Deuterium Substitution Effect on the Excited-State Dynamics of Rhodopsin†

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We investigated the excited-state dynamics of the *cis*−*trans* photoisomerization of rhodopsin by analyzing deuterium substitution effects for hydrogen atoms bonded to C_{11} and C_{12} of the retinal chromophore by the method of Fourier transform of optical absorption spectra (FTOA). Plotting the absolute value of the time correlation function of modified vibrational wave packet, we found that the deuterium substitution effects do not appear in the excited-state dynamics until about 20 fs after photon absorption, weakly appear in the time range 20−60 fs, significantly appear in the time range 70−110 fs, and complicatedly appear in the time range 110−170 fs. By analyzing those deuterium substitution effects, we obtained a result that the concerted motions of hydrogen out-of-plane (HOOP) waggings at C_{11} and C_{12}, which are found to exist in native rhodopsin in the time range 20−60 fs, do not contribute to the excited-state dynamics in its time range appreciably and that the coupled motions of hydrogen atoms at C_{11} and C_{12}, which are significantly coupled with the skeletal twisting motion of the chromophore in the time range 70−110 fs, contribute to the excited dynamics in its time range substantially. The hydrogen motions after 110 fs contribute to the excited-state dynamics in a complicate way. This *cis*−*trans* photoisomerization process of rhodopsin is basically similar to that of bacteriorhodopsin, which was obtained by the comparative analysis of the FTOA of 13-*trans-locked*-bacteriorhodopsin with native bacteriorhodopsin.

Introduction

The pimary process of vision is the *cis*−*trans* photoisomerization of retinal chromophore in rhodopsin. This photoisomerization has some specific features: the isomerization takes place exclusively from 11-cis form to all-*trans* form of the chromophore, the isomerization rate is ultrafast[1,2] (in less than 200 fs) and the quantum yield is high (0.67). Furthermore, the coherent nature of the vibrational wave packet propagation in the excitation continues even after transition to the ground state, suggesting a very smooth propagation of the vibrational wavepackect on the excited potential energy surface.[3] These specific photochemical properties of rhodopsin are not found in retinal in solution. Therefore, it has been considered that the protein environment surrounding the chromophore must play a special role in the photoisomerization.[4−9] However, this photoisomerization process, namely, the excited-state dynamics, is so fast that the detailed mechanism by which the microenvironment of the protein works for realizing this specific, ultrafast, and coherent nature of photoisomerization of rhodopsin is not clarified well up to the present time.

Under these situations, we have developed a new analytical method to catch the molecular reality of the excited-state dynamics in much detail. Theoretical study of the time-dependent description of molecular motions was extensively made by Gordon.[10] He related the time correlation function of the permanent dipole moment of orientation-fluctuating molecules with the Fourier tranform of the infrared absorption spectrum.[10] Later in 1978, Heller formally showed that the optical absorption spectrum can be related to the Fourier transformation of the data of excited-state dynamics of the

† Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; PC, L-α-phosphatidylcholine from fresh egg yolk; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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vibrational wave packet.[11,12] Our method is succeeding to the Heller's method but the procedure is opposite to his. We obtain the information of the excited-state dynamics by a Fourier transform of optical absorption spectra, and so this method is called FTOA. Much detail of the theoretical framework was given by one of the authors,[13] based on the Kubo theory for the Brownian motion. In the FTOA analysis, we use the experimentally obtained optical absorption spectrum of the pigment. Fourier transform of the spectrum is made to obtain the time correlation function (tcf) of the modified vibrational wave packet (transition dipole moment times the vibrational wave function) propagating on the excited-state potential surface. The tcf is generally a complex function.[13] We calculate the amplitude $|C(t)|$ and phase of the tcf. The decrease of $|C(t)|$ shows how far the wave packet propagated from the Franck−Condon state as time elapses. This method has an advantage that we can obtain much accurate information for the position of the wave packet at the shorter time region.[13] This FTOA method was first applied to benzene in cyclohexane and hexane.[13,14] We could visualize a beat of tcf amplitude and phase with a 35−36 fs period due to the breathing vibration of the ring. From these data we could evaluate the vibrational relaxation rate for the breathing mode and that of the total vibrations together. This FTOA method was then extensively applied to rhodopsin[15−17] and bacteriorhodopsin.[18−20] In rhodopsin, the amplitude $|C(t)|$ of the tcf of rhodopsin at 273 K decreased by 3 or 4 orders of magnitude in the initial 20 fs and decreased with a slower slope in the time region 20−170 fs (at most 3 orders decrease in this 150 fs time region).[15] Although no significant structure was seen in $|C(t)|$ until 20 fs, prominent vibrational structures appeared in the time region 20−170 fs. The FTOA analysis was also made for rhodopsin at 93 K.[15] The result for $|C(t)|$ is quite similar to that of rhodopsin at 273 K except that the specific structure is a little modified. Much detailed analysis of the temperature dependence of tcf was made for bacteriorhodopsin.[18]

Theoretical analyses of these $|C(t)|$ were made[16,18] by assuming harmonic approximation for the vibration and using the values of frequencies and displacements of normal coordinates of 25 and 29 vibrational modes, for rhodopsin and bacteriorhodopsin, respectively, which were determined by the resonance Raman scattering experiments.[21,22] The result was that the theoretically obtained $|C(t)|$ of rhodopsin decreased by 1.5 orders of magnitude (the ordinate of the graphs of Figure 2 in ref 16 was erroneously written in $\log_{10}$ unit instead of $\log_e$ unit) in 20−50 fs and remained almost constant after it.[16] Therefore, the experimental data of the initial steep decrease of $|C(t)|$ (about 4 orders of magnitude in 20 fs) and the overall large decrease of $|C(t)|$ by 7 orders of magnitude could not be reproduced by the theoretical calculations based on the simple harmonic oscillators model of vibrations. When we incorporated considerable amount of inhomogeneous broadening (ca. 900 cm^{-1}) with Gaussian form in the absorption spectrum, we could really reproduce the initial steep decrease of $|C(t)|$ in agreement with the FTOA data. However, this steep decrease of $|C(t)|$ continued after it, and $|C(t)|$ became much smaller than the FTOA data after 20 fs. This phenomenon holds true in both of rhodopsin and bacteriorhodopsin. Therefore, the shape of the inhomogeneous broadening cannot be a smooth Gaussian. If we add random noise to the smooth Gaussian curve, the contribution of the inhomogeneous broadening to the tcf stops after a certain time, which is determined by the amplitude of the random noise. This random noise effect on tcf was examined in detail in the previous paper.[15] The random noise might come from the situation that each protein environment of the chromophore can be at a number of conformational substates but the number of these substates is finite. We call this inhomogeneous broadening the random-noise-wearing Gaussian model. This model is favorable to interpreting the FTOA data. If we adopt the random-noise-wearing Gaussian model, the inhomogeneous broadening effect appears only in the steep decrease region of tcf until about 20 fs in rhodopsin and 30 fs in bacteriorhodopsin. The behavior of the tcf after this initial stage reflects the intrinsic property of the excited-state dynamics. This aspect is consistent with the result of the temperature dependence of the tcf of bacteriorhodopsin, whose initial stage had a remarkable temperature dependence, but the tcf in the later stage was nearly temperature independent and had much vibrational structure.[18]

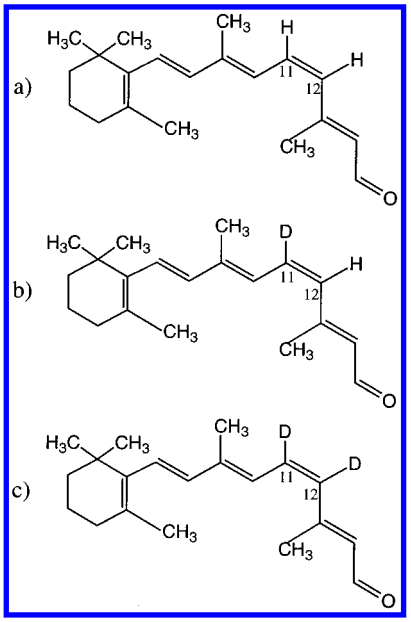

Figure 1. (a) 11-*cis*-Retinal, (b) [11-D]retinal in 11-*cis* form, and (c) [11,12-D_2]retinal in 11-*cis* form.

On the basis of these considerations, we aim to extract the molecular picture of the *cis*−*trans* photoisomerization around C_{11}═C_{12} from the tcf of rhodopsin. For this objective, in this paper, we make the FTOA analysis for the modified rhodopsins where [11-D]retinal and [11,12-D_2]retinal in 11-cis form (see Figure 1) are incorporated into opsin and compare those results with the FTOA data of native rhodopsin. The deuterium substitution for hydrogen atoms at C_{11} and C_{12} must alter the effective mass for the twisting motion around the C_{11}═C_{12} bond. At the stage where the dynamical motion of the twisting around the C_{11}═C_{12} bond becomes large, the deuterium substitutions might cause a significant effect on $|C(t)|$. From such a study, we determine to what extent and in what stage of the excited-state dynamics the hydrogen atoms contribute to the C_{11}═C_{12} bond twisting.

Materials and Methods

Preparation of Retinals. 11-*cis*-Retinal was purified from isomeric mixture produced by irradiation of all-*trans*-retinal in acetonitrile using high-performance liquid chromatography.[23] [11-D]Retinal and [11,12-D_2]retinal in 11-*cis* form were synthesized as described previously.[24]

Preparation of Unmodified Rhodopsin, [11-D]Rhodopsin, and [11,12-D_2]Rhodopsin. Bovine opsin was purified in CHAPS/PC buffer [0.6% CHAPS, 0.8 mg/mL PC, 50 mM HEPES, 20% (w/v) glycerol, pH 6.6] using concanavalin A

affinity colum chromatography as described previously.[25] It was mixed with 3−4 times molar excess of unmodified or deuterated retinal dissolved in a small amount of ethanol, followed by incubation at 23 °C in the dark overnight. Formed unmodified or deuterated rhodopsin preparation was applied to a DEAE-Sepharose column (Pharmacia). It was washed with the buffer supplemented with 10 mM hydroxylamine to remove the excess retinal, and then hydroxylamine was removed by washing the column with the buffer. Rhodopsin was then eluted with the buffer containing 140 mM NaCl. One-twentieth volume of 1 M hydroxylamine (neutralized by NaOH) was added to the sample before the measurements.

Spectroscopy. Absorption spectra were recorded with a Shimadzu MPS-2000 spectrophotometer. The scanning speed and the spectral shift width of the probe beam were set at "slow" and 0.5 nm, respectively. For each sample, 50 absorption spectra of rhodopsin sample or those of the buffer were recorded at 0.2 nm intervals and averaged, followed by calculation of their difference spectra to obtain the precise absorption spectrum of rhodopsin. The temperature of the sample was maintained at 4 °C by circulating ethylene glycol/water mixture. After recording 50 absorption spectra, 1.4% of rhodopsin in the sample was bleached by the probe beam. However, hydroxylamine in the sample prevents the photoproduct from being accumulated by immediately converting it into retinal oxime, which has no absorbance in the α-band region of the absorption spectrum of rhodopsin. Therefore, the inaccuracy caused by the bleach of rhodopsin is less than 0.03%.

FTOA Analysis. Although the optical absorption spectra were obtained experimentally as carefully as possible, further refinement is made by smoothing the spectrum in a moderate level. Then, we subtract the contribution from the β-band and make the extrapolation of the spectrum in such a way suitable for the Fourier transformation as previously reported.[15] The optical absorption spectrum thus refined is used for the Fourier transform.

Results

The observed absorption specra of wild-type rhodopsin, [11-D]rhodopsin, and [11,12-D_2]rhodopsin, and the difference spectra among them in the wavelength region 420−650 nm, which we use in the analysis, are shown in Figure 2, parts a and b, respectively. Here, the amplitudes and wavelengths of the maximum absorption are adjusted to coincide among the three spectra. From Figure 2b, we can see a clear difference in shape of the absorption spectra among these three kinds of rhodopsins, although it is not so much evident in Figure 2a.

The calculated absolute values $|C(t)|$ of the tcf for rhodopsin, [11-D]rhodopsin, and [11,12-D_2]rhodopsin in the time region 0−260 and 0−180 fs are plotted in Figures 3 and 4, respectively. The curve for rhodopsin (solid line) is essentially the same as the one previously reported[15] even if the preparation condition differs substantially. It has remarkable vibrational structures with a rough period of 50 fs. Before 20 fs, no deuterium substitution effect appears. The curve for [11-D]rhodopsin deviates appreciably from that of rhodopsin in the time range 20−60 fs and more evidently in 70−170 fs. The curve for [11,12-D_2]rhodopsin is quite similar to that of rhodopsin until about 60 fs except a small difference at 39 and 42 fs, and it is intermediate between those of rhodopsin and [11-D]rhodopsin after 70 fs. The deuterium substitution effect in [11-D]rhodopsin is larger than that of [11,12-D_2]rhodopsin as a whole. The deuterium substitution effects after 170 fs are not evidently seen.

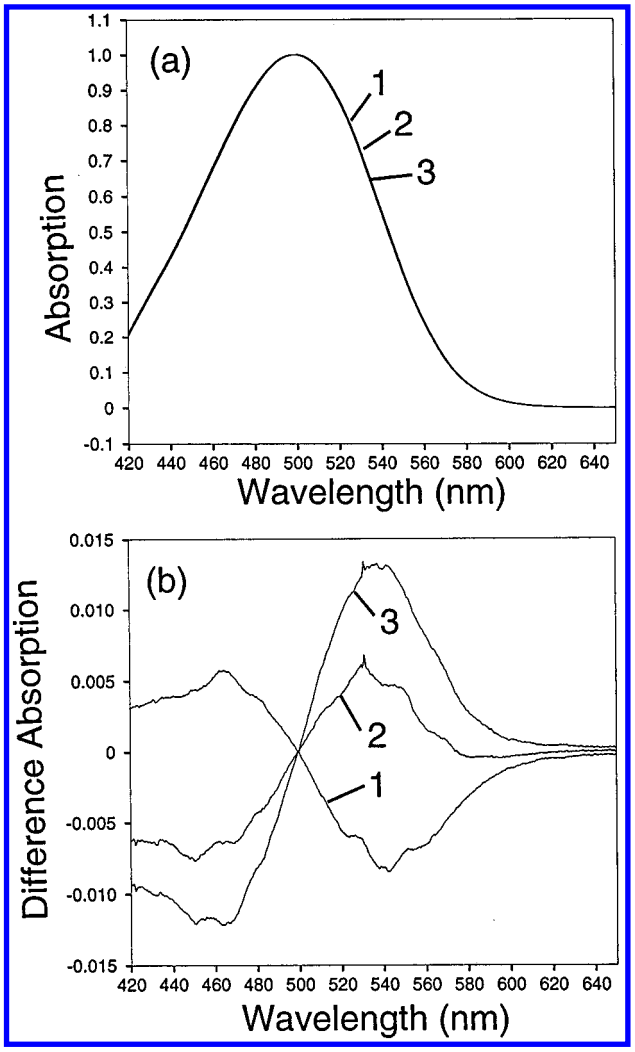

Figure 2. (a) Absorption spectra in the wavelength region 420−650 nm, which we use in the FTOA analysis of wild-type rhodopsin (1), [11-D]rhodopsin (2), and [11,12-D_2]rhodopsin (3). Originally the experimental data of the absorbance were 0.8−0.9 for these spectra. The maximum absorption wavelengths of these spectra were in the range 500.0−499.6 nm. For ease of comparison, the maximum absorption is normalized to 1.0 and the maximum absorption wavelength is adjusted to 500.0 nm. (b) The calculated difference spectra using Figure 2(a): [11-D]rhodopsin *minus* wild-type rhodopsin (1), [11,12-D_2]rhodopsin *minus* wild-type rhodopsin (2), and [11,12-D_2]rhodopsin *minus* [11-D]rhodopsin (3).

The calculated phase of $C(t)$ in the time region 0−260 fs is plotted in Figure 5. Here, the phase is defined as $\Theta(t) = \arctan(\mathrm{Im}\ C(t)/\mathrm{Re}\ C(t))$.

We see that the deuterium substitution effect on the phase is relatively small. No deuterium substitution effect is seen before about 20 fs. In the time range 20−170 fs, the phase propagation of [11-D]rhodopsin is a little decreased compared to that of rhodopsin. After 120 fs, the phase propagation of [11,12-D_2]-rhodopsin starts to deviate from that of rhodopsin. In all the rhodopsins, the slopes of the curves increased abruptly at 170 fs. Generally speaking, as long as the wave packet propagates on the excited-state potential surface, the phase propagation proceeds with a constant rate on average.[13] However, when the wave packet goes into the mixed state between the ground and excited state in the diabatic picture, the phase propagation is greatly perturbed. Therefore, the abrupt change of the phase propagation at 170 fs will indicate that the transition of the modified vibrational wave packet to the ground state start taking place at about 170 fs.

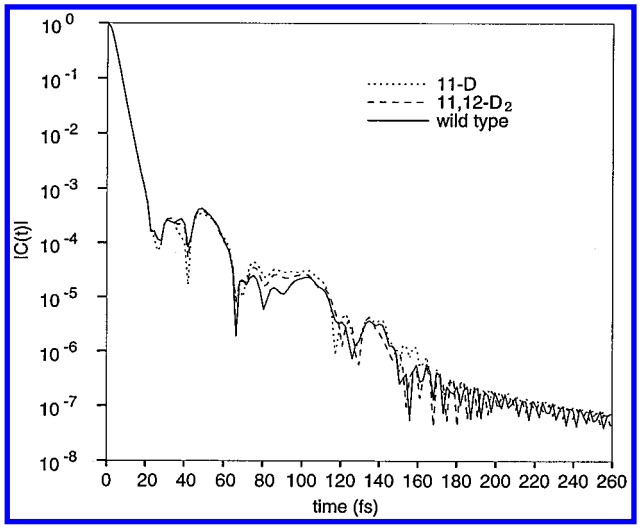

Figure 3. Absolute value of tcf $|C(t)|$ for rhodopsin, [11-D]rhodopsin, and [11,12-D_2]rhodopsin in the time range 0−260 fs.

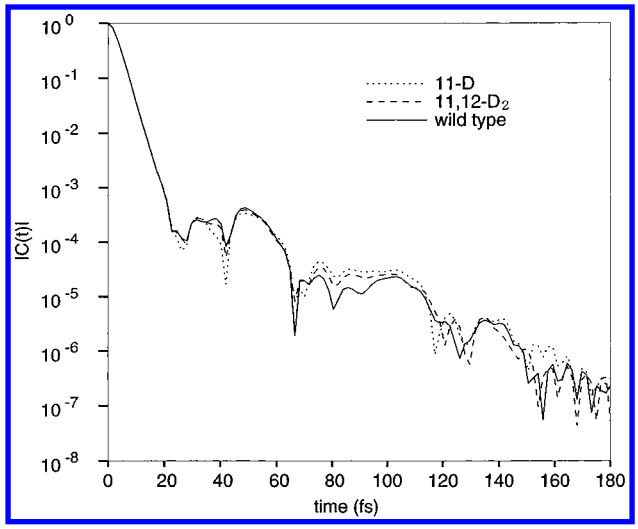

Figure 4. Absolute value of tcf $|C(t)|$ for rhodopsin, [11-D]rhodopsin, and [11,12-D_2]rhodopsin in the time range 0−180 fs.

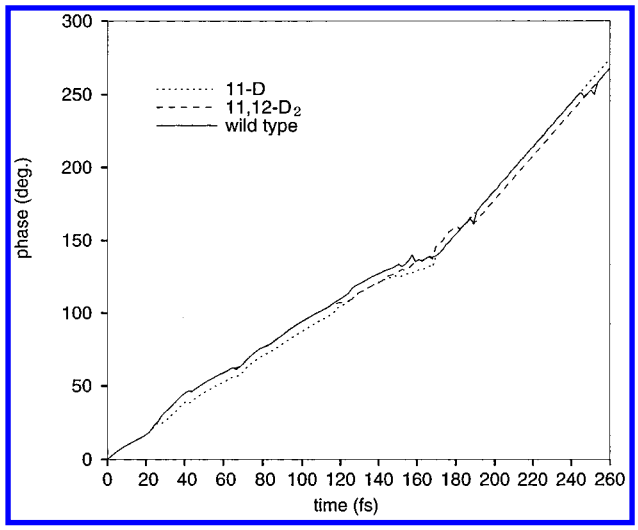

Figure 5. Phase of tcf for rhodopsin, [11-D]rhodopsin, and [11,12-D_2]rhodopsin in the time range 0−180 fs.

While the curve of [11,12-D_2]rhodopsin is similar to that of rhodopsin before about 60 fs, the curve of [11-D]rhodopsin appreciably deviates. This fact would indicate that an isolated motion of the HOOP wagging mode works in the excited-state dynamics while concerted motions of the HOOP wagging modes do not effectively work in the excited-state dynamics before about 60 fs. In the time range 70−110 fs, on the other hand, the curve of [11,12-D_2]rhodopsin is elevated significantly from that of rhodopsin but its effects is still smaller than that of [11-D]rhodopsin. This fact indicates that concerted motions of hydrogen atoms at C_{11} and C_{12} contribute to the total wave packet propagation very well. In the time range 110−170 fs, however, complicated deuterium substitution effect appears in tcf.

TABLE 1: Theoretical and Experimental Values of the Time When tcf Increases or Decreases by the Mono-Deuterium Substitution to the Chromophore of Rhodopsin

theory		experiment	
time (fs)	increase or decrease[a]	time (fs)	increase or decrease[a]
19	↑	(20)	(↑)
27	↓	25	↓
38	↓	38−42	↓
54	↑	60	↑

[a] The up-arrow and down-arrow represent increase and decrease of tcf, respectively.

Discussion

We try to analyze the deuterium substitution effect on tcf before about 60 fs in Figure 4. Although the deuterium substitution effect is not large in this time range, it is reproducible by many ways of manipulating the optical absorption spectra and FTOA procedures. Since the tcf of [11,12-D_2]rhodopsin is almost the same as that of rhodopsin, we are led to consider that the concerted motion of hydrogen atoms in the HOOP wagging mode and the concerted motion of deuterium atoms in the deuterium-out-of-plane (DOOP) wagging mode exist in native rhodopsin and [11,12-D_2]rhodopsin, respectively, play no substantial role in the tcf in the time range until 60 fs. The uncoupled motion of H and D in [11-D]rhodopsin can work in the tcf in the time range 20−60 fs. On the basis of the vibrational analysis of the HOOP mode of bathorhodopsin,[28] we assume that the wavenumber of this uncoupled HOOP wagging motion in the excited state is 880 cm^{-1}. The period is 38 fs. Considering that the mass of the DOOP motion is roughly twice the HOOP motion, we assume that the wavenumber of uncoupled DOOP motion in the excited state is 620 cm^{-1}. Its period is 54 fs. The vibrational wave packet elevated to the Franck−Condon state by photon absorption starts moving along the excited-state potential surface of each mode. The wave packet moves to the farthest place in a half-period of time and comes back to the closest place to the original Franck−Condon place in a period of time. Therefore, the tcf is smallest in a half-period and largest in a period. We consider the difference of tcf of [11-D]rhodopsin from tcf of rhodopsin. Theoretically we expect the increase of the difference of tcf at 19 and 54 fs and decrease of it at 27 and 38 fs. This result is listed in Table 1 and compared with the experimental data read from Figure 4. The theoretical values are almost consistent with the experimental data. Experimentally the increase of tcf at 20 fs is not so obvious, but the tcf has a hump around 20 fs and slight increase of tcf is found when Figure 4 is enlarged. There remains in Figure 4 a slight increase of the difference at 33 fs and a slight decrease of the difference at 49 fs, which were not explained by the above theoretical calculations. Therefore, a slightly more complex thing might happen than the above theoretical model. However, since the basic agreement between

the theory and experiment was obtained, we may say that the deuterium substitution effect on tcf before about 60 fs can be explained mostly by a model that the uncoupled HOOP and DOOP wagging motions in [11-D]rhodopsin take part in the excited-state dynamics substantially but the concerted HOOP and DOOP motions do not take part in appreciably.

The deuterium substitution effect on tcf in the time range 70−110 fs is quite different from the above situation. The level of tcf is lower in the order of rhodopsin, [11,12-D_2]rhodopsin, and [11-D]rhodopsin. The deuterium substitution effect in this time range is always elevating the level of tcf, and its magnitude is much larger than the other time ranges. A consistent interpretation of this result is that coupled motions of the hydrogen atoms at C_{11} and C_{12} take part most significantly in the excited-state dynamics for the time range 70−110 fs. Coupled motions of the deuterium atoms in [11,12-D_2]rhodopsin also take part in the excited-state dynamics, and they are more effective than the uncoupled motions of the hydrogen and deuterium atoms in [11-D]rhodopsin because the tcf level of [11,12-D_2]rhodopsin is lower than that of [11-D]rhodopsin. The reason why vibrational characters of the hydrogen motions change after 70 fs would be that pure HOOP wagging motions are not good vibrational modes in such a later time range but that the wagging and bending motions of the hydrogen atoms would be strongly coupled with the carbon skeletal motion of the chromophore at this stage. Although we cannot say explicitly how much the C_{11}═C_{12} bond is twisted in this time range, it is certain that the coupled motions of hydrogen atoms at C_{11} and C_{12} became coupled strongly with the carbon skeletal motion of the chromophore due to considerable twistings of the C_{11}═C_{12} bond and other bonds.

At this stage, it may be instructive to compare the above data with the resonance Raman scattering data of rhodopsin and bathorhodopsin.[27,28] In the Raman scattering experiment, the concerted HOOP wagging motions at C_{11} and C_{12} were observed in rhodopsin.[27] The uncoupled HOOP wagging motions at C_{11} and C_{12} were observed in bathorhodopsin.[27,28] Apparently the character of the uncoupled motions of hydrogen atoms, which were active in the excited-state dynamics of [11-D]rhodopsin for the time range 20−60 fs, looks like that of bathorhodopsin. However, there is a clear difference between them in the point that deuteration of the two hydrogen atoms had almost no effect on the excited-state dynamics while the Raman spectrum shifted and had considerable intensity. The character of the concerted motions of hydrogen atoms at C_{11} and C_{12}, which were active in the excited-state dynamics of rhodopsin for the time range 70−110 fs, looks like that of rhodopsin in the ground state. However, the motional behavior of the hydrogen atoms is rather different between them, because the hydrogen motions in the former case are thought to be strongly coupled with the skeletal motion of the chromophore while those in the latter case were not.[27]

After 110 fs, the deuterium substitution effect is complicated. This fact will reflect the following situation. In the last stage of the excited-state dynamics, where the C_{11}═C_{12} bond will be twisted very much, the coupling between motions of hydrogen and/or deuterium atoms at the C_{11}═C_{12} bond and motions of the other degrees of vibrational freedom including C_{11}═C_{12} twisting can change as the excited-state dynamics proceeds.

It would be intriguing to compare the present result with that of FTOA analysis for the 13-*trans*-*locked*-bacteriorhodopsin. This bacteriorhodopsin incorporates the retinal analogue where the C_{13}═C_{14} and C_{12}−C_{13} bond twistings are forbidden due to the formation of a five-membered ring with these two bonds.[20] Comparing the tcf of 13-*trans*-*locked*-bacteriorhodopsin with the tcf of native bacteriorhodopsin, we found that the initial decay of $|C(t)|$ until about 20 fs and the following slower decrease until about 80 fs are affected only slightly except for the vibrational structure by locking the C_{13}═C_{14} and C_{12}−C_{13} bond twistings. This initial change of $|C(t)|$ for both native and 13-*trans*-*locked*-bacteriorhodopsins is consistent with the experimental observation of the very quick absorption spectral shift (in within 10−30 fs) after excitation for both of the native and locked bacteriorhodopsins.[29] This experimental data directly testify the reliability of the FTOA analysis. The locking effect gradually appears after about 80 fs until 200 fs where $|C(t)|$ decreases by about 1 order of magnitude. This result indicates that the C_{13}═C_{14} bond twisting does not occur appreciably in less than about 80 fs even in the native bacteriorhodopsin and it occurs substantially after 80 fs. These results are consistent with the results obtained in the present study on rhodopsin. It is reasonable that the similar molecular mechanism works between the excited-state dynamics of rhodopsin and that of bacteriorhodopsin.

Recently detailed molecular models of the structure of visual pigments were presented by three groops.[30−32] Those models accommodate basically the previous model of Birge et al.[33,34] for the active site of the *cis*−*trans* isomerization reaction. Namely, the carboxylate anion was put in the proximity of C_{12} as well as of C_{13}, C_{15}, and the Schiff base nitrogen. We guess that this carboxylate anion will contribute to give rise a highly favorite potential energy surface for the *cis*−*trans* isomerization of rhodopsin. To make this picture concrete, the FTOA analysis for rhodopsin mutant produced by the site-directed mutagenesis is useful. This problem is under investigation.

Recently ab initio molecular orbital calculations were made for the excited-state minimum energy paths of the *trans*−*cis* isomerization reaction of a short protonated Schiff base polyene.[35] They showed that the central double bond is greatly prolonged first after excitation and the central bond starts twisting after the maximum elongation of the double bond is attained. If this property of the short polyene applies to the long polyene as well, we can expect that the very rapid drop of $|C(t)|$ in rhodopsin until about 20 fs may partly correspond to the rapid elongation of the double bond of the chromophore. However, we need more analysis using suitably modified rhodopsins to confirm it.

The fluorescence of rhodopsin containing eight-membered-ring-retinal decayed with a time constant of 60 fs and almost completely decayed in 200 fs, and its time course was independent of the fluorescence wavelength.[2] If we assume that this fluorescence decay process corresponds to the *cis*−*trans* photoisomerization process of eight-membered rhodopsin, its excited-state dynamics proceeds with a much more rapid (about twice) rate than that expected by the present FTOA analysis of rhodopsin. This fact indicates that the *cis*−*trans* photoisomerization of rhodopsin will proceed more slowly (with about a half-rate) than that of eight-membered rhodopsin. The fs up-conversion experiments on rhodopsin are now under investigation.

So far, many experimental studies have been done for the excited-state dynamics of retinal proteins. Most of them were resonance Raman scattering methods,[21,36] femtosecond pump−absorption probe method,[1,3,29,37] and up-conversion fluorescence method.[2,38] Among them, the resonance Raman scattering spectra provide information as to the motion of the vibrational wave packet of each mode in the region not as far from the Franck−Condon state. The femtosecond time-resolved absorp-

tion and fluorescence spectra provide information as to the time course of the electronic energy gap biased by the vibrational state under the wave packet propagation in the excited state. In this context, the data analyzed by the FTOA method provide information as to the propagation of the modified vibrational wave packet in the excited state by means of the overlap between the modified vibrational wave packet at the Franck−Condon state and that of the propagating modified vibrational wave packet. Therefore, the FTOA method and the resonance Raman scattering method are useful to obtain direct infomation of the vibrational wave packet propagation. The advantage of the FTOA method is that we can obtain very accurate absorption spectra in the ground state and so we can obtain the information of the excited-state dynamics not only at the very early time region, ca. 20−40 fs, but also up to about 200 fs. Indeed, we observed slow decrease of tcf with much vibrational structure after about 20 fs for rhodopsin in this paper and after about 30 fs for bacteriorhodopsin.[18] On the other hand, there is a disadvantage that we obtain the information of the wave packet propagation of all the vibrational freedoms together. In such a way, the FTOA method may be used in complement with the other methods for the study of the excited-state dynamics.

Conclusion

We made the FTOA analysis of rhodopsins incorporating deuterated retinal chromophores, by adopting the random-noise-wearing Gaussian model for the inhomogeneous broadening. Before about 20 fs, no substantial deuterium substitution effect on tcf was seen. In the time range 20−60 fs, the tcf of [11-D]rhodopsin slightly deviated from that of rhodopsin while the tcf of [11,12-D_2]rhodopsin was quite similar to that of rhodopsin. In the time range 70−110 fs, the deuterium substitution effect was substantial: the amplitude of tcf is smaller in the order rhodopsin, [11,12-D_2]rhodopsin, and [11-D]rhodopsin. In the time range 110−170 fs, the deuterium substitution effect appeared in a complicated way. Analyzing those deuterium substitution effects, we obtained the following results. Before about 20 fs, hydrogen atoms at C_{11} and C_{12} play no significant role in the excited-state dynamics. The concerted motions of HOOP waggings at C_{11} and C_{12}, which are found to exist in rhodopsin in the time range 20−60 fs, do not contribute to the excited-state dynamics in its time range. If the HOOP wagging motions were decoupled by the deuterium substitution at C_{11}, both the HOOP and DOOP waggings contribute slightly to the excited-state dynamics in the time range 20−60 fs. The coupled motions of hydrogen atoms at C_{11} and C_{12}, which are strongly coupled with the skeletal twisting motion of the chromophore in the time range 70−110 fs, play a significant role in the excited-state dynamics in its time range. After 110 fs, most drastic motion of the chromophore including the large amplitude twisting of the C_{11}═C_{12} bond would be taking place. This molecular picture for the *cis*−*trans* photoisomerization dynamics of rhodopsin is qualitatively similar to that of the *cis*−*trans* photoisomerization in bacteriorhodopsin, which we obtained previously by the comparative study of the FTOA of 13-*trans*-*locked*-bacteriorhodopsin with that of native bacteriorhodopsin.[20]

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