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Equilibrium and Kinetics of the Allosteric Transition of GroEL Studied by Solution X-ray Scattering

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Introduction

ATP-induced allosteric transitions of GroEL appear to play a key role in the chaperone function of this protein. Therefore, studies on the ATP-induced structural transitions of GroEL are important if we wish to fully understand the functional mechanisms of GroEL, and the kinetics of the structural transition must be an important aspect of those studies.

In the present study, we have thus performed structural analysis of each allosteric states of GroEL in solution by SAXS. The use of the 2D CCD-based X-ray detector, in combination with a stopped-flow technique, has also allowed us to directly observe the kinetics of the ATP-induced allosteric transition of GroEL [1].

Experimental

The SAXS experiments were performed at BL15A. Scattering patterns were recorded by a CCD-based X-ray detector. The experimental details and the analysis of the scattering data were essentially the same as described previously. Stopped-flow SAXS measurements were carried out using a stopped-flow apparatus specially constructed by Unisoku, Inc. The dead time of the mixing was 7 ms.

Results and Discussion

The results of SAXS show that the three allosteric states (the TT, the TR, and the RR states) are structurally different from each other. (data not shown)

Next we studied the kinetics of the ATP-induced structural change of GroEL from the TT state to the TR state by stopped-flow SAXS measurements. Fig. 1(a) shows the kinetics of the structural transition of GroEL (3.9 μM) when mixed with ATP (85 μM). When the time course of integral intensity (I_{int}) was fitted to a single exponential function, the apparent rate was 3.4 s^{-1}.

Fig. 1(b) shows the scattering curves of GroEL measured at early and late stages of the ATP-induced kinetics measured by I_{int}. The averaged scattering curve between 10 and 310 ms shows the same scattering pattern as that in the absence of ATP. Thus, GroEL remains in the TT state at the early stage of the kinetics. After completion of the exponential kinetics, the averaged scattering curve shows the characteristic scattering pattern, which is coincident with those observed in the static scattering pattern of GroEL at 85 μM ATP, indicating that GroEL is in the TR state. These results afford clear evidence that the observed kinetics represent the ATP-induced structural transition from the TT to the TR state of GroEL.

The results obtained by stopped-flow SAXS, in combination with fluorescence spectroscopy, propose that kinetic allosteric model, which is a combination of the conventional transition state theory and the MWC model, well explain the allosteric transition of GroEL.

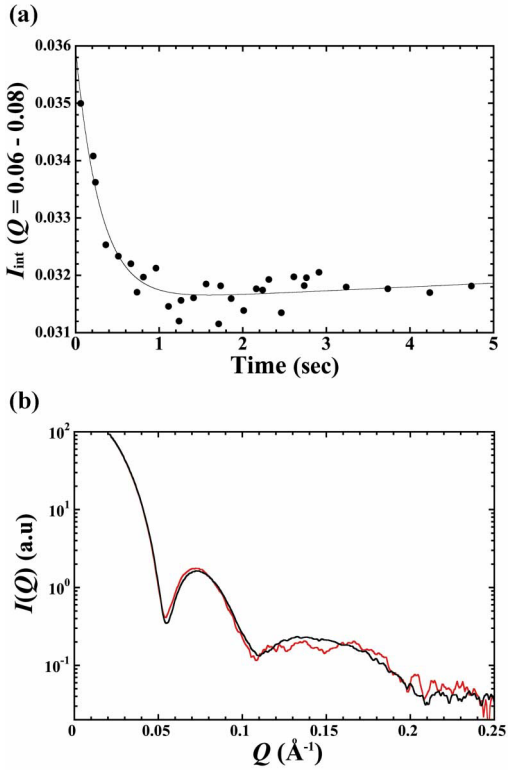

Fig. 1 (a) A kinetic curve of the ATP-induced structural change of GroEL at 4.8°C monitored by I_{int}. The integral region of Q employed was from 0.06 Å^{-1} to 0.08 Å^{-1}. The solid line shows a theoretical kinetic progress curve assuming a single exponential. (b) The SAXS patterns were averaged between 10 ms and 310 ms (solid line) and between 1.5 sec and 3 sec (dashed line).

References

[1] T. Inobe et al., J. Mol. Biol. 327, 183 (2003).

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