A study of distance dependence on vibrational energy flow in proteins taking advantage of the periodic character of α helices

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We investigated vibrational energy transfer from heme in heme proteins by observing intensity changes of anti-Stokes Raman spectra of a tryptophan (Trp) residue [1-2]. In our previous study, we observed energy transfer using Trp residues at different distances from heme in globular proteins [1]. However, it is impossible to observe distance dependence without altering orientation between heme and Trp, because globular protein has complex folding structure. In this study, we systematically observed distance dependence on energy transfer in protein taking advantage of the periodic character of α helices.

Cytochrome b_{562} has four parallel helices (Figure 1a). Taking advantage of the periodic character of α helices, distance between heme and Trp can be changed with equal intervals by introducing a Trp residue to one-turn separated positions of the same helix. The schematic structures of the cytochrome b_{562} mutants are shown in Figure 1b. Anti-Stokes UV resonance Raman (UVRR) spectra were measured using 230 nm probe pulse light after photoexcitation of heme at 405 nm pump pulse.

In time-resolved anti-Stokes spectra of R98W, L94W and A91W, W18, W17, and W16 bands due to the introduced Trp residue were observed at 770, 877, and 1010 cm^{-1}, respectively. In the time-resolved difference spectra of R98W and L94W, positive bands were observed at the bands due to the excited Trp residue from 0 to 30 ps, resulting from the energy flow from heme to the Trp residue. We compared the temporal changes in the anti-Stokes W18 band intensities for cytochrome b_{562} mutants (Figure 1c). The intensity changes of W18 band decreased as the heme-Trp distance increased. The rise of W18 band intensity of L94W was slower than that of R98W. These results are consistent with the prediction from the classical thermal diffusion. However, intensity changes of W18 band of A91W is weaker than those calculated on the basis of a 1-dimensitonal classical diffusion model, which suggests more realistic diffusion model is required to describe the energy transfer in proteins.

References

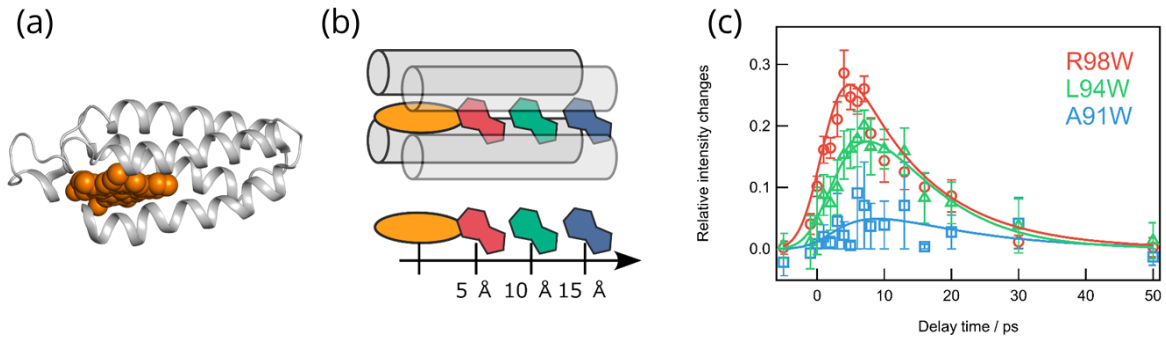

Figure 1. (a) Crystal structure of the cytochrome b_{562}. Orange spheres represent heme. (b) Schematic structure of the cytochrome b_{562}. Heme is shown as an orange ellipse, and Trp is represented by a polygon. The distance between heme and Trp for R98W (red), L94W (green) and A91W (blue) are about 5, 10, 15 Å, respectively. (c) Temporal changes of relative intensity in the W18 bands of R98W (red), L94W (green) and A91W (blue). Solid curves show the best fit using a bi-exponential function convoluted with the instrumental response function.

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