Femtosecond Fluorescence Study of the Rhodopsin Chromophore in Solution

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Time-resolved fluorescence spectroscopy was used to study the excited-state dynamics of a protonated Schiff base of 11-*cis*-retinal (PSB11), which plays a central role in vision as the chromophore of rhodopsins. The femtosecond time resolution enabled us to determine the fluorescence lifetime of PSB11 in methanol, which was composed of two components. Both femtosecond and picosecond components are interpreted as being due to the relaxation processes along the isomerization coordinate, which was revealed by the comparative measurements of 11-*cis*-locked 5-membered retinal (PSB5).

Rhodopsin (Rh) is a photoreceptor protein present in the eyes. The primary photochemical process is a cis–trans isomerization of the protonated Schiff base of 11-*cis*-retinal (PSB11; Figure 1a) in the protein.[1] It is well known that the photochemistry of Rh is unique; the quantum yield of the photoreaction (0.67 for bovine Rh)[2] is temperature-independent and is also independent of the excitation wavelength,[3] and its fluorescence quantum yield is very low ($\phi \sim 10^{-5}$).[4] These observations suggest a rapid isomerization along a barrierless excited-state potential surface. In fact, recent femtosecond transient absorption spectroscopy on Rh revealed that the isomerization is an event in the femtosecond regime,[5–7] which is common for other retinal proteins.[8,9] We thus know that one of the fastest reactions in photochemistry occurs in our eyes.

How is such a rapid reaction realized in the protein? We may give an answer by determining the photophysical and photochemical properties of the chromophore (PSB11) in different environments. In rhodopsin, the chromophore interacts specifically with the surrounding amino acid residues that are fixed in the protein coordinate, and both chromophore and the amino acid residues possess little inhomogeneity. On the other hand, in solution, the chromophore–solvent interaction leads to the most stable structure with perhaps greater heterogeneity in the environment.

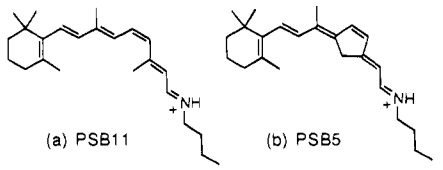

Figure 1. Molecular structures of (a) a protonated Schiff base of 11-*cis*-retinal (PSB11) and (b) 11-*cis*-locked 5-membered retinal (PSB5).

The previous picosecond time-resolved emission[10] and absorption[11] experiments of PSB11 in methanol revealed that the fluorescence decay and product formation are complete within 10 ps. These facts indicate that the fast isomerization observed in rhodopsin is essentially part of the nature of the chromophore itself. However, these experiments could not observe the isomerization processes in the real time scale because of the limited time resolution,[10,11] since femtosecond time resolution is necessary to investigate the isomerization dynamics. In this Communication, we present an approach to elucidate the isomerization dynamics of PSB11 in methanol by means of femtosecond fluorescence spectroscopy. We also performed measurements on a protonated Schiff base of 11-*cis*-locked 5-membered retinal (PSB5; Figure 1b). Since cis–trans isomerization is completely prohibited[12] in this compound, unlike in protonated Schiff bases of flexible 7- and 8-membered retinals,[12,13] the fluorescence decay of PSB5 is due only to relaxation processes, except those along isomerization coordinate.

The PSB samples were formed from 11-*cis*-retinal[14] and the 11-*cis*-locked 5-membered retinal[15] by mixing with excess amount of 1-butylamine, followed by protonation in methanol by addition of HCl.[12] The λ_{max} values are 445 and 466 nm for PSB11 and PSB5, respectively, while the steady-state fluorescence emission spectra (excitation at 444 nm) showed that the emission maxima are located at 660 (PSB11) and 645 nm (PSB5). The fluorescence quantum yields of PSB11 and PSB5 were determined as 2.8×10^{-4} and 1.3×10^{-3}, respectively, by use of a standard dye molecule, fluorescein, in 0.1 N NaOH ($\phi = 0.90$).[16]

A femtosecond up-conversion apparatus[17,18] was used to measure the fluorescence decay of PSB11 and PSB5 in methanol. The PSB samples, which were flowed through a 1-mm path cell, were excited with the pump pulse of 444 nm wavelength. Only about 0.8% of the PSB molecules are excited under these conditions. By combining sum-frequency mixing between the fluorescence from the sample and the femtosecond gate pulse (888 nm), and a single-photon counting, we realized a sensitive detection of the time-resolved fluorescence signal with a femtosecond resolution (fwhm of the instrumental response, 170 fs).

According to the fluorescence emission spectra, we chose five wavelengths covering the whole emission region and applied femtosecond up-conversion. The typical fluorescence

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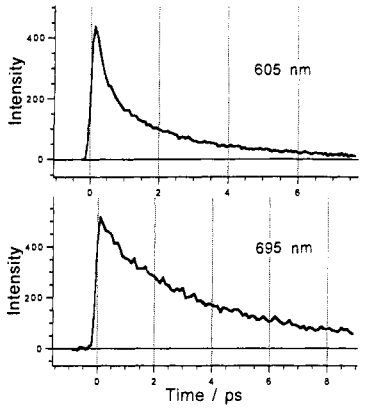

Figure 2. Fluorescence decay kinetics of a protonated Schiff base of 11-*cis*-retinal (PSB11) in methanol. The present instrumental response function possesses a Gaussian shape whose fwhm is 170 fs. The decays of PSB11 are nonexponential and single-exponential at 605 and 695 nm, respectively, and the lifetimes are 0.5 ps (60%) and 2.0 ps (40%) at 605 nm, and 3.1 ps at 695 nm.

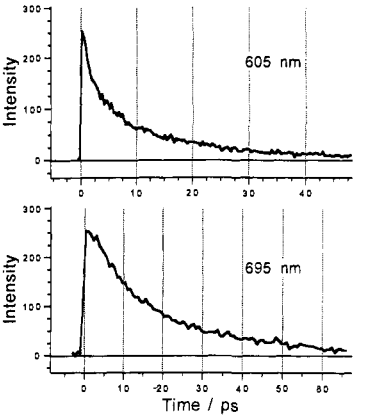

Figure 3. Fluorescence decay kinetics of a protonated Schiff base of 11-*cis*-locked 5-membered retinal (PSB5) in methanol. The decays of PSB5 are nonexponential and single-exponential at 605 and 695 nm, respectively, and the lifetimes are 2.0 ps (55%) and 12.6 ps (45%) at 605 nm, and 15.8 ps at 695 nm.

decays of PSB11 are shown in Figure 2. The decay kinetics of PSB11 are nonexponential or single-exponential at the wavelengths shorter (605 nm) or longer (695 nm) than the emission maximum (660 nm), respectively, and the lifetimes were found to be 0.5 and 2.0 ps at 605 nm and 3.1 ps at 695 nm. The obtained lifetimes for five wavelengths were classified by two features, the fast femtosecond (90−600 fs) and the slow picosecond (2−3 ps) components. We roughly estimated the populations of fast and slow components to be 25 and 75%, respectively. The whole emission from PSB11 diminished within 10 ps, in good agreement with the low fluorescence quantum yield (2.8×10^{-4}) obtained here and the previous picosecond emission study.[10]

The kinetic profiles obtained for PSB11 are very similar to those for the protonated Schiff base of *all-trans*-retinal in methanol (AT-PSB)[17] that is also the chromophore of retinal proteins such as bacteriorhodopsin. In both PSB11 and AT-PSB, the femtosecond component is not due to the intramolecular relaxation from the Franck−Condon state, because a rise component cannot be detected at the longer wavelength side. Thus, the origin of two decay components is possibly the inhomogeneous distribution in the ground state. It should be noted that the lifetimes are ~1.2−1.4 times shorter in PSB11 than those in AT-PSB.[17] Slightly faster decay of PSB11 may be reflected by their molecular structures, namely the initial steric hindrance between C_{10}−H and C_{13}−CH_3 in PSB11 (Figure 1) which may be accelerating the fluorescence decay.

The previous transient absorption of PSB11 revealed that the product formation time due to cis−trans isomerization is <8 ps.[11] It is therefore reasonable that the excited-state dynamics observed for PSB11 is directly coupled to the relaxation processes along the isomerization coordinate. To test the contribution of excited-state isomerization in more detail, we compared the decay kinetics of PSB5 to those of PSB11. Figure 3 shows that the kinetic profiles of PSB5 at 605 and 695 nm look similar to those of PSB11, whereas the lifetimes were about 5 times longer. The fast and slow components of the nonexponential decay at 605 nm were 2.0 and 12.6 ps, respectively, and a single-exponential decay time constant at 695 nm was 15.8 ps for PSB5. The estimated populations were about 25 and 75% for the fast and slow components, respectively, identical to those in PSB11. We therefore interpreted both components of PSB11 to become ~5 times longer by locking the 11-*cis* form, suggesting that both fast and slow components are due to the rotational relaxation around the C_{11}−C_{12} double bond.

Although 11-*cis* is locked, the excited state of PSB5 possesses a very short lifetime (<15 ps). It may be caused by efficient nonradiative relaxation processes from its excited state. In other words, to realize the efficient isomerization in rhodopsin, the isomerization may have to be an event in the femtosecond regime so that the isomerization overcomes the relaxation processes. From this point of view, it is interesting to compare the processes between solution and protein. In methanol, the fluorescence lifetime of PSB5 is 5 times longer than that of PSB11. On the other hand, in the protein, the fluorescence lifetime of a rhodopsin analog possessing 5-membered retinal (85 ps)[12] is probably 2 orders of magnitude longer than that of the native rhodopsin, where the latter was estimated from the transient absorption results.[5] This suggests that the protein moiety enhances the isomerization rates of the chromophore and suppresses alternative relaxation mechanisms that do not follow the isomerization coordinate.

In the present study, femtosecond and picosecond components were observed for PSB11 in methanol, and both appear to be coupled to the relaxation along isomerization coordinate. It is noted that picosecond stimulated emission (2−3 ps) was also observed for octopus rhodopsin[7] as well as the femtosecond processes.[5−7] These facts suggest the similarity of the excited-state dynamics of PSB11 between protein and solution. Nevertheless, the present results show the following differences: (1) the femtosecond component in rhodopsin is much faster (<200 fs)[5] than that in methanol (90−600 fs) and (2) the femtosecond component is predominant in rhodopsin and minor in methanol (~25%). They indicate the role of the protein as the environment of PSB11, probably leading to more efficient isomerization in protein ($\phi = 0.67$)[2] than in solution ($\phi \sim 0.15$).[19] A more detailed study of this will be our future focus.

In conclusion, the present femtosecond measurements determined the fluorescence lifetimes of PSB11 in methanol. The two components obtained are related to the relaxation processes along isomerization coordinate. The present results suggest that the specific protein moiety of rhodopsin aids the comfortable cis−trans isomerization by enhancing the isomerization speed and suppressing the competitive relaxations.

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