Time-resolved resonance Raman observation of the chromophore structure in primary intermediates of microbial rhodopsins

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Microbial rhodopsins consist of transmembrane seven α-helices and a retinal chromophore covalently linked to the conserved lysine residue. Photoisomerization of the chromophore from all-*trans* to 13-*cis* form and following structural changes in the chromophore trigger structural changes in the protein moiety, leading to various functions. It is crucial to explore primary structural changes in the chromophore following the photoisomerization. In this study, to explore the primary structural changes, we measured time-resolved resonance Raman (TRRR) spectra of the photointermediates appearing within a few picoseconds for two ion-pumping rhodopsins; halorhodopsin from *Natronomonas pharaonis* (*Np*HR), an inward chloride ion pump and *Gloeobacter* rhodopsin (GR), outward proton pump.

Figure 1 shows TRRR spectra of *Np*HR (panel A) and GR (panel B) for time delays from −10 and 100 ps measured with 575-nm pump and 500-nm probe pulses. A top trace in each panel is the spectrum without pump pulse irradiation, representing the spectrum of the unphotolyzed state. The other traces are the time-resolved difference spectra obtained by subtracting the spectral contribution of the unphotolyzed state from the spectra with pump pulse irradiation. In the difference spectra, we observed an unusual doublet feature in the ethylenic C=C stretching region, 1517 and 1595 cm^{-1} for *Np*HR, 1535 and 1597 cm^{-1} for GR, and a strong band at high frequency in the C–C stretching region, 1252 cm^{-1} for *Np*HR, 1254 cm^{-1} for GR at 0, 2, and 5 ps. All of these bands disappeared at 20 ps. The spectra at 20 and 50 ps were close to those of K intermediates both for *Np*HR and GR. The present data show the photocycle involves a primary intermediate which has been spectrally unidentified. Structural features of the chromophore in the primary intermediate will be discussed.

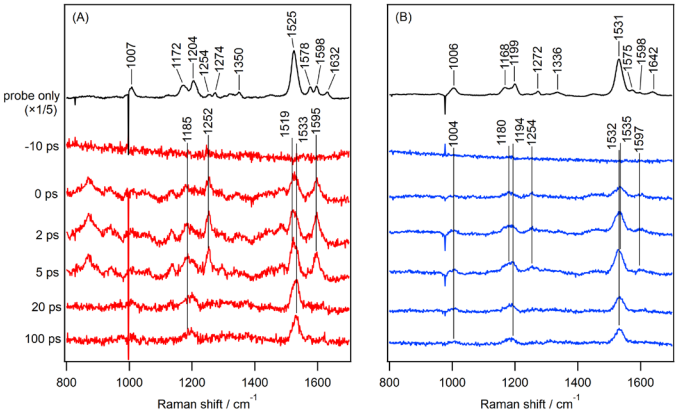

Figure 1. Time-resolved resonance Raman spectra of *Np*HR (A) and GR (B). Probe and pump wavelengths were 500 nm and 575 nm, respectively. The instrumental response time was 6.3–6.5 ps. Black traces represent the spectra of the unphotolyzed state. Red and blue curves represent time-resolved difference spectra at each delay time. The spectrum of buffer has been subtracted from all the spectra.