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 Time-Resolved Thermodynamics: Heat Capacity Change of Transient Species during Photo-Reaction of PYP

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Heat capacity changes of short lived transient species in different time ranges were measured for the first time by using the thermal component of the transient grating and transient lens signals at various temperatures. This method was applied to the transient intermediates of Photoactive Yellow Protein (PYP). The temperature dependence of the enthalpy change shows that the heat capacity of the short lived intermediate pR_2 (also called I_1 or PYP_L) species is the same as that of the ground state (pG) species within our experimental accuracy, whereas that of the long lived intermediate pB (I_2 or PYP_M) is much larger (2.7±0.4 kJ/mol K) than that of pG. The larger heat capacity is interpreted in terms of the conformational change of the pB species such as melted conformation and/or exposure of the non-polar residues to the aqueous phase. This technique can be used for photochemical reaction in general to investigate the conformational change and the hydrophobic interaction in time domain.

II-E-2 Conformational Changes of PYP Monitored by Diffusion Coefficient: Effect of N-Terminal α-Helices

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Conformational changes in the light illuminated intermediate (pB) of photoactive yellow protein (PYP) were studied from a view point of the diffusion coefficient (D) change of several N-truncated PYPs, which lacked the N-terminal 6, 15, or 23 amino acid residues (T6, T15, and T23, respectively). For intact PYP (i-PYP), D of pB (D_{pB}) was *c.a.* 11% lower than that (D_{pG}) of the ground state (pG) species. The difference in D ($D_{pG} - D_{pB}$) decreased upon cleavage of the N-terminal region in the order of i-PYP > T6 > T15 > T23. This trend clearly showed that conformational change in the N-terminal group is the main reason for the slower diffusion of pB. This slower diffusion was interpreted in terms of the unfolding of the two α-helices in the N-terminal region, increasing the intermolecular interactions due to hydrogen bonding with water molecules. The increase in friction per one residue by the unfolding of the α-helix was estimated to be 0.3×10^{-12} kg/s. The conformational change in the N-terminal group upon photo-illumination is discussed.

II-E-3 Diffusion Coefficient and the Secondary Structure of Poly-L-Glutamic Acid in Aqueous Solution

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The diffusion coefficients (D) of poly-L-glutamic acid (PLG) at various pH were investigated by the laser induced transient grating method with a new photoreactive probe molecule. The pH dependence of D was compared with that of the helical content of PLG measured by the circular dichroism. It was found that the pH dependences of both quantities are very similar. Since the frictions of the translational diffusion of charged and protonated carboxyl group were found to be similar each other, it was concluded that the conformation of the main polymer chain is a main factor to determine the diffusion process; that is, the α-helix conformation makes the molecular diffusion faster. This result indicates that the conformational change of a protein can be detected by monitoring the diffusion coefficient.