Collapse and Search Dynamics of Apomyoglobin Folding Revealed by Submillisecond Observations of α-Helical Content and Compactness

Proteins can quickly form their folded structures from randomly unfolded conformations through numerous intraprotein interactions among the mainchains and sidechains. Two important classes of interactions contribute to the stabilization of protein structures. The hydrogen-bonding interactions between the amide groups of mainchains mainly stabilize secondary structures. In contrast, the hydrophobic interactions between sidechains are mainly responsible for the stabilization of tertiary structures. The characterizations of protein-folding dynamics based on kinetic observations of secondary and tertiary structures are important for the understanding of intraprotein interactions that enable the protein-folding phenomena.

In this study [1], we chose apomyoglobin (apoMb) as a representative of α-helical proteins. At neutral pH, apoMb possesses seven helices labeled A~E, G and H, which amount to 55 % of α-helical content (f_H). The helices assemble into a globular shape with a radius of gyration (R_g) of 18.2 Å. The acid-unfolded state of apoMb is observed at pH 2.2, and possesses a small f_H (5 %) and a large R_g (29.7 Å). A simple model of the folding of apoMb assumes that the formation of helices precedes that of tertiary contacts, and implies the importance of local hydrogen bonding in the initial stage of the folding. However, the observation that no peptide fragments of the helix regions of apoMb except for H-helix can maintain helical conformations suggests a collapse of the mainchain before the formation of the secondary and tertiary structures. The difficulty in observing secondary and tertiary structures with a submillisecond resolution precludes us from resolving and analyzing these processes.

We have recently developed an experimental strategy to explore the submillisecond folding dynamics of proteins based on the combined use of a solution mixing device, circular dichroism (CD), and small-angle X-ray scattering (SAXS) [2,3], as shown in Fig. 1 (**BL45XU**). The CD signal monitored at 222 nm gives f_H. The SAXS method is useful for monitoring R_g and shape of proteins. We utilized the developed strategy to induce the folding of apoMb upon a pH jump from pH 2.2 to 6.0, and to observe the folding behavior of apoMb in the time domain from 300 μs to 1 s.

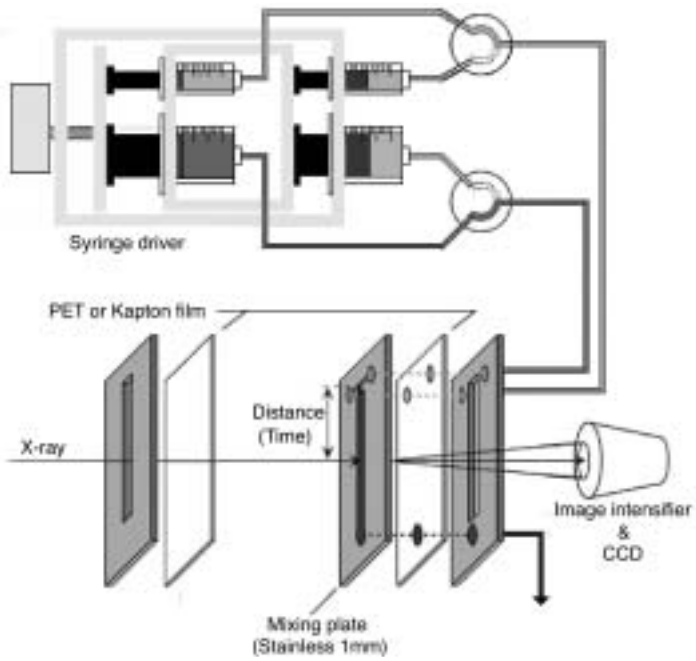

Fig. 1. Developed system for kinetic observations of protein folding by SAXS in submillisecond domain.

The first important finding of this study is the rapid and significant collapse and helix formation that occurs within 300 μs. The amount of collapsed domain estimated from SAXS measurements is more than 80%, indicating that the most of the mainchain is collapsed. The interpretation seems to contradict a previous consensus on the intermediate, i.e., that the collapsed domain is composed of only the A, G and H helices located in the N and C terminal regions [4]. We conclude that the initial collapse involves not only the terminal domain but also the central domain of apoMb. The second important observation of this study is the processes after the initial collapse, which can be described as the stepwise helix formation within the collapsed conformation. Interestingly, several previous reports on the folding of apoMb suggested changes in tertiary contacts in this time domain [5]. The folding after the initial collapse can be interpreted as a stepwise process during which apoMb searches for the correct tertiary contacts within the collapsed conformation to stabilize the helical structures.

We examined the folding trajectories of apoMb and cytochrome *c* (cyt *c*) in the conformational space defined by R_g and f_H as shown in Fig. 2. These are the only proteins without disulfide linkages whose folding dynamics have been characterized in terms of R_g and f_H. Both proteins similarly exhibit cooperative acquisitions of f_H and R_g after the initial collapse, indicating that the secondary and tertiary structures are largely formed together during the protein folding. However, while the folding of apoMb is characterized by a large-scale collapse, corresponding to ~50% of the overall change in R_g from the unfolded state to the native state, the initial collapse of cyt *c* corresponds to only ~ 35 % of the total. Thus, the comparison of the characterized trajectories of these proteins suggests the importance of the initial hydrophobic collapse for the conformational search during protein folding.

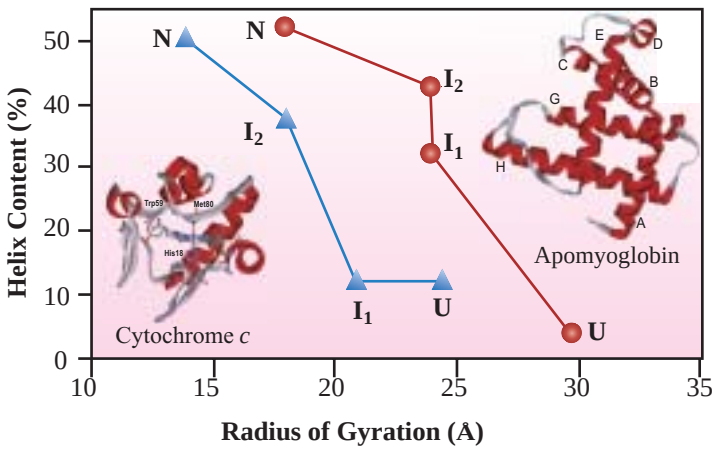

Fig. 2. Folding trajectories of apoMb and cyt *c* in two-dimensional conformational space defined by f_H and R_g.

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