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Rhodopsin Emission in Real Time: A New Aspect of the Primary Event in Vision

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Fluorescence from visual rhodopsin is observed in real time. Bovine rhodopsin is excited at 430 nm and its spontaneous fluorescence is detected by up-conversion apparatus with femtosecond time resolution. Fluorescence decay is nonexponential and can be expressed approximately by superposing exponential functions. Further, we have found that the decay shows dependence on monitoring wavelength and becomes slower at longer wavelength. Lifetimes and fractions of components in the decay curves are determined to be 146 fs (80%), 1.5 ps (16%), and 50 ps (4%) and 330 fs (70%), 1.7 ps (22%), and 36 ps (8%) at 578 and 635 nm, respectively. The contributions of the fastest components are much larger than those of the slower ones, which seems to be correlated with the efficient cis−trans isomerization of rhodopsin in the protein environment, and on the other hand, our finding of the slower decay at the longer wavelength seems to suggest a new aspect in the dynamics of primary event in vision as discussed below.

Rhodopsin (Rh), a photoreceptor protein present in our eyes, is an excellent molecular switch to convert a light signal to the electrical response of the photoreceptor cells.[1] The extremely fast and very unique nature of the photochemistry of Rh has been demonstrated on the basis of the large quantum yield of the photoreaction (0.67 for bovine Rh),[2] which is temperature and exciting wavelength independent,[3] the very low fluorescence quantum yield ($\phi \sim 10^{-5}$),[4] the results of the previous ultrafast transient absorption spectroscopy which revealed that the primary photoreaction process is indeed cis−trans isomerization[5] because of no product formation in the case of five-membered rhodopsin, and formation of the primary product occurring within 200 fs.[6] Moreover, it has been confirmed that the cis−trans isomerization yield of the Rh chromophore, protonated Schiff base of 11-cis retinal (PSB 11) in solution (~ 0.15),[7] is much smaller compared with that in protein.[2]

The isomerization in the femtosecond regime is probably achieved by a barrierless reaction from the Franck−Condon (FC) excited state to the primary product by the coherent process in view of the fact that the phase information is confirmed to remain in the primary product.[8] It has been emphasized that the ultrafast coherent process from the excited FC state will lead to the very efficient photoproduct formation.[8] If that is the case, direct observation of the fluorescence in the femtosecond regime, which can probe also dynamics on the excited-state potential energy surface, should give useful information on the reaction mechanisms. Nevertheless, none could have time-resolved the Rh *fluorescence* until now.

On the other hand, studies on the photoisomerization of the Rh analogue possessing 11-cis-locked retinal chromophore with an eight-membered ring (Rh8) have revealed that photoexcitation of Rh8 results in the formation of reaction intermediates corresponding to photorhodopsin and bathorhodopsin with a quantum yield similar to those of Rh[9] and its fluorescence lifetime has been determined by means of the femtosecond up-conversion method to be 60 fs throughout the probed wavelengths, suggesting that the isomerization of Rh8 from the fluorescence state occurs in a coherent manner.[10] Of course, the reaction mechanisms of Rh8 are not the same as those in native Rh. Rh8 does not undergo complete photoisomerization reactions and decomposition but returns to the original ground state from the intermediate state. In addition, 60 fs may be much shorter than the product formation time (<200 fs)[6,8] of the native Rh, which has prompted us to undertake femtosecond fluorescence up-conversion studies on native Rh.

In the present study, bovine Rh was solubilized by 0.6% CHAPS, and purified by concanavalin A-Sepharose column chromatography.[11] Hydroxylamine (100 mM) was mixed before measurement. Absorbance of the Rh sample was 0.75 at 500 nm per 1 mm path length, and the optical purity ($A_{280\ nm}/A_{500\ nm}$) was 1.6−2.2. The Rh sample was flowed through a 1 mm cell and a femtosecond up-conversion apparatus for the measurement of fluorescence dynamics of Rh was similar to that described elsewhere.[12] The full width at half-maximum of the instrumental response was 210 fs. Before the measurement of the native Rh, we examined Rh8 by this apparatus, results of which clearly reproduced the previous measurements.[10] The Rh sample was excited at 430 nm and the sum frequency signal of the fluorescence and the gate pulse (860 nm) was collected for 2 s per each delay time by single photon counting. Absorption spectra were monitored before and after the measurement, and the bleaching of Rh during the measurement was corrected. Irradiation for 1 s yielded 0.12% of Rh bleached under the present conditions. The emission signal from completely bleached sample was comparable to the background level.

Figure 1 shows the fluorescence decay curves of Rh at 578 (a) and 635 nm (b). The decay kinetics are found to be nonexponential and fitted approximately by three exponentials. However, the contributions of the slowest components with a few 10 ps lifetimes are much smaller compared with the most dominant and the fastest components with hundreds of fs lifetimes and those with somewhat smaller contributions and ps lifetimes.

It should be noted that the repeated excitation with 76 MHz for the slowly flowing Rh sample might cause the fluorescence

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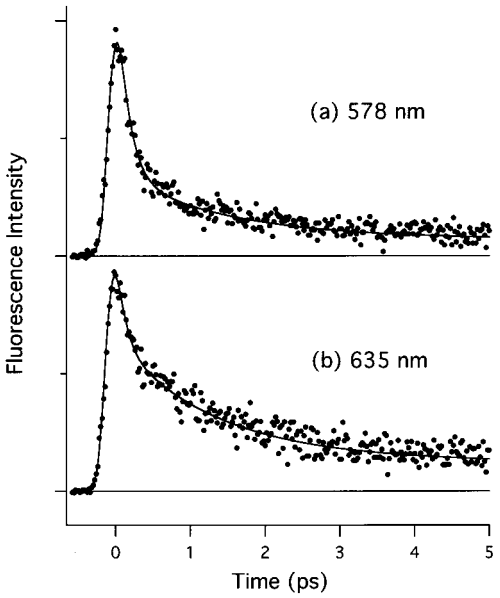

Figure 1. Fluorescence decay kinetics of bovine rhodopsin recorded at 578 (a) and 635 nm (b). Solid lines are best fitting curves, which were obtained by deconvolution procedure with the instrumental response function (fwhm; 210 fs). Fluorescence lifetimes were 146 fs (80%), 1.5 ps (16%), and 50 ps (4%) at 578 nm (a) and 330 fs (70%), 1.7 ps (22%), and 36 ps (8%) at 635 nm (b).

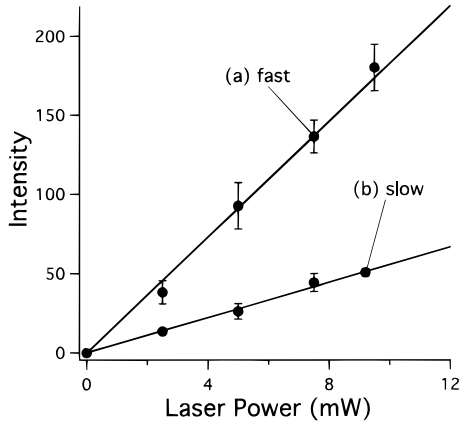

Figure 2. Excitation power dependence of the fluorescence from rhodopsin. Fluorescence intensities observed at 578 nm and at 0 ps (a) and those observed at 635 nm and at 1.5 ps (b) are plotted versus excitation laser power at 430 nm (in mW units).

observation from reaction intermediates of Rh, which prompted us to examine the excitation power dependence of the fluorescence intensity. Namely, if the fluorescence originates exclusively from the excited state of Rh, the linear relationship is expected between the excitation power and the fluorescence intensity. However, if emission from the intermediate state contributes to the observed fluorescence intensity, the power dependence of the fluorescence intensity will be more enhanced than the linear relationship with an increase of the laser power, because the emission from the intermediate state is a multiphoton process. The fluorescence intensities were detected at the peak of the fluorescence time profile and at the delay time of 1.5 ps, respectively, for the observation at 578 (a) and 635 nm (b). The linear relations indicated in Figure 2 warrant that only the Rh fluorescence was detected in our decay curve measurements.

Thus, we have confirmed that the bovine Rh shows the fluorescence decay curves in the hundred fs to ps regime and the decay shows dependence on the monitoring wavelength and becomes slower at longer wavelength. The latter fact indicates the slower reaction processes on the excited state potential energy surface competing with fluorescence transition at the longer wavelength region as discussed below. It should be noted here that even the fastest components of the fluorescence decay curves in the case of the native Rh are considerably slower than the 60 fs decay time observed throughout probed wavelengths for the Rh8 fluorescence dynamics.[10] The difference between Rh8 and native Rh suggests that the presence of the covalent bond around the C_{11}═C_{12} position presumably helps rapid deformation from the original configuration.

At any rate, the fast components of the native Rh might originate from the fast coherent reaction dynamics on the excited-state potential energy surface. Previous transient absorption spectroscopy of Rh detected oscillations in the product ground state, and from their phase analyses ultrafast coherent isomerization was proposed.[6,8] It should be noted here that the fluorescence spectra of Rh observed by a stationary method show a considerable shift depending upon the excitation wavelength according to a recent investigation.[13] Namely, the spectra undergo a considerable blue shift upon shifting the excitation wavelength to a shorter one, which seems to indicate that the fluorescence arises from the vibrationally unrelaxed state in the course of the relaxation process from the excited FC state toward the primary photoisomerization product.[13] Moreover, the study of the excited state dynamics of Rh by means of the time correlation function of the vibrational wave packet produced by the Fourier transform of experimentally obtained optical absorption spectra (FTOA) has led to the conclusion that the reaction takes place at 170 fs after the coherent motion from the excited FC state along the reaction coordinate.[14] However, if such ultrafast coherent reaction is responsible exclusively for the primary process in vision, it is difficult to understand why the fraction of 0.36 of excited Rh remains unreactive. Our results here seem to suggest a possible mechanism to solve this problem.

Namely, the fluorescence decay time of the main component observed at 578 nm (146 fs) is rather close to the formation time of the primary product obtained by fs pump−probe experiment (<200 fs)[6,8] and the lifetime of the coherent wave packet on the excited-state potential surface obtained by FTOA (170 fs).[14] However, the corresponding value observed at 635 nm (330 fs) is considerably longer than those values.[6,8,14] In general, the relaxation process of the coherent wave packet from the excited FC state along the reaction coordinate seems to be coupled not only with the twisting motion but also with many other vibrational modes including high-frequency ones.[14−16] In the almost relaxed (or critically damped) state, the coherent crossing to the product state may become difficult leading to the longer life fluorescence at longer wavelength regions. From such a state, a considerable part of the transitions might lead to the reactant ground-state surface, resulting in the decrease of the photoisomerization yield. It should be noted here that similar observation of the wavelength dependence of the multiexponential fluorescence decay was reported on PSB11 in methanol solution.[17]

To establish the validity of such reaction mechanisms, more detailed and critical studies are necessary and systematic experimental studies including the measurements of fluorescence dynamics with higher time resolutions are now going on in our laboratory.

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