Proceedings of the 2nd International Conference on Fluid Flow, Heat and Mass Transfer
Ottawa, Ontario, Canada, April 30 – May 1, 2015
Paper No. 162

Protein Diffusion of Reaction Intermediates under Crowded Condition

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Extended Abstract

In order to elucidate molecular mechanism of chemical reactions, the kinetics of the reactions are important information and have to be investigated. For kinetic measurement of chemical reactions, a variety of spectroscopic methods have been developed so far. For example, time-resolved absorption and emission detections, or the time-resolved resonance Raman scattering technique have been applied to many chemical reactions. However, we sometimes encounter difficulties to use these conventional spectroscopic techniques for elucidating the reactions, in particular, for those of proteins. In order to solve this difficulty, we have developed a time-resolved detection of the translational diffusion coefficient in time-domain based on a pulsed laser induced transient grating (TG) technique (Terazima, 2011a, 2011b). Molecular translational diffusion is the process by which matter is transported from one part of position to another as a result of random molecular motions. The diffusion coefficient (D) reflect the diffusing molecular size. Hence, if we can monitor the D changes in time-domain, we can see how the molecular size changes by the reaction. More importantly, not only the size, but also the intermolecular interaction affects the magnitude of D. If the friction between the diffusing solute and the solvent molecules increases, D should decrease. Therefore, measurement of D in time-domain would be useful and powerful technique to study the time development of not only the molecular size but also intermolecular interaction, both of which are spectrally silent.

By using this diffusion detection method, we have studied variety of reactions of proteins, such as a blue light sensor protein phototropin, which controls the phototropism of a plant. We will present results of reactions of LOV2 (Light-Oxygen-Voltage 2) domain of phototropin in crowded environment. We measured the TG signals to study the reaction dynamics of the diffusion coefficient and reaction rate of the protein. In a buffer solution, we observed a significant D change from 9.2×10^{-11} m^2/s to 6.0×10^{-11} m^2/s with a time constant of 1.0 ms. This time dependent D was interpreted in terms of the unfolding of α-helices in the linker region. In order to investigate the crowding effect, we measured the diffusion behaviour under the crowding condition which was constructed with a high concentration of polysaccharide (Ficoll PM70). We found that the diffusion coefficients of the reactant and product molecules were influenced differently by this crowding environment: D of the photoproduct is less sensitive to the viscosity of the crowded solution. We think that this difference comes from the different structural flexibility of reactant and product molecules. From the transient lens measurement, it was found that the rate of unfolding reaction mainly depended on solution viscosity but the conformation is influenced by the concentration of the crowder. Based on these results, we will discuss the crowding effect on the reaction dynamics of the phototropin LOV2 domain.

TerazimaM. (2011a). Time-Dependent Intermolecular Interaction during Protein Reactions, *Phys. Chem.*, 13, 16928-16940.

TerazimaM. (2011b). Studies of photo-induced protein reactions by spectrally silent reaction dynamics detection methods: Applications to the photoreaction of the LOV2 domain of Phototropin from *Arabidopsis Thaliana*, *Biochim.Biophys.Acta*, 1814, 1093‑1105.