II-E Structure and Energy Changes during Protein Reaction Dynamics

The thermodynamic properties (enthalpy, thermal expansion coefficient, compressibility, partial molar volume, *etc.*) as well as the transport property (diffusion coefficient) of proteins are of fundamental importance to understand the structural fluctuation and the dynamics of protein molecules. Traditional techniques that can access to these quantities are certainly useful and powerful to characterize the proteins. However, knowledge of these properties of time-dependent or unstable (intermediate) species during biological reactions is very limited. It is most desirable to develop and use a method that can measure these properties in time domain so that reaction intermediates can be characterized in a similar way. In this project, we try to construct a method to probe energies and conformational changes as well as the diffusion coefficients of biological proteins in time domain. One of interesting applications of this technique is to detect spectral silent kinetics in reactions of biological proteins.

II-E-1 Hydrogen Bonding Dynamics During Protein Folding of Reduced Cytochrome *c*: Temperature and Denaturant Concentration Dependence

NISHIDA, Shinpei[1]; NADA, Tomokazu[1]; TERAZIMA, Masahide[2]
([1]Kyoto Univ.; [2]IMS and Kyoto Univ.)

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Folding dynamics of reduced cytochrome *c* triggered by the laser induced reduction method is investigated from a view point of the intermolecular interaction change. Change of the diffusion coefficient of Cyt *c* during the refolding process is traced in time domain from the unfolded value to the native value continuously at various denaturant (guanidine hydrochloride (GdnHCl)) concentrations and temperatures. In the temperature range of 288 K–308 K and GdnHCl concentration range of 2.5 M–4.25 M, the diffusion change can be analyzed well by the two state model consistently. It was found that the $m^{\ddagger}$-value and the activation energy of the transition state from the unfolded state for the hydrogen bonding network change are surprisingly similar to that for the local structural change around the heme group monitored by the fluorescence quenching experiment. This agreement suggests the existence of common or similar fundamental dynamics including water molecular movement to control the refolding dynamics. The nature of the transition state is discussed.

II-E-2 Conformational Dynamics of Phototropin 2 LOV2 Domain with the Linker upon Photoexcitation

EITOKU, Takeshi[1]; NAKASONE, Yusuke[1]; MATSUOKA, Daisuke[2]; TOKUTOMI, Satoru[2]; TERAZIMA, Masahide[3]
([1]Kyoto Univ.; [2]Osaka Pref. Univ.; [3]IMS and Kyoto Univ.)

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Conformational dynamics of LOV2 domain of phototropin, a plant-blue-light photoreceptor, is studied by the pulsed laser induced transient grating (TG) technique. The TG signal of LOV2 without the linker part to the kinase domain exhibits the thermal grating signal due to the heat releasing from the excited state and a weak population grating by the adduct formation. The diffusion coefficients of the adduct product after forming the chemical bond between the chromophore and Cys residue is found to be slightly smaller than that of the reactant, which fact implies that the core shrinks slightly on the adduct formation. After that change, no significant conformational change was observed. On the other hand, the signal of LOV2 with the linker part to the kinase domain clearly shows very different diffusion coefficients between the original and the adduct species. The large difference indicates significant global conformational change of the protein moiety upon the adduct formation. More interestingly, the diffusion coefficient is found to be time dependent in the observation time range. This dynamics representing the global conformational change is a clear indication of a spectral silent intermediate between the excited triplet state and the signaling product. From the temporal profile analysis of the signal, the rate of the conformational change is determined to be 2 ms.