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Fs photo-isomerization in bacteriorhodopsin by few-cycle pulses

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Abstract

We have build up non-collinear optical parametric amplifier (NOPA) laser system with adaptive pulse tailoring, which enables to generate sub-4-fs laser pulses. Using the ultrashort laser pulses generated by NOPA, we have performed pump–probe measurement of bacteriorhodopsin (bR) using ultrashort pulses, which can observe real-time frequencies of molecular vibrations. Both of the spectra of pump and probe pulses are nearly the same and cover a spectral range from 500 to 710 nm with a nearly constant phase. The time-gated Fourier transform of the measured absorbance change trace revealed that the frequency of the C=C stretching mode was modulated along with the torsion motion around $C_{13}=C_{14}$ during the photoisomerization of bR. It shows the bond length of $C_{13}=C_{14}$ is modulated on the deformation around $C_{13}=C_{14}$ during the photoisomerization. The hydrogen-out-of-plane (HOOP) mode and the in-plane C=C–H bending mode coupled with C–C stretching mode are found to be intermixed with each other in the same period of the torsion motion. It also shows us the real-time conformation change of the molecule, which makes both of the modes indistinguishable.
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Keywords: Ultrafast; Real-time spectroscopy; Pump–probe spectroscopy; Difference absorption spectrum

1. Introduction

Photoisomerization of rhodopsin is the key process in light detection system of eyes of living matters. The process was studied for a long time using various measuring methods. To know the details of the process, it is necessary to observe the ultrafast dynamics of the process. Development of ultrashort pulse lasers enabled us to observe real-time dynamics of the chemical reactions. The group of Zewail [1] succeeded to directly observe transition state of a simple molecule during its chemical reaction, and found the dynamical motion of a wave packet along a reaction coordinate and the crossing between two potential surfaces described by the Landau–Zener (LZ) tunneling mechanism [2,3] and more recently in terms of conical intersection of the surfaces [4]. Researches on ultrafast phenomena followed his works using ultrafast laser pulses, and gave us new insight of chemical reactions, which are now being considered as nuclear wavepacket in quantum mechanics. However, the rhodopsin is fragile and easily damaged by ultrafast laser pulses [5–9], which makes it difficult to measure the ultrafast dynamics. Alternatively, the ultrafast dynamics of bacteriorhodopsin (bR) was observed using ultrafast laser pulses, because bR is more rigid than rhodopsin and its photoisomerization process is closely relevant to that of rhodopsin. bR is a retinal protein with the function of a light-driven proton pump in a halobacterial cell membrane. The photocycle of bR is one of the most investigated biological photoinitiated reactions. The primary process in the cycle is the all-*trans* to 13-*cis* isomerization of the retinal moiety of bR. Because of the ultrafast photoreaction of bR, bR is a possible candidate of the optical memory device. The clarification of the ultrafast dynamics of the bR photoisomerization elucidates essential

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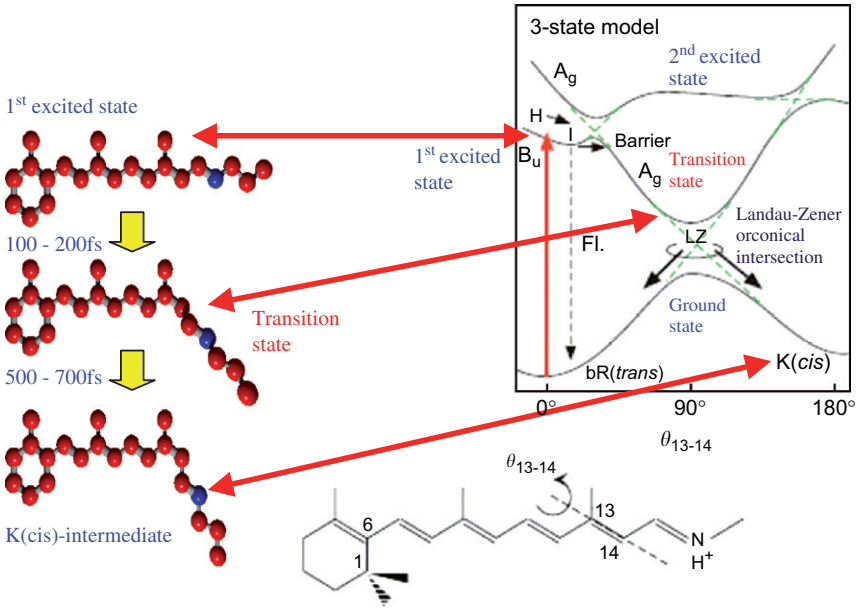

Fig. 1. Schematic diagram of the photoisomerization process of bR.

reaction in bR during the photoisomerization, which helps us to understand the primary process of the vision process and find a possible usage of bR as optical memory device. Many research groups used ultrashort laser pulses to observe the ultrafast functionality of bR, however it is still controversial.

Fig. 1 shows the schematic diagram of the photoisomerization process of bR. As partly shown in the Fig. 1, bR undergoes the following photochemical cycle:

$$bR_{568} \xrightarrow{h\nu} H \rightarrow I_{460} \rightarrow J_{625} \rightarrow K_{610} \rightarrow L_{543} \rightarrow M_{412} \rightarrow N_{560} \rightarrow O_{640} \rightarrow bR_{568}.$$

The subscripts in the photo chemical cycle means the wavelength (in nm) of absorption maxima of the species. H is 1st excited state with a chromophore of an all-trans conformation, which is excited from the ground state (bR_{568}) by Frank–Condon excitation. Researches on the vibrational spectroscopy shows that H and I_{460} are all-*trans* excited state species and K_{610} is a ground state species with a chromophore already transformed into the 13-*cis* conformation. However, the ultrafast conversion from H to K_{610}, which finish earlier than 1 ps, is not known clearly. The group of Atkinson claims that bR is not photoisomerized in the primary process, and the group of Ruhman [10,11] found that locked-retinal in bR also shows photo-induced spectral change, which is similar to that of ordinary bR. The close resemblance in the initial transient spectral evolution of both native and the artificial pigments indicates that the ultrafast process from H to I_{460} dose not involve $C_{13}{=}C_{14}$ torsion, so I_{460} is still on all-*trans* conformation. Furthermore, recent papers claimed that the first event is not the *trans–cis* isomerization but a skeletal stretching from the H state [10–13]. Recent *ab-initio* calculations of photoisomerization dynamics have suggested that skeletal deformation takes place about 50 fs before $C_{13}{=}C_{14}$ torsion takes place [14,15]. In the field of theory, there are some controversial discussions, as a two-state, two-mode model [12,16,17] and a three-state model [18–21] of the photoisomerization of bR are proposed independently. Some research groups performed pump–probe measurements of bR and showed the absence of the spectral change in the stimulated emission spectrum during the period of the excited state lifetime (30 fs–1 ps delay time region) [18–20]. In the measurements of spontaneous fluorescence lifetime, it was found that there is almost no dynamic Stokes shift during the period of the excited state lifetime [22]. If the photoisomerization process of bR follows the two-state model, the K intermediate state should appear during the period of the excited state lifetime, which modifies the stimulated emission spectrum and causes the dynamics Stokes shift. So, these experimental results cannot be explained by the two-state model but instead support the three-state model. In our previous work, we have observed transient absorption signal at several probe wavelengths, and monitored the change of the vibrational spectra of the transition state. It showed that despite photoexcitation of the anti-bonding molecular orbital involved, isomerization does not occur instantly. The experimental result also agrees with the three-state photoisomerization model and firmly discounts the initially suggested two-state model for this process.

2. Materials and methods [23]

In the present work, we have performed pump–probe measurement of bR using ultrashort pulses, which can observe real-time frequencies of molecular vibrations. The ultrashort pulse were generated from our home-made non-collinear optical parametric amplifier (NOPA) [23–26] with

adaptive pulse tailoring, which permits a factor-of-30 compression of the pump pulses from a standard Ti:sapphire chirped-pulse-amplification system. The pump source of the NOPA is a regenerative amplifier CPA1000 (MXR Clark), of which pulse duration, central wavelength, and repetition rate are 120 fs, 790 nm, 1 kHz, respectively. Both of the spectra of pump and probe pulses are nearly the same and cover a spectral range from 500 to 710 nm with a nearly constant phase. The compressor, which was used for the adaptive pulse tailoring, consisted of a diffraction grating telescopic dispersion line, specially designed multiplayer dielectric chirped mirrors, and a computer-controlled flexible mirror. In addition, we have combined the ultrafast pump–probe measurement system with a 128 channel lock-in amplifier, which was developed by our group. From the experimental result measured with the system, we observed real-time spectral change of the difference absorption spectrum, which gives us intuitive view of the wavepacket motion on the potential energy surface of excited state of bR. It enables us to clearly understand the ultrafast photoisomerization process of bR, which has never been observed with the other pump–probe measurements only measuring at several probe wavelengths.

3. Results and discussion

The absorption spectrum of the sample measured in the present work was shown as thick line in Fig. 2(A) with the laser spectrum, which is drawn in thin line. The sample solution of bR was in a 1 mm cell, and has a strong absorption peak around 560 nm, which is well overlapped with the shorter wavelength part of the laser spectrum.

Fig. 2(B) shows the measurement result of the real-time absorbance change spectra, which has 900 data points in time region between −100 and 800 fs with 1 fs step and 128 data points in spectral region between 505 and 665 nm with 1.25 nm step. Black lines show the contour line of $\Delta A = 0$.

In the middle spectral range, from 540 to 600 nm, the sign of ΔA was negative. This spectral range is well overlapped with the peak of absorption spectrum of bR, therefore the negative ΔA signal was caused by the bleaching effect, which means that the ground state was depleted when bR was photoexcited by the pump pulse. The negative ΔA signal decays following the recovery of the ground state depletion caused by the decay of the electronic excited state. In the shorter spectral range, from 505 to 530 nm, the sign of ΔA was positive and its amplitude decays within 100 fs. The positive ΔA signal was caused by the induced absorption of the 1st excited state, as is also assigned in previous works by other groups [18,27,28]. The internal conversion from the 1st electronic excited state to the other intermediate states causes the decay of the positive ΔA signal. In the longer spectral range, from 610 to 665 nm, the transient absorbance change shows negative value ($\Delta A < 0$) in the delay time earlier than ~200 fs. On the other hand, ΔA has positive value ($\Delta A > 0$) in the delay

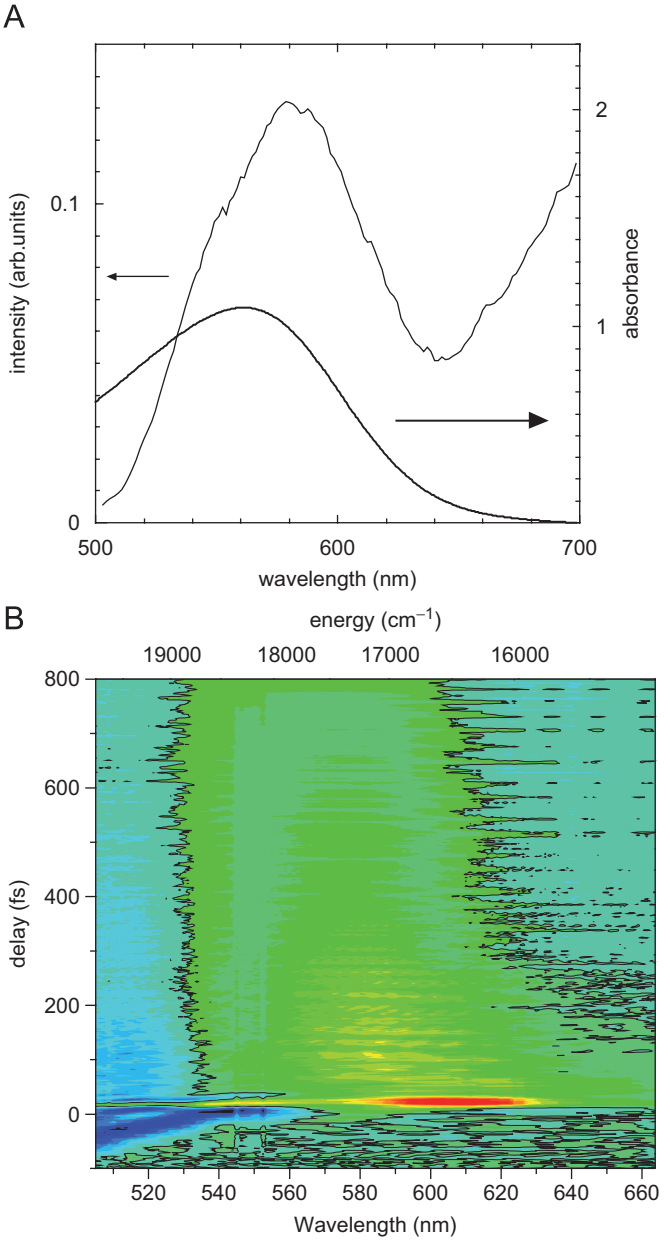

Fig. 2. (A) Laser spectrum (thin line) and absorption spectrum of bR (thick line). (B) Real-time absorbance change spectra, which has 900 data points in time region between −100 and 800 fs with 1 fs step and 128 data points in spectral region between 505 and 665 nm with 1.25 nm step. The contour line of $\Delta A = 0$ is shown as black lines.

time later than ~200 fs. This phenomenon can be explained as follows. Just after the photoexcitation of bR, the ground state depletion appears and decreases the absorbance of the sample compared with the case without pump pulse ($\Delta A < 0$). About 200 fs after the photoexcitation of bR, the 1st excited state which was generated just after the photoexcitation will be transformed into J intermediate state, which causes induced absorption from the intermediate state and makes the sign of ΔA to be negative.

The time traces of the absorbance change spectra show oscillation on the slow dynamics of the decay of the electronic excited state. These oscillating modes are

corresponding to Raman-active modes observed in Refs. [28–32]. The time-gated Fourier transformation of the 2D ΔA spectra at an arbitrary delay time t_d gives the frequency and amplitude of the molecular vibrations at t_d. Therefore, the time-gated Fourier transformation calculated scanning t_d, which is called spectrogram, enables us to directly observe the real-time frequency and amplitude of those vibrations.

Strong signal was observed in the 1500–1550 cm^{-1} region, which corresponds to the molecular vibration frequency of the ethylene-like symmetric C=C stretching mode. In all of the probe spectral range, the frequency of C=C mode was modulated with a oscillation period of around 200 fs. The C=C stretching frequency was also modulated in previous work using 5 fs visible pulses [24–33] and the modulation period was synchronized with the frequency of the C=C–C bending mode. On the analogy of the phenomena observed in the previous work, the modulation observed in the present work can be explained with the torsion motion around the $C_{13}=C_{14}$ bond during the *trans–cis* isomerization around the $C_{13}=C_{14}$ bond. Utilizing an empirical equation representing the relation between the bond length and frequency [34], the real-time bond length of $C_{13}=C_{14}$ in bR during the photoisomerization was calculated from the real-time frequency of C=C stretchig mode, which shows the change of C=C bond length is 40 m Å at most.

In the 800–1050 cm^{-1} region of the spectrogram, we found the hydrogen-out-of-plane (HOOP) modes. And also the in-plane C=C–H bending modes coupled with C–C stretching modes appear in the 1150–1300 cm^{-1} region which is called the fingerprint region. Both of the modes are sensitive to the rotation angle around the C=C bond, that is to say both of the modes become inseparable when the rotation angle is about 90°, which is the intermediate state during the trans–cis photoisomerization. It will well explain that the decreased HOOP mode frequency and the increased C=C–H mode frequency are intermingled in the time-period of the torsional motion around the C=C bond during the *trans–cis* isomerization around the C=C bond. By our recent analysis, it has clarified that the primary process of the photoisomerization of bR starts with a very short-life C=N stretching mode, which reflects the ultrafast deformation of retinal configuration near the protonated Schiff basse and the detail analysis is going to be published.

4. Conclusion

Ultrafast time-resolved spectroscopy of bR was performed by pump–probe method using sub-5-fs visible pulses. The time-gated Fourier transform of the measured absorbance change trace revealed that the frequency of the C=C stretching mode was modulated along with the torsion motion around $C_{13}=C_{14}$ during the photoisomerization of bR. It shows the bond length of $C_{13}=C_{14}$ is modulated on the deformation around $C_{13}=C_{14}$ during the photoisomerization. The HOOP mode and the in-plane C=C–H bending mode coupled with C–C stretching mode are found to be intermixed with each other in the same period of the torsion motion. It also shows us the real-time conformation change of the molecule, which makes both of the modes indistinguishable.

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