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 studied the structure of the GroEL-GroES complex under physiological conditions by small-angle X-ray scattering, which is a powerful technique to directly observe the structure of the protein complex in solution.

1. Asymmetry of the GroEL-GroES Complex under Physiological Conditions as Revealed by Small-Angle X-Ray Scattering[1)]

In spite of the well-known functional importance of GroEL-GroES complex formation during the chaperonin cycle, the stoichiometry of the complex has not been clarified. The complex can occur either as an asymmetric 1:1 GroEL-GroES complex or as a symmetric 1:2 GroEL-GroES complex, although it remains uncertain which type is predominant under physiological conditions. To resolve this question, we studied the structure of the GroEL-GroES complex under physiological conditions by small-angle X-ray scattering, which is a powerful technique to directly observe the structure of the protein complex in solution. We evaluated molecular structural parameters, the radius of gyration and the maximum dimension of the complex, from the X-ray scattering patterns under various nucleotide conditions (3 mM ADP, 3 mM ATPγS and 3 mM ATP in 10 mM $MgCl_2$ and 100 mM KCl) at three different temperatures (10 °C, 25 °C, and 37 °C). We then compared the experimentally observed scattering patterns with those calculated from the known X-ray crystallographic structures of the GroEL-GroES complex. The results clearly demonstrated that the asymmetric complex must be the major species stably present in solution under physiological conditions. On the other hand, in the presence of ATP (3 mM) and beryllium fluoride (10 mM NaF and 300 μM $BeCl_2$), we observed the formation of a stable symmetric complex, suggesting the existence of a transiently formed symmetric complex during the chaperonin cycle.

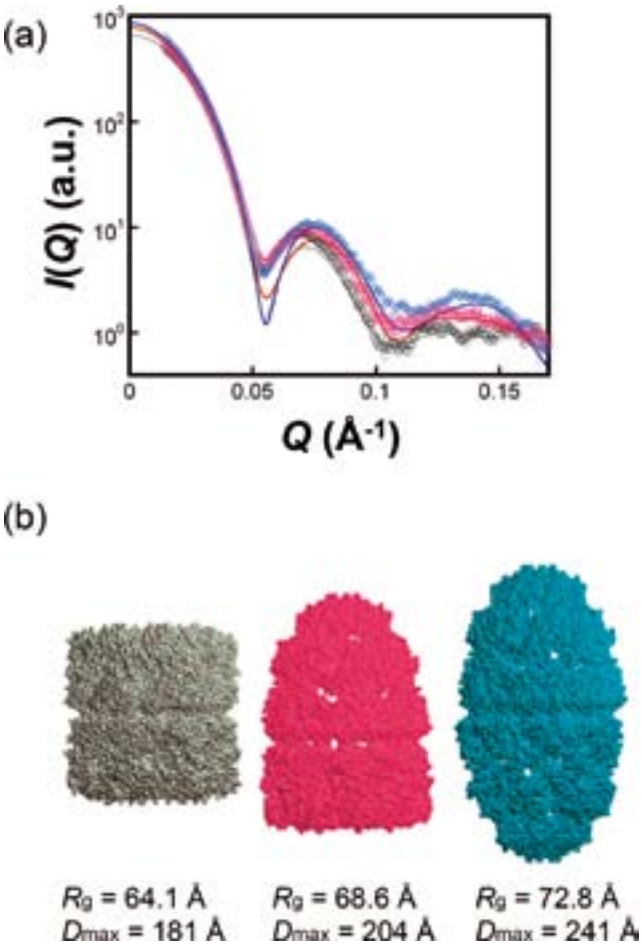

Figure 1. (a) Small-angle X-ray scattering patterns of molecular chaperones, GroEL and the GroEL-GroES complexes. (b) The three-dimensional structures of GroEL (gray), the bullet-type GroEL-GroES complex (magenda), and the football-type GroEL-GroES complex (cyan) (Inobe *et al*., *Biophys. J.* **94**, 1392–1402 (2008)).

Reference

1) T. Inobe, K. Takahashi, K. Maki, S. Enoki, K. Kamagata, A. Kadooka, M. Arai and K. Kuwajima, *Biophys. J.* **94**, 1392–1402 (2008).

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