Scaling Relationship in Initially Collapsed Conformation of Protein Folding

Proteins spontaneously collapse from the extended conformations of the unfolded state to the compact conformation of the native state. Polypeptide collapse is therefore an essential dynamics in protein folding; however, its molecular mechanism is largely unknown due to the scarcity of experimental data on the collapse. We have been investigating the collapse process in protein folding using time-resolved small-angle X-ray scattering (SAXS) analysis [1-3]. Our strategy is unique in that it enables the characterization of transient conformations of proteins with a lifetime shorter than a millisecond. We characterized the folding process of three proteins with different topologies. The characterized dynamics demonstrated a common folding mechanism termed the "collapse and search" mechanism, in which the initial collapse occurring within a millisecond promotes the search for the correct secondary and tertiary structures (Fig. 1). The observation that the folding of the three proteins with different topologies commonly demonstrated the initial and significant collapse suggested that the collapse is caused not by the individual properties of proteins but by the physical properties of proteins. In this research paper, we will give an explanation of the initial collapse on the basis of Flory's theory of polymers [4].

The basic theory of polymer conformations, in which the transition of polymers from an extended coil to a collapsed globule termed the coil-globule transition was developed by Flory [5]. The transition might explain the collapse of proteins; however, no clear evidence for the explanation was presented. According to Flory's theory, a polymer without short-distance interactions has radius of gyration (R_g) described by the scaling law (1):

$$R_g = a N^{\nu} \qquad (1)$$

where N is the number of monomers, a is a parameter and ν is a scaling exponent. At temperatures below the transition temperature (T_θ), the polymer is classified as a globule whose scaling exponent equals 1/3. In contrast, at temperatures above T_θ, the polymer is classified as a coil whose scaling exponent is close to 3/5. Denatured proteins in the presence of denaturants possess a scaling exponent close to 3/5, and are classified as coils. To determine whether the initially collapsed intermediates observed in protein folding are globules, the scaling relationship and the exponent for the intermediates should be examined.

To examine the scaling relationship of collapsed intermediates, it is necessary to investigate proteins with various chain lengths. Thus, we observed the folding process of heme oxygenase (HO), which is the longest protein (263 residues) ever characterized by time-resolved SAXS analysis (Fig. 2). Time-resolved SAXS analysis was performed at beamline **BL45XU**. HO demonstrated a complicated folding mechanism due to a *cis-trans* isomerization of X-proline peptide bonds and oligomer formation. By carefully conducting various double-jump experiments and examining concentration dependencies, we obtained the R_g for the initially collapsed conformation of HO. The R_g values for HO and other proteins in the initially

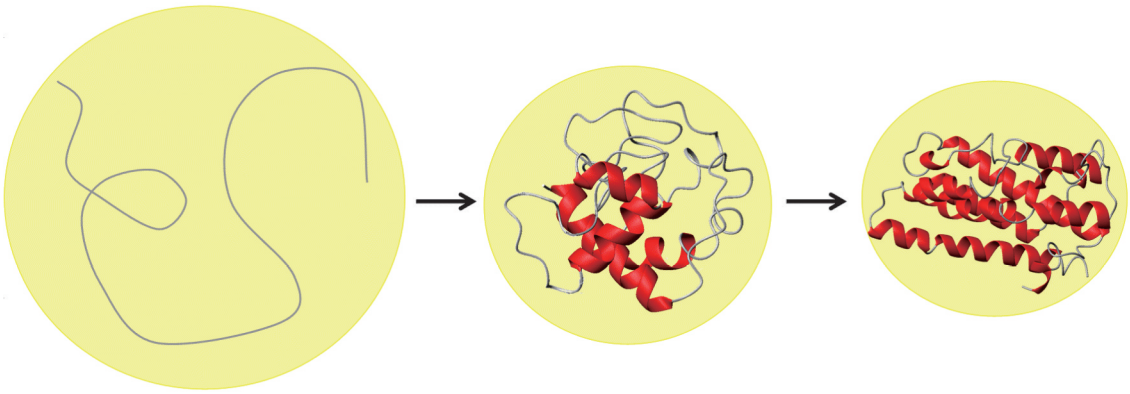

Fig. 1. Collapse and search mechanism of protein folding generally observed for proteins with more than 100 residues. The unfolded protein (left) collapses to the initial intermediate (middle) within a millisecond after initiating the protein folding reaction. The collapsed intermediate searches for the correct secondary and ternary contacts and convert to the native structure (right). The time constant for the native structure formation is in the range of milliseconds to seconds.

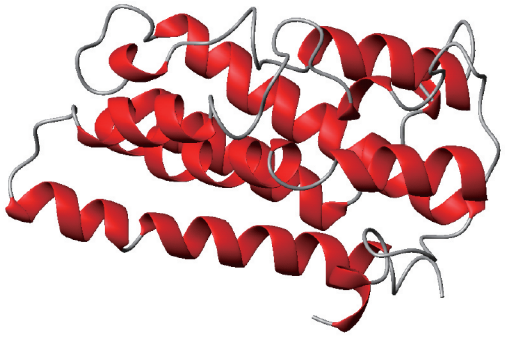

Fig. 2. Folded structure of heme oxygenase.

collapsed conformation were plotted against chain lengths in Fig. 3. The R_g values for the seven proteins are well fitted by the scaling law with $\nu = 0.35 \pm 0.11$, which is close to 1/3 of the predicted value for globules. The observation clearly suggests that the collapsed intermediates correspond to the globules explained by Flory's theory, and that the properties of proteins as polymers likely determine the initial collapse dynamics of protein folding.

The collapse and search mechanism is generally observed for proteins with more than 100 residues. Interestingly, predicting the structure of proteins that are larger than 100 residues is still extremely difficult despite the significant advances in the recent prediction methods. A better understanding of the mechanism of the collapse will give an important insight that will help improve our ability to predict protein structure. Improvements in the time-resolution and quality of SAXS data for the characterization of the chain collapse should reveal rich structural events involved in the process.

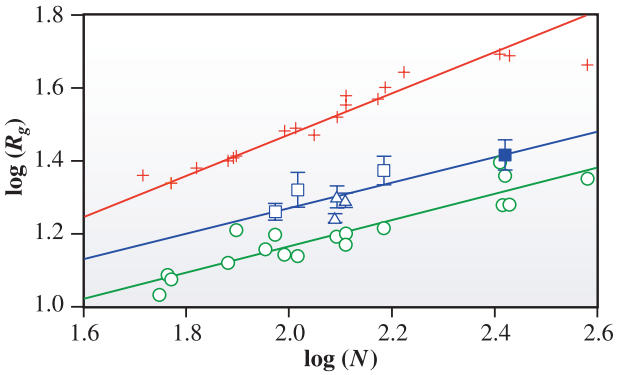

Fig. 3. Relationship between radius of gyration (R_g) and number of amino acid residue (N) for proteins in various conformational states. The blue rectangles and triangles denote the R_g values for the initially collapsed conformation. The filled blue square is the data of HO obtained in this study. The red crosses and green circles are the R_g data of the unfolded and native proteins, respectively.

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