. As a result, I have shown that at least two kinetic folding kinetic intermediates were equivalent for or at least closely related to the equilibrium intermediate populated at pH 4. The results show that GFP folds in a hierarchy mechanism through the productive folding intermediates that have the characteristics of the molten globule state. The observation was analogous to those in certain other globular proteins such as apomyoglobin, although the backbone topology of GFP (β-sheet) is very different from the topology of apomyoglobin (α-helical). It is suggested that the hierarchical folding is a general mechanism of folding of globular proteins that have a variety of different backbone structures.