Probing Ultrafast Structural Dynamics of Photoactive Yellow Protein with Femtosecond Time-Domain Raman Spectroscopy

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Abstract: Ultrafast dynamics of photoactive yellow protein was investigated by time-resolved impulsive stimulated-Raman spectroscopy. Time-Domain vibrational data revealed rapid change of the hydrogen-bonding structure in the excited state and vibrational structure of the first ground-state intermediate.
OCIS codes: (300.6500) Spectroscopy, time-resolved; (300.6450) Spectroscopy, Raman

1. Introduction

Photoactive yellow protein (PYP) is a water-soluble, cytosolic protein, which was discovered in a halophilic purple phototrophic bacterium, *Halorhodospira halophila.* PYP is widely considered to function as a blue-light photoreceptor for negative phototactic response of this organism. The function of PYP is realized by a photocycle, which is triggered by the photo-induced trans-to-cis isomerization of the chromophore, p-coumaric acid (pCA). The isomerization reaction has been considered to occur on the femto-to-picosecond time scale, and it gives rise to the formation of the first ground-state intermediate called the I_0 state. The I_0 state eventually transforms into the pR state on the ns time scale, followed by a transition to the long-lived, putative signaling state pB. Since the discovery of PYP, a great deal of effort has been made to elucidate the photocycle and to characterize intermediates, using various experimental techniques, and these studies provided detailed insights into the structure of each intermediate, that is, the conformation of the chromophore, the hydrogen bonding between the chromophore and surrounding amino acid residues, and so forth [1]. However, these experimental approaches have provided information about the events that take place on time scales longer than 100 ps. Therefore, the structural dynamics of PYP on the femto- to picosecond region have been still veiled largely, regardless of its fundamental importance in activating the function.

Aiming to shed new light on the ultrafast primary photoreaction process of PYP, we used time-resolved impulsive stimulated Raman spectroscopy (TR-ISRS [2,3]) to track the structural evolution of PYP from the excited state down to the I_0 state on the femto- to picosecond time scale.

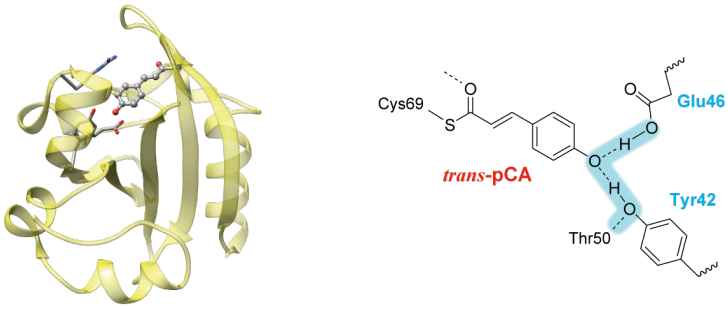

Fig. 1. (Left) Crystallographic structure of PYP. (Right) Chemical structure around the chromophore cavity.

2. Experimental

PYP was dissolved in 10-mM Tris-HCl buffer at pH 7. Time-resolved impulsive stimulated Raman spectroscopy (TR-ISRS) was carried out using a setup based on noncollinear optical parametric amplifiers (NOPA) that were driven by the output of the Ti:sapphire regenerative amplifier (1 mJ, 1 kHz, 780 nm). The output of the first NOPA was used as the actinic excitation pulse (P1, 450 nm, 250 fs). The output of the second NOPA (500-700 nm, 6.5 fs) was divided into two, and they were used as the impulsive excitation pulse (P2) to induce coherent nuclear wavepacket motion in the excited state through resonant impulsive stimulated Raman process and the probe pulse (P3) to monitor the transient absorbance change, respectively.

3. Results and discussion

In the left panel of Figure 2, oscillatory components of TR-ISRS signals measured at various P1-P2 delay times (ΔT) are shown after the subtraction of slowly-varying population components from the raw data. The oscillatory features are due to the nuclear wavepacket motion on the excited state. Fourier transform analysis of these oscillatory components yields frequency-domain vibrational spectra of excited-state PYP at respective delay times (ΔT) as shown in the right panel of Figure 2. The observed vibrational spectrum of the excited state exhibits

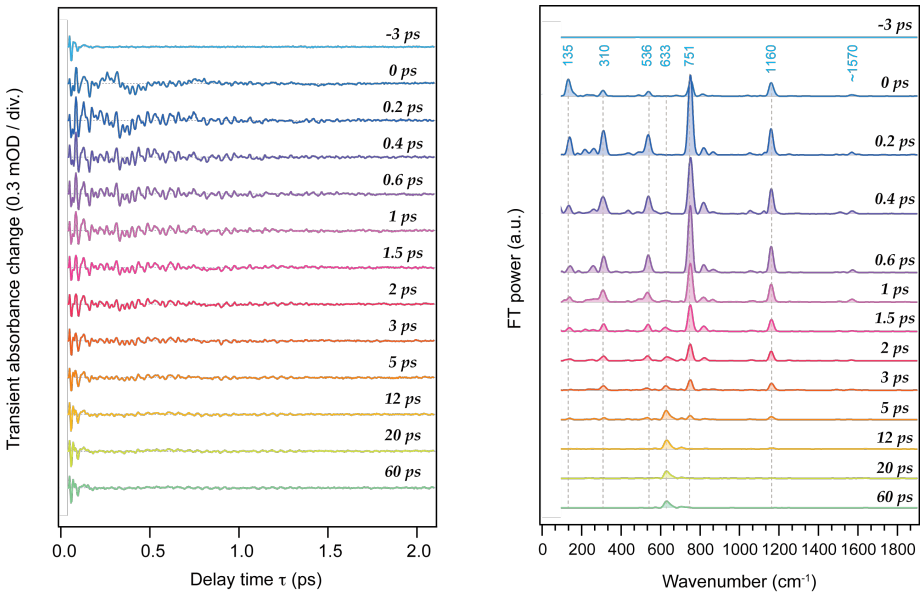

Fig. 2. (Left) Oscillatory components of TR-ISRS signals obtained at various delay time ΔT. (Right) FT power spectra of the oscillatory components.

prominent vibrational features at 1160 cm^{-1} (in-plane bending vibration of C_{ph}-H and O-H of the phenolic part of pCA), 751 cm^{-1} (C_{ph}-O and C_{ph}-C_{et} stretch), and 536 cm^{-1} (phenolic-ring deformation). Furthermore, several noticeable bands were observed in the lower-frequency region (100-400 cm^{-1}). Remarkable feature in the obtained time-resolved vibrational spectra in Figure 2 is threefold. First, although the decay rate constants of the higher-frequency bands are consistent with those of the population decay of the excited state (~2 ps), the 135-cm^{-1} band was found to decay within 1 ps. Second, the 1160-cm^{-1} band showed a gradual upshift by ~4 cm^{-1} towards 5 ps. Notably, the spectral signatures of these bands have been proposed as a sensitive marker of the hydrogen-bonding (HB) structure around pCA. Therefore, the observed dynamics of these bands indicate the rapid change of the HB structure in the excited state upon photoexcitation of the chromophore. Third, a growth of the 633-cm^{-1} band can be clearly recognized in the time-resolved spectra in Figure 2, which could be attributable to the formation of the product I_0 state. The obtained spectrum of the I_0 state exhibits pronounced bands around 630 cm^{-1}, with several weaker bands in the other frequency region. Importantly, the obtained vibrational spectrum of the I_0 state significantly differs from those of the pG or pR state, whose chromophore configuration is planar trans and cis, respectively. This indicates the distinct structure of the I_0 state. All these experimental data reveal the first comprehensive overview of the primary structural dynamics of PYP that includes the ultrafast change of the HB structure followed by the isomerization of the chromophore.

4. References

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