Protein Dynamics of Hemoglobin and Myoglobin: Time-resolved resonance Raman study

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Protein dynamics are intimately connected to the structure/function relationship of biological systems. The molecular mechanism of cooperativity in oxygen binding of hemoglobin (Hb) is one of the classical problems in this aspect. The binding of small molecular ligands to the hemes in Hb is a highly localized perturbation. Nonetheless, this localized perturbation initiates a sequence of propagating structural events that culminates in a change of quaternary structure. Myoglobin (Mb) is structurally very similar to a subunit of Hb and serves as a model system for the tertiary relaxation processes. In this talk, I will discuss the structural dynamics of Hb and Mb following ligand dissociation from the heme.

Resonance Raman (RR) spectroscopy is a versatile spectroscopic technique for studying the structure of proteins. For Hb and Mb, Raman bands of the heme moiety are selectively enhanced when the excitation wavelength around 400 nm is employed. On the other hand, when the excitation wavelength is tuned between ~195 and 260 nm, strong resonance Raman scattering from the peptide backbone and aromatic amino acids provides vibrational information on local protein structure and environmental changes. Thus, we can selectively obtain structural information for the heme moiety and protein by tuning the excitation wavelength for RR measurements. We have constructed time-resolved visible and ultraviolet RR spectrometers to study protein dynamics of Hb and Mb. Protein dynamics upon ligand dissociation was investigated by examining temporal changes of RR spectra of some specific parts of the proteins. The RR bands due to the heme vibrations showed almost instantaneous changes upon the ligand dissociation. On the other hand, frequency shift in the picosecond time range was observed for a stretching mode of iron-histidine bond, which is the only covalent linkage between the heme and the protein. RR bands of tryptophan and tyrosine residues also showed temporal changes in the picosecond time region. Based on the time-resolved RR data, I will discuss the sequence of propagating structural events which is driven by the ligand dissociation. The following topics will be focused on.

1. Protein dynamics of Hb
    a. Dynamics of Hb and its isolated chains
    b. Dynamics of Hb encapsulated in porous sol-gels
2. Protein dynamics of Mb
    a. Dynamics of Mb and its mutant which lacks covalent linkage between the heme and the polypeptide
    b. Ultrafast protein dynamics upon ligand dissociation revealed by picosecond time-resolved ultraviolet RR spectroscopy