Vibrational Energy Flow in Hemeproteins

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Abstract: We demonstrate that time-resolved anti-Stokes ultraviolet resonance Raman spectroscopy is a powerful tool for studying the vibrational energy flow in proteins with a spatial resolution of an amino acid residue.
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1. Introduction

Flow of excess energy from a reacting molecule is one of the key issues for understanding how chemical reactions take place in the condensed phases, such as liquid and protein. Excess energy is often deposited in many degrees of freedom right after photoreactions or internal conversions. Many experiments have been performed to study the dissipation processes of excess energy after photoexciting the chromophores. Particularly, hemeproteins have been extensively studied because the heme exhibits ultrafast internal conversion (< 100 fs) and, hence, large amount of excess energy is deposited by photoexcitation. The cooling processes of the heme [1] and the heating of solvent molecules [2] have been well characterized by ultrafast spectroscopy. However, the energy flow within protein moiety has not been directly observed.

In this study, we succeeded in observing the vibrational energy flow in photoexcited hemeproteins by using anti-Stokes ultraviolet resonance Raman (UVRR) spectroscopy. UVRR spectroscopy can selectively monitor Raman bands of aromatic amino acid residues, such as tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) [3]. Because anti-Stokes Raman intensity reflects the population in vibrationally excited states, it can be a direct probe of vibrational energy of residues in a protein. In many hemeprotein, the distance and relative orientation between heme and the amino acid residues are well characterized based on X-ray crystallographic data. Because the distance can be as long as 20 Å, it is possible to observe how the energy deposited to the heme migrates to surrounding residues by measuring the anti-Stokes band intensities of the residues. Accordingly, studies using the present technique based on hemeproteins will provide us new insights for understanding the mechanism of vibrational energy transfer in condensed phases.

2. Vibrational energy flow in cytochrome *c* [4]

Vibrational energy flow in ferric cytochrome *c* has been examined by picosecond time-resolved anti-Stokes UVRR measurements. By taking advantage of the extremely short nonradiative excited state lifetime of heme in the protein, excess vibrational energy of ~25000 cm^{-1} was optically deposited selectively at the heme site. Subsequent energy relaxation in the protein moiety was investigated by monitoring the anti-Stokes UVRR intensities of the Trp59 residue, which is a single tryptophan residue involved in the protein and is located close to the heme group. It was found from temporal changes of the anti-Stokes UVRR intensities that the energy flow from the heme to Trp59 and the energy release from Trp59 took place with the time constants of 1−3 and ~8 ps, respectively. These data are consistent with the time constants for vibrational relaxation of the heme [1] and heating of water [2] reported for hemeproteins. Comparison of the data generated upon excitation to the two different electronic excited states showed that the kinetics of the energy flow were not affected by the amount of excess energy deposited to the heme group. These results demonstrate that the present technique is a powerful tool for studying the vibrational energy flow in proteins.

3. Vibrational energy flow in myoglobin

We investigated distance dependence of energy flow from the heme to discuss the energy transport mechanism in protein moiety, measuring time-resolved anti-Stokes UVRR spectra of myoglobin mutants upon the excitation of heme. In the mutants, two original Trp residues were replaced with Phe and Tyr residues, to prepare Trp-free myoglobin mutants. Then, a Trp residue was introduced at a desired position. Thus, we prepared several mutants in which a Trp residue located at different distance from the heme. For all mutants we studied, anti-Stokes bands attributed to the Trp residue at 760 (W18 band) and 1012 cm^{-1} (W16 band) were observed. These bands appeared in a few picoseconds after the photoexcitation and diminished in tens of picoseconds. The increase and decrease of band intensities can be ascribed to energy transfer from the heme and energy release to the surrounding residues, respectively. In the time-resolved spectra, the anti-Stokes band intensities due to the transient species became lower

as the distance between the Trp residue and the heme was longer. This is consistent with the simple idea that spatial density of the excess energy decreases as the energy diffuses. The time evolution of anti-Stokes intensities were compared among the myoglobin mutants. The intensity rise of the anti-Stokes band became slower as the distance between the Trp residue and the heme was longer. This means that it takes longer time for the excess energy to arrive at the position farer from the heme. We analyzed the data to see if a classical heat transport model reproduces the data. The model was not able to reproduce whole set of the observed anti-Stokes data, suggesting that the molecular-level description is necessary to account for the energy transport in the protein.

4. Advantages of the present technique for studies on intermolecular vibrational energy transfer in condensed phases

Despite extensive experimental and theoretical investigations, intermolecular vibrational energy transfer between two different polyatomic molecules in solution has received very little attention due to experimental difficulties. Vibrational energy transfer between a solute molecule and solvent molecules has been studied by various pump-probe techniques [5-7]. For instance, Iwaki and Dlott studied the vibrational energy relaxation of a methanol-CCl_4 mixture using mid-IR pump and anti-Stokes Raman probe experiments [6]. They excited the C-H and O-H stretching modes of methanol and probed the low-wave number Raman bands of solvent. In these studies, however, the averages of energy transfer processes to many solvent molecules were observed. Selective observation is impossible for energy transfer between a specific pair of solute and solvent molecules for which distance and orientation are well-defined.

An attempt was made to observe the energy transfer between a pair of molecules by utilizing molecular heater-thermometer integrated systems, where two different molecules, namely, a heater that absorbs the visible radiation and a thermometer that probes the temperature by changing the absorption in the vicinity of its hot band, are brought into proximity by covalently linking the two discrete molecules [8-10]. However, for systems with longer linkers, the flexibility of the linker makes it difficult to keep the distance and relative orientation between the heater and thermometer molecules well-defined.

In hemeprotein, the distance and relative orientation between heme and the amino acid residues are well characterized based on X-ray crystallographic data, and the distance between heme and the amino acid residues can be as long as 20 Å. It is possible to observe how the energy deposited to the heme migrates to surrounding residues by measuring the anti-Stokes band intensities of the residues. Excess energy as great as 25000 cm^{-1} can be deposited into the heme by photoexcitation via the Soret transition. Moreover, we can investigate distance dependence of energy flow from the heme by introducing the probe residue at the desired position by site-directed mutagenesis. Accordingly, studies using the present technique based on hemeproteins will provide us new insights for understanding the mechanism of vibrational energy transfer in condensed phases.

5. References

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