Elucidation of the Molecular Mechanisms of Protein Folding

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions

KUWAJIMA, Kunihiro	Professor
MAKABE, Koki	Assistant Professor
NAKAMURA, Takashi	IMS Research Assistant Professor
CHEN, Jin	OIIB Research Assistant Professor
TAKENAKA, Toshio	Post-Doctoral Fellow
MIZUKI, Hiroko	Technical Fellow
IKEDA, Yukako	Technical Fellow
TANAKA, Kei	Secretary

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 equilibrium and kinetics of bimolecular $MgATP^{2-}$ binding to GroEL.

1. Dissecting a Bimolecular Process of $MgATP^{2-}$ Binding to the Chaperonin GroEL

The chaperonin GroEL from *Escherichia coli*, a tetradecameric protein complex consisting of two heptameric rings stacked back to back with a central cavity, is one of the best characterized molecular chaperones that facilitate protein folding *in vivo*. The ATP^{-} dependent control of the affinity for its target protein and the co-chaperonin GroES is essential for its molecular chaperone function, and this control occurs through a series of cooperative allosteric transitions of GroEL induced by $MgATP^{2-}$. The equilibria and kinetics of the allosteric transitions of GroEL have thus been studied for some time by a variety of techniques. However, the initial bimolecular step of $MgATP^{2-}$ binding to GroEL, which must precede the allosteric transitions, remains to be clarified.

Here, we studied the equilibrium and kinetics of $MgATP^{2-}$ binding to a variant of GroEL, in which Tyr485 was replaced by tryptophan, via isothermal titration calorimetry (ITC) and stopped-flow fluorescence spectroscopy (Figures 1 and 2). In the absence of K^+ at 4 ~ 5 °C, the allosteric transitions and the subsequent ATP hydrolysis by GroEL are halted, and hence, the stopped–flow fluorescence kinetics induced by rapid mixing of $MgATP^{2-}$ and the GroEL variant solely reflected $MgATP^{2-}$ binding, which was well represented by bimolecular noncooperative binding with a binding rate constant, k_{on}, of 9.14×10^4 M^{-1} s^{-1} and a dissociation rate constant, k_{off}, of 14.2 s^{-1}, yielding a binding constant, K_b (= k_{on}/k_{off}), of 6.4×10^3 M^{-1}. We also successfully performed ITC to measure

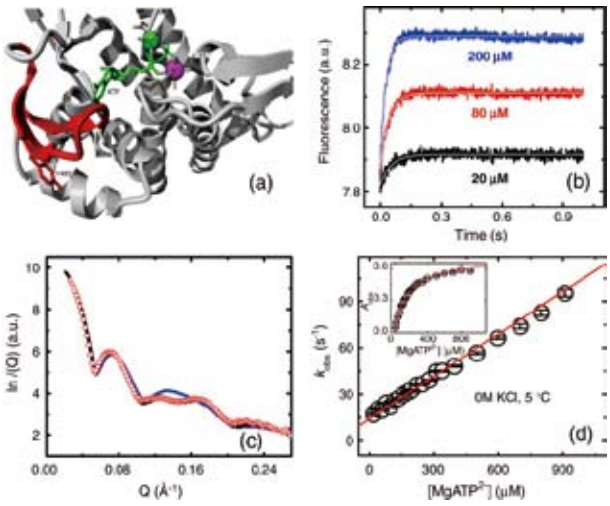

Figure 1. The structure around the $MgATP^{2-}$-binding site of GroEL (a), the SAXS patterns of wild-type GroEL in the different allosteric states (b), and the binding kinetics of $MgATP^{2-}$ to GroEL(Y485W) (c and d).

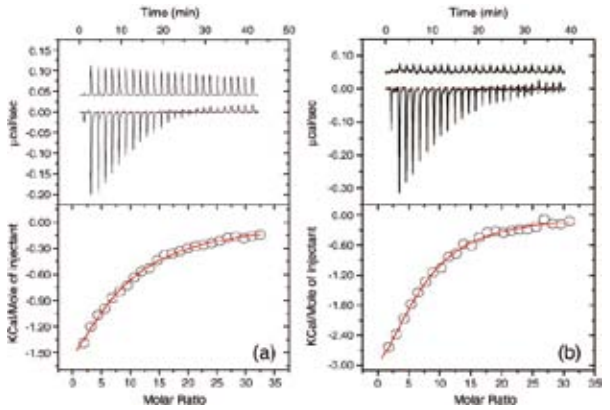

Figure 2. $MgATP^{2-}$-binding isotherms to GroEL(Y485W) (a) and GroEL(wild type) (b) measured by ITC in the absence of K^+ at 5 °C.

binding isotherms of $MgATP^{2-}$ to GroEL and obtained a K_b of 9.5×10^3 M^{-1} and a binding stoichiometric number of 6.6 (Figure 2). K_b was thus in good agreement with that obtained by stopped-flow fluorescence. In the presence of 10 ~ 50 mM KCl, the fluorescence kinetics consisted of three to four phases (the first fluorescence-increasing phase, followed by one or two exponential fluorescence-decreasing phases, and the final slow fluorescence-increasing phase), and comparison of the kinetics in the absence and presence of K^+ clearly demonstrated that the first fluorescence-increasing phase corresponds to bimolecular $MgATP^{2-}$ binding to GroEL. The temperature dependence of the kinetics indicated that $MgATP^{2-}$ binding to GroEL was activation-controlled with an activation enthalpy as large as 14 ~ 16 kcal mol^{-1}.

To further elucidate what kind of activation ($P^*L \rightarrow PL^‡$) takes place during $MgATP^{2-}$ binding to apo GroEL, we investigated the X-ray crystallographic structures of the $MgATP^{2-}$-binding site of apo GroEL (PDB code: 1OEL) and $MgATP^{2-}$-bound GroEL (PDB code: 1KP8). The $MgATP^{2-}$-bound GroEL, originally complexed with ATPγS, assumed the T-state conformation, and hence provides an excellent model of the $MgATP^{2-}$-bound complex (PL) in the present study. As a result, the two structures were almost superimposable to each other. All atoms other than the $O^γ$ of Thr38 are not shifted more than 1.9 Å (Figure 3(a)).

If there is no essential difference in the binding-site structure between apo and $MgATP^{2-}$-bound GroEL, how can we explain the $\Delta H^‡$ of 14 ~ 16 kcal mol^{-1} that is involved in the activation step from P*L to PL (Figure 3(b))? A possible explanation is given by partial dehydration and conformational strain in the transition-state complex ($PL^‡$) that exists between P^*L and PL. Both $MgATP^{2-}$ and the binding groove of GroEL are highly hydrated in P^*L, but these hydrated water molecules must be completely removed from the binding surface between $MgATP^{2-}$ and the binding groove in PL, except for the two caves underneath the groove. In $PL^‡$, $MgATP^{2-}$ and the binding groove are thus only partially dehydrated, and this partial dehydration increases the energy level of $PL^‡$ as the final stabilization requires full dehydration. Furthermore, there may be conformational strain imposed on the binding groove in $PL^‡$ when specific interactions steer the ligand into the binding groove, and probably some openings at the entrance of the groove are required for accommodating $MgATP^{2-}$. Such conformational strain also increases the energy level of $PL^‡$. As a result, the activation from P^*L to PL is accompanied by a $\Delta H^‡$ as large as 14 ~ 16 kcal mol^{-1}. Interestingly, a very similar $\Delta H^‡$ (16 kcal mol^{-1}) was observed in the reversed activation from P^*L to PL. In the reversed process, the partial hydration and the conformational strain similarly occur in $PL^‡$, leading to the similar $\Delta H^‡$ in the reversed activation process.

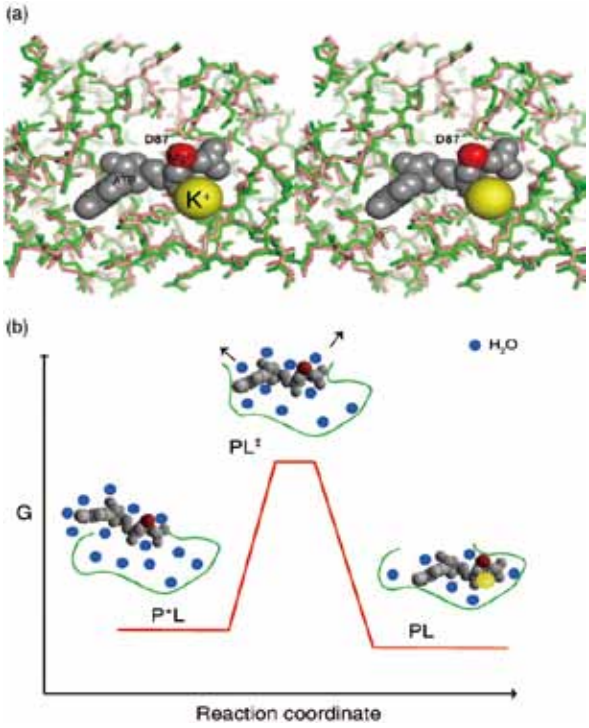

Figure 3. (a) A stereo view of superposition of X-ray structures of apo GroEL (green) (PDB code: 1OEL) and $MgATP^{2-}$-bound GroEL (pink) (PDB code: 1KP8) in a region around the binding site. (b) A reaction diagram of $MgATP^{2-}$ binding to GroEL and schematic representations for the diffusional encounter complex (P^*L), the transition–state complex ($PL^‡$), and the final stable complex (PL).

Reference

1) J. Chen, K. Makabe, T. Nakamura, T. Inobe and K. Kuwajima, *J. Mol. Biol.* **410**, 343–356 (2011).