クライオストップトフロー法による蛋白質フォールディング初期過程の研究
The Early Events of Protein Folding Studied by Cryo-Stopped-Flow Method

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The fastest event of protein folding starts in 10 ns region. As it is so fast and the folding is the complex events, it is not easy to investigate the early processes of protein folding. Laser temperature-jump method has been applied successfully, which gave some events; (1) alpha-helix formation is as fast as 10 ns – 200 ns region. (2) beta-hairpin is formed faster than a few μs. (3) Fastest event of long-range interaction is c.a. several μs region. However, due to the limitation of the measured probes, the obtained view is seriously limited. Circular dichroism and x-ray scattering can give more direct information on the secondary structure formation and the global conformation. In addition, temperature-jump method can only be applied to cold-denaturation,

We have developed cryo-stopped-flow method for applying to the protein folding with three probes; circular dichroism, x-ray scattering and fluorescence. With this method, the protein dissolved in the denaturant is diluted with the folding buffer by stopped-flow. Thus, we monitor the folding process by the probels mentioned above. The main problem of this method is that the mixing time cannot be so short. Therefore, we can monitor only late processes of folding slower than ms region. We then cool down and monitor the folding process at subzero temperature (0C to -28C). To prevent from freezing, we use antifreeze chemicals such as ethylene glycol.

With this method, we have obtained the followings;

(1) The alpha-helix formation is so fast that we cannot monitor even at -55C.
(2) In apomyoglobin folding, the early events finished so fast that we cannot monitor as low as -20C.
(3) We could observe the alpha-helical burst formed in the early stage of beta-lactoglobulin, as already reported.
(4) In addition, we observed alpha-helical bursts in all proteins so far investigated including SH3, ubiquitin and FHS domain proteins. The amplitude of the burst is proportional to the helix propensity calculated by Helix2 program. This suggests that the following folding scheme.

The unfolded protein forms alpha-helix only in short time at submicro-second region. When the alpha-helices are trapped with each other, they become more stable and they form the folding core. This might be the part of the native conformation in case of alpha-helix-rich proteins, whereas the core is disrupted in case of beta-rich proteins.

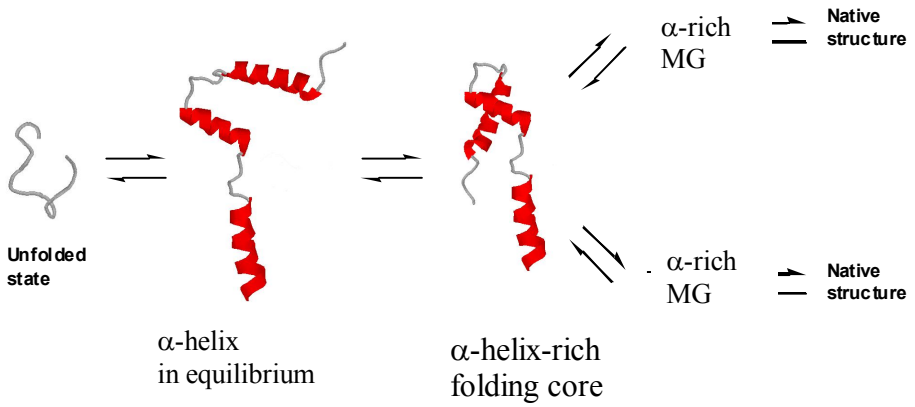

Figure1. Scheme of the Early Events of Protein Folding

References.

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