Vibrational Dynamics in Photoactive Yellow Protein Revealed by Mid-IR Pump / Visible Probe Spectroscopy

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Abstract: Vibrational dynamics of the chromophore in photoactive yellow protein is studied by mid-IR-pump-visible-probe spectroscopy. So-called 'ground state intermediate', which is believed to be a *cis* isomer, is directly generated by vibrational excitation.
OCIS codes: (300.6530) Spectroscopy, ultrafast; (170.1420) Biology

1. Introduction

Photoactive yellow protein (PYP) is a 125-residue, 14 kDa photoreceptor protein isolated from *Ectothiorhodospira halophile*. The chromophore of PYP is a 4-hydroxycinnamic acid which is covalently bound to Cys69 through a thioester linkage. In the ground state, the chromophore is in a deprotonated *trans* form stabilized through a hydrogen-bonding network with Glu46, Tyr42, and Cys69 (see Fig. 1(a)). After photoexcitation, PYP undergoes a photocycle with a number of intermediate states (I_0, I_1, I_2), which involves *trans-cis* isomerization of the chromophore, rearrangement of the hydrogen-bonding network surrounding the chromophore, and large structural changes of the protein. PYP returns from the I_2 state to its initial ground state in a few hundred milliseconds [1]. Pump-dump-probe spectroscopy [2] and visible pump / mid-IR probe spectroscopy [3] suggested the existence of 'ground state intermediate (GSI)', which is formed from the electronic excited state on the different pathway from the I_0-state formation. It has been proposed that the GSI and I_0 are a *cis* isomer, but only the I_0 intermediate has a chromophore with a broken hydrogen bond with Cys69.

In this paper, vibrational dynamics in the electronic ground state of the chromophore in photoactive yellow protein is studied by mid-IR pump / visible probe spectroscopy. Since a protein has large absorption bands in the mid-IR region, it is difficult to selectively obtain vibrational information on the chromophore by using conventional mid-IR pump-probe spectroscopy. Therefore, we use a visible pulse for selective probing of the chromophore structural signature.

2. Experimental

90% of the output from a Ti:sapphire regenerative amplifier (800 nm, 120 fs, 900 mW, 1kHz) was introduced into an optical parametric amplifier (OPA), while a portion of the remaining output was used to generate a broadband probe pulse with a CaF_2 plate. A mid-IR pump pulse was generated by difference frequency generation in a type-I $AgGaS_2$ crystal of the signal and idler pulses from OPA. The spectral width and the pulse energy of the pump pulse used in this study were 150 cm^{-1} and 2 μJ, respectively. The probe pulse after the sample was dispersed onto a linear image sensor with a spectrometer. The output signals were digitized and collected at the repetition rate of the laser system (1 kHz). The mid-IR pump beam was modulated at 500 Hz by a mechanical chopper, which was frequency locked to the laser pulse train.

PYP were prepared as described previously [4]. We used a PYP sample held in a rotation cell with two BaF_2 windows (50 μm of sample thickness) with an optical density of 0.5 OD at 445 nm in heavy water solution.

3. Results and Discussion

A blue line on the top panel in Fig. 1(b) shows the raw data of an absorbance change upon excitation at 1620 cm^{-1}. Both the transient absorption (positive signal) and the ground-state bleach (negative signal) were observed. To obtain the transient absorption spectrum, we subtracted the bleach signal by using the ground-state absorption spectrum (black line). As shown in the bottom in Fig. 1(b), the transient absorption extracted has a peak at 467 nm, which is 1080 cm^{-1} lower than the peak energy of the ground-state absorption. We also measured the transient absorption spectrum upon excitation at 1400 cm^{-1}. However, there is no detectable difference of the spectra between 1620 and 1400 cm^{-1} experiments.

Time profiles of the transient absorption bands measured with pump pulses at 1620 and 1400 cm^{-1} are shown in Fig. 1(c). Both time profiles have rise and decay components. We obtained a rise time of 3.0 ps and a decay time of 6.0 ps for 1620 cm^{-1} pumping, and a rise time of 0.5 ps and a decay time of 5.8 ps for 1400 cm^{-1} pumping.

These results indicate that the vibrational excited states prepared by 1620 and 1400 cm^{-1} pulses relax to the identical state with different time constants depending on the initial state. The relaxed state has a lifetime of about 6

ps, and a characteristic absorption spectrum peaking at 467 nm. These temporal and spectral properties of the relaxed state are quite identical to characteristics of the GSI state reported so far [2,3]. Therefore, it can be concluded that vibrational excitation at 1620 and 1400 cm^{-1} induces distortion of the chromophore, which is likely to be a *cis* isomer, and goes back to the initial equilibrated ground state with a time constant of 6 ps.

4. Conclusion

Mid-IR pump / visible probe spectroscopy is effective to reveal vibrational dynamics in the chromophore embedded in a protein environment. Vibrational excitation at 1620 and 1400 cm^{-1} induces distortion of the chromophore, the structure of which has been suggested to be a *cis* isomer [3].

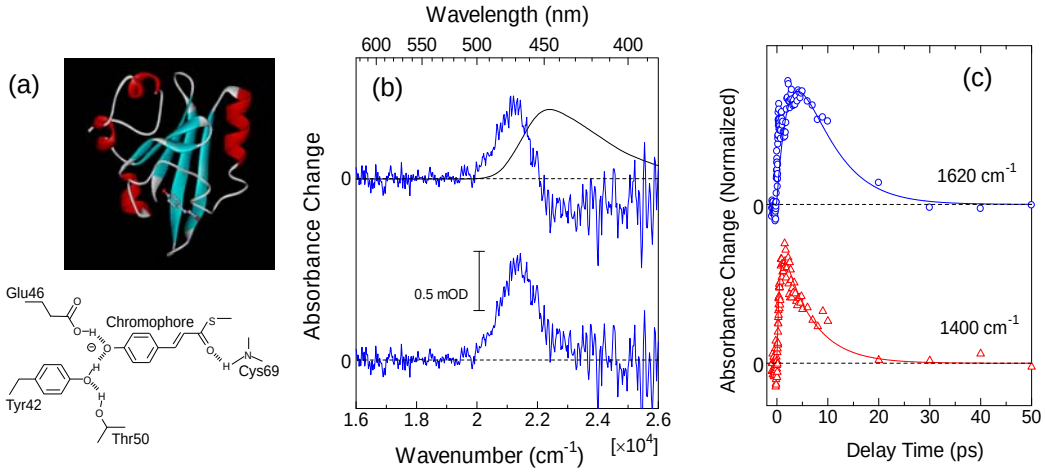

Fig.1. (a) Schematic depiction of the chromophore with its surrounding hydrogen-bonding network for PYP. (b) Top: Raw data of an absorbance change induced by a 1620 cm^{-1}-pump pulse at a delay time of 3.0 ps (blue). Stationary ground-state absorption spectrum (black). Bottom: Transient absorption spectrum obtained by subtracting the ground-state bleach signal (see text). (c) Time profiles of the absorbance changes at 480 nm measured at 1620 cm^{-1}-pump pulse (blue circle) and 1400 cm^{-1}-pump pulse (red triangle). Solid lines are results of fitting.

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