Excited-State Dynamics of a Protonated Retinal Schiff Base in Solution

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The dynamics of *all-trans* and 13-*cis* retinal protonated Schiff base (RPSB) were studied in different solvents by means of picosecond transient spectroscopy. The decay time of the excited state absorption was found to be wavelength dependent due to the contribution of the faster decay of stimulated emission. The stimulated emission has a lifetime of a 2.5−4 ps while the excited state absorption decay is biexponential with lifetimes of 2.5−4 and 10−12 ps. The fluorescence quantum yield is strongly temperature dependent, but viscosity has a small effect on both excited-state lifetime and fluorescence quantum yield. This leads to the conclusion that there is a ∼600 cm^{-1} barrier in the excited-state which results from intramolecular electronic factors and not from the solvent viscosity. The comparison of these results with those for the retinal in rhodopsin and bacteriorhodopsin is discussed in terms of the protein catalysis for the retinal photoisomerization.

Introduction

Retinal protonated Schiff base (RPSB) is the chromophore of retinal proteins that is responsible for vision or light-driven transmembrane ion pumping.[1] The photoexcited RPSB molecule undergoes photoisomerization which initiates energy transduction in visual complexes or bacteriorhodopsin.[1,2] It is known that the photoisomerization of RPSB in protein takes place very fast (200 fs in rhodopsin[3] and 500 fs in bacteriorhodopsin (bR)[4]). It is generally accepted that such rapid and efficient photoisomerization (quantum yield of photoisomerization is 0.6 in bR[5]) is catalyzed by the surrounding protein.[6] The photoproducts of the excitation of *all-trans* RPSB are mostly the 11-*cis* in retichrome and 13-*cis* in bacteriorhodopsin.[7,8] These observations suggest the importance of the protein environment in the retinal photoisomerization process. We showed previously that the rate of photoisomerization in bR can be significantly lowered by specific replacement of the charged amino acid residues within the retinal cavity, while the photoisomerization quantum yield remains unaffected.[9,10] Additional information about photoisomerization of retinal can be obtained by investigating its dynamics in an environment different from that of the protein. In solution, solvent molecules have more degrees of freedom and different surrounding charges compared to the protein media, which implies that the dynamics of RPSB in solution may be significantly different from that in proteins.

The previous picosecond time-resolved emission and absorption experiments of RPSB in methanol[11,12] showed that the fluorescence decay and product formation are completed within 10−15 ps. However, in these experiments, the photoisomerization process could not be observed in real time due to the limited time resolution.[11,12] Recently fluorescence dynamics data of *all-trans* and 11-*cis* RPSB were reported in methanol with femtosecond time resolution.[13,14] It was found[13,14] that fluorescence decay was a nonexponential process at the blue side of fluorescence band (605 nm, 90−600 fs, and 2−3 ps) and monoexponential at the red side of the fluorescence band (695 nm and 3.1 ps). In both *all-trans* and 11-*cis* RPSB, the femtosecond component did not originate from the intramolecular relaxation process from the Franck−Condon state since a rising component cannot be detected at the longer wavelength at longer time. It was concluded[13,14] that the two components are the result of inhomogeneous distribution of molecules in the ground state.

Another explanation of the biexponential decay of the fluorescence may be attributed to the possible efficient coupling between two low lying π, π^* states: the optically allowed $^1B^+{}^*_u$ state and the forbidden $^1A^-{}^*_g$ state. The $^1B^+{}^*_u$ state is believed to be lower in energy in RPSB in solution.[15] The recent studies on the fluorescence of *all-trans* retinal with unprotonated Schiff base (SB)[16] also suggest the existence of two fluorescent excited states, most likely S_1 ($^1A^-{}^*_g$) and S_2 ($^1B^+{}^*_u$) states. The optically forbidden S_1 state of SB is formed from the allowed S_2 state within 1 ps and then decays with a lifetime of 35 ps.[16] Spectroscopic studies suggested[15,17] that protonation of the Schiff base results in a strong mixing of the $^1B^+{}^*_u$ and $^1A^-{}^*_g$ states.

In the present paper, we report the dynamics of *all-trans* and 13-*cis* RPSB in different solvents by subpicosecond transient spectroscopy. We found that the dynamics of stimulated emission of *all-trans* and 13-*cis* RPSB in methanol, acetonitrile, and 1-butanol have lifetimes of 2−3 ps and are significantly shorter than the observed lifetime of the excited-state absorption which was found to be equal to 10−12 ps. This situation is similar to the observed dynamics of photoisomerization of dyes.[18,19] The dynamics of triphenylmethane dyes (TMD) were interpreted in terms of barrierless photoisomerization[20−22] because of the observed negative activation energy and a linear dependence of the excited-state lifetime on viscosity. In contrast to the results on TMD, we found a positive activation energy in the excited state of RPSB and a weak viscosity dependence which suggests the presence of a barrier in the excited state. A comparison of the results observed for PSB in these solvents with those observed in retinal proteins is discussed in terms of the role of protein in changing the dynamics of the retinal photoisomerization.

Experimental Section

The Schiff base of 13-*cis* and *all-trans* retinal were prepared from 13-*cis* and *all-trans* retinal and *n*-butylamine as described previously,[7,8] followed when necessary by protonation of solution by addition of HCl. The isomeric composition of RPSB was checked on an HPLC instrument before the measurements and was found to be 90−95% of the original retinal isomer. The absorption and emission spectra were recorded with conventional absorption (Beckman) and fluorescence (PTI, Inc.) spectrometers, respectively. Variable-temperature measurements were completed by using helium cryostat (CTI-

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TABLE 1: Position of Absorption and Emission Spectra Maxima and Quantum Yield of Photoisomerization for RPSB and SB in Hexane

	absorption, nm		fluorescence, nm		quantum yield of isomerization	
solvent	*all-trans*	13-*cis*	*all-trans*	13-*cis*	*all-trans*	13-*cis*
methanol	447	438	630	630	0.17	0.13
acetonitrile	449	444	650	652	0.27	0.19
1-butanol	453	447	610	603	N/A	N/A
SB in hexane	356		410		<0.01	

Cryogenic). The excitation level for temperature dependence of fluorescence quantum yield measurements was kept as low as possible in order to eliminate the photoproduct accumulation. The laser system was similar to that described previously.[9,10] Briefly, the laser system consists of a commercial Coherent Satori dye laser pumped by an Antares mode-locked YAG laser. As a result, pulses with an energy of about 1 mJ and 350 fs pulse duration were obtained at a wavelength of 595−615 nm at a 10 Hz repetition rate. This output was mixed with the residual fundamental radiation (1064 nm) of a regenerative amplifier in a KDP crystal in order to obtain radiation at 380−390 nm. The output energy at 380 nm was typically about 0.2 mJ.

The optical density of the samples used in the transient absorption measurements was about 1.5−2 in a 2 mm optical path length at the excitation wavelength. The transient absorption setup was described previously.[10] The energy of the pump beam at 380 nm was typically less than 30 μJ, resulting in excitation of 10% of the SB or RPSB molecules. The reference beam had an energy of less than 50 nJ. The reference and probe beams were passed through a monochromator or polychromator and were detected by two photodiodes or a CCD detector (Princeton Instruments, EUV-1024, controller ST-130, respectively). The kinetics were analyzed by the least-squares method.

Results

Steady-State Measurements. The absorption spectra of 13-*cis* and *all-trans* RPSB in methanol, acetonitrile, hexane, and 1-butanol are very similar. They have absorption maximums at 447 nm for *all-trans* and 438 nm for 13-*cis* RPSB. The unprotonated SB has an absorption maximum at 356 nm. The fluorescence spectra of the RPSB samples have slightly different positions and Stokes shifts. *all-trans* RPSB in acetonitrile, methanol, and 1-butanol have a maximum of fluorescence at 650, 630, and 610 nm, respectively. 13-*cis* RPSB has a fluorescence spectrum similar to that for *all-trans* RPSB. The position of absorption and emission maxima for RPSB in different solvents are shown in Table 1. Large Stokes shifts in more polar solvents could be due to large induced dipole moments in the excited state.[23] The decrease in temperature leads to an increase in the fluorescence quantum yield (Figure 1). Generally, the fluorescence yield can be written as

$$\Phi_{fl} = K_r/(K_r + K_{nr} + K_{iso}) \quad (1)$$

where Φ_{fl}, K_r, K_{nr}, and K_{iso} are the quantum yield of fluorescence and the rate constants of the radiative, nonradiative, and photoisomerization processes, respectively. In the case of RPSB, $K_r \ll K_{nr}$, K_{iso}, so

$$(K_{nr} + K_{iso}) \sim K_r/\Phi_{fl} \quad (2)$$

In the proposed simple model, we assume that the rate constant for the nonradiative process has a small temperature dependence and the isomerization rate constant is determined by the presence of a barrier along torsional coordinate:

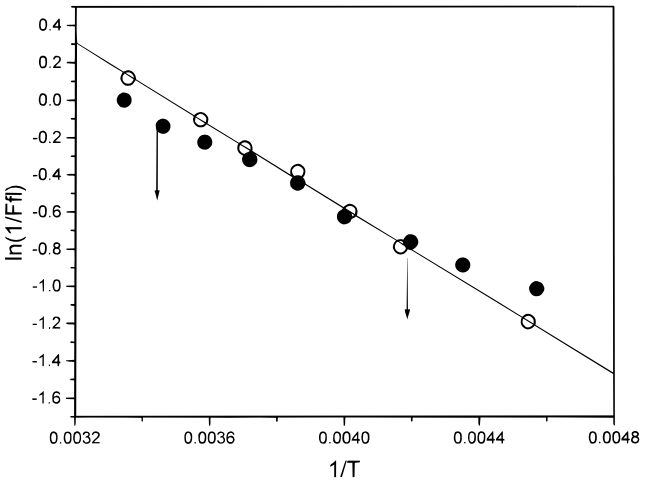

Figure 1. Dependence of $\ln(1/\Phi_{fl})$ of RPSB in methanol (solid circles) and in 1-butanol (open circles) on inverse temperature, where Φ_{fl} is the quantum yield of fluorescence. The solid lines are best linear fits. Arrows indicate the position of the points with equal viscosity for these two solvents.

$$K_{iso} = K_0 \exp(-\Delta G_a/K_B T) \quad (3)$$

where K_0 is the photoisomerization rate constant in the absence of a barrier, ΔG_a is the barrier height, and K_B is the Boltzmann constant. If the nonradiative rate constant is smaller than the rate of photoisomerization, a plot of $\ln(1/\Phi_{fl})$ vs $1/T$ should give a straight line. The slope of the line (corresponding to ΔG_a) is found to be 600 cm^{-1} in the excited state (Figure 1). This barrier, however, could be a combination of the electronic barrier and the viscosity barrier. To evaluate the viscosity dependence of the photoisomerization process we made similar measurements with 1-butanol as a solvent. The data are shown in Figure 1. The slope for 1-butanol ($\sim$760 cm^{-1}) is slightly higher than that for methanol ($\sim$600 cm^{-1}), suggesting a small viscosity effect. The viscosity of methanol and 1-butanol at room temperature are 0.44 and 2.54 cP, respectively, but the quantum yields of fluorescence are almost equal. Assuming that the hydrogen bonding is about the same in these two solvents, one can conclude that the positive activation energy of $\sim$600 cm^{-1} is largely due to the intramolecular electronic barrier present in the excited state of RPSB.

Time-Resolved Measurements. Photoexcitation of *all-trans* RPSB in methanol by 0.3 ps 380 nm laser pulse leads to the formation of broad spectrum in the wavelength region of 450−750 nm (Figure 2). This spectrum consists of ground-state bleach, a positive absorption due to the excited-state absorption, and a negative peak due to stimulated emission. The transient spectrum reflects fast dynamics due to the ground-state recovery, decay of the excited state, and possible formation of the product absorption. Figure 3 shows the kinetics as measured at wavelengths 520 (maximum of excited-state absorption) and 650 nm (maximum of stimulated emission). These two kinetics are not coincidental. The signal due to stimulated emission disappears faster than excited-state absorption decay. Normalized kinetics are shown in the same figure (inset in Figure 2). The decay of the stimulated emission can be fitted to single-exponential process with a lifetime of 3.5 ps. There is also some contribution of a very fast component with a lifetime of <0.3 ps. These lifetimes are close to those observed in time-resolved fluorescence experiment previously,[13] except that we could not resolve the components with a lifetime of 90 fs found in ref 13 due to the limited time resolution (300 fs). The decay of the absorbance changes at 520 nm was fitted to double-exponential kinetic with lifetimes of 3.5 and 10 ps. The fitting parameters are collected in Table 2. A similar effect was

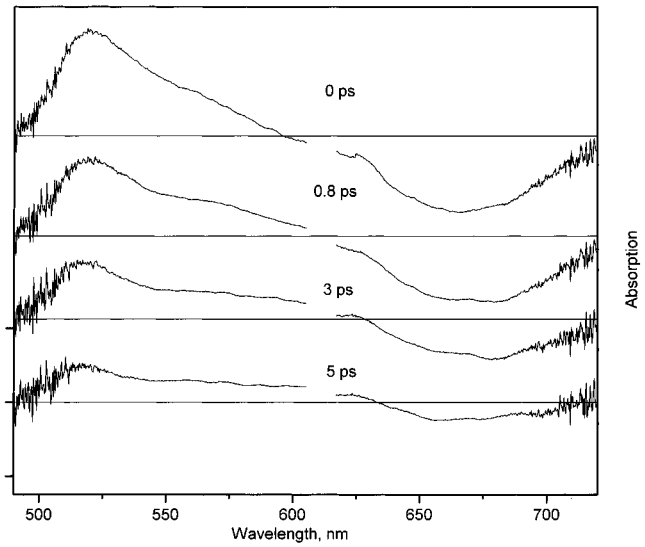

Figure 2. Transient absorption spectra of *all-trans* RPSB in methanol obtained after excitation by a 0.3 ps 380 nm laser pulse.

TABLE 2: Kinetics Parameters of the Fits for Absorption Changes for RPSB in Different Solvents

sample	A_1	A_2	τ_1, ps	τ_2, ps
all-trans, methanol, 520 nm	0.6	0.4	3.5	10.0
all-trans, methanol, 650 nm	0.5	0.5	<0.2	4.2
13-*cis*, methanol, 520 nm	0.7	0.3	2.0	13.0
13-*cis*, methanol, 650 nm		1		2.5
all-trans, acetonitrile, 520 nm	0.7	0.3	2.5	11.0
all-trans, acetonitrile, 650 nm		1		2.9
13-*cis*, acetonitrile, 520 nm	0.5	0.5	3.0	11.0
13-*cis*, acetonitrile, 650 nm		1		3.0
all-trans, 1-butanol. 520 nm	0.6	0.4	3.0	10.0
all-trans, 1-butanol. 650 nm		1		3.0
13-*cis*, 1-butanol. 520 nm	0.65	0.35	2.5	12.0
13-*cis*, 1-butanol. 650 nm		1		2.5

observed with 13-*cis* RPSB in methanol (Figure 4). The decay of the a positive absorption at 520 nm for 13-*cis* RPSB in methanol was also slower than decay of the stimulated emission. In the case of 13-*cis* RPSB, however, the ground-state absorption of the *all-trans* RPSB (photoproduct of 13-*cis* photoisomerization) can contribute as positive signal at wavelength 520 nm.[12,24] This contribution is small and can be observed as positive offset in the kinetics of ΔA_{520} (Figure 5). The best fits for stimulated emission decay and absorption at 520 nm are shown in Table 2, and the respective lifetimes are equal to 2.5 ps for emission and 2.0 and 13 ps for absorption at 520 nm. The values of excited-state lifetimes for 13-*cis* RPSB in methanol are close to that for *all-trans* RPSB.

The different dynamics of the transient spectrum at different wavelengths lead to the slight change of the spectrum shape within a few picoseconds in the spectral range 550−625 nm. This spectral difference can be attributed to two factors, first is the formation of the new intermediate in this spectral range and second is different dynamics of photoinduced absorption at wavelengths of 520−600 nm and stimulated emission signals in the spectral range from 600 to 700 nm. To evaluate the second possibility the transient spectrum at time zero and time delay of 8 ps was compared with steady-state fluorescence and absorption spectra (Figure 6). It can be shown that change of the shape of transient absorption change 0 and 8 ps may be explained by wavelength-dependent dynamics of the absorption changes. It suggests that the only feature observed is $S_1 \rightarrow S_n$ absorption. In any case contribution of the possible "photoproduct" different from the excited state at 520 nm should be minor.

We also studied dynamics of SB retinal in hexane. The positions of the absorption maximum and fluorescence spectra are very different from those of RPSB (Table 2). The photoexcitation by 0.3 ps 380 nm laser pulse leads to the formation of the spectrum shown at Figure 8. This spectrum is similar to that measured previously.[25] Because the position of fluorescence spectra for SB in hexane is very different from that of RPSB, the stimulated emission band is not observed (Figure 7). As it was suggested previously[16] the $^1B^{+}{}^*_u$ (S_2) state converts to $^1A^{-}{}^*_g$ (S_1) states within 1 ps. We did not observed significant changes of the transient spectra in this time scale, which indicates that excited-state absorptions for the $^1B^{+}{}^*_u$ and $^1A^{-}{}^*_g$ states are not very different. The spectrum in the wavelength region from 470 to 750 nm represents an excited-

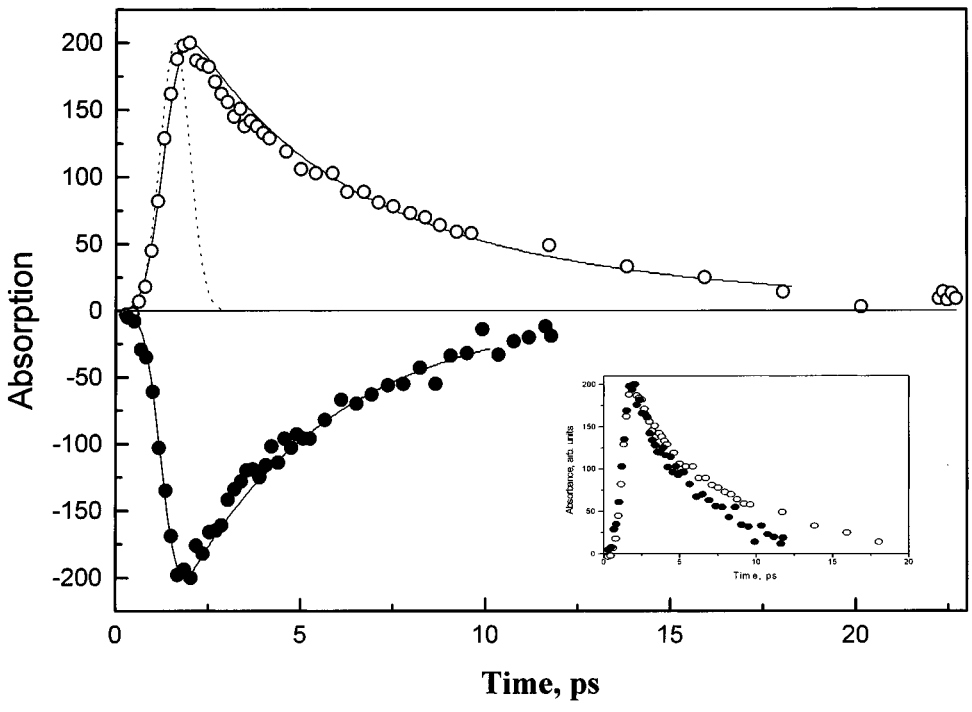

Figure 3. Kinetics of the transient absorption changes of *all-trans* RPSB in methanol at 500 (open circles) and 650 nm (solid circles). The solid lines are best fit with parameters shown in Table 2. Inset shows the comparison of normalized kinetics at 650 and 500 nm.

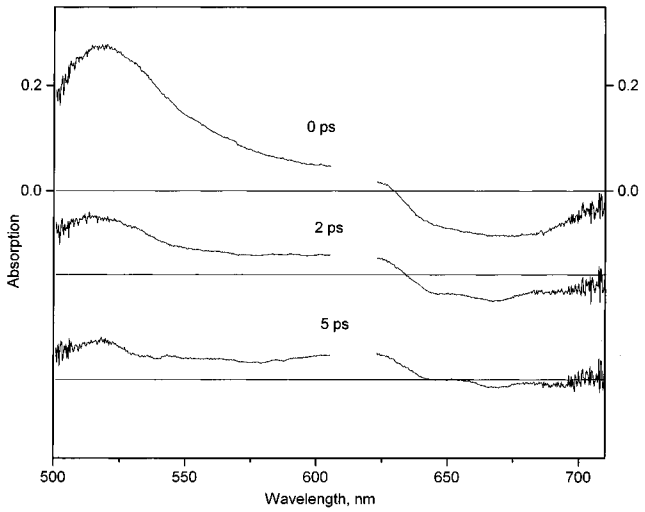

Figure 4. Transient absorption spectra of 13-*cis* RPSB in methanol obtained after excitation by a 0.3 ps 380 nm laser pulse.

state absorption for SB, which is not much different from $S_1 \rightarrow S_n$ absorption for RPSB (Figure 6). The decay of the SB excited state is much slower than that of RPSB and is in a range 30−35 ps, which is in agreement with the fluorescence lifetime measurements studied previously.[16] All these data support an assumption that the 520 nm absorption signal is a singlet excited state.

The temperature dependence of the RPSB stimulated emission lifetime in methanol is shown in Figure 8. The kinetics were fitted as single exponential at all used temperatures. There is a gradual increase of stimulated emission lifetime with temperature decrease. The slop of $\ln(1/\tau_{fl})$ vs $1/T$, where τ_{fl} is stimulated emission lifetime, is very similar to that of fluorescence quantum yield. Taking into consideration that for the single-exponential process $\Phi_{fl} \sim \tau_{fl}$ the activation energy from the slop of $\ln(1/\tau_{fl})$ vs $1/T$ was found to be $\sim$620 cm^{-1} (Figure 8). The data for stimulated emission lifetime measured here are in excellent agreement with fluorescence quantum yield data reported in the previous section.

To study the effect of solvent polarity and viscosity, we used acetonitrile and 1-butanol solvents with viscosities of 0.44 and 2.54 at room temperature, respectively. The dynamics of *all-trans* and 13-*cis* RPSB in 1-butanol and acetonitrile were found to be similar to those in methanol. The fitting parameters of the kinetics are collected in Table 2. Again, the decay of stimulated emission band is treated as a monoexponential process and is faster than the decay of absorption at 500 nm which is represented by a double-exponential process. The decay parameters are similar to those in methanol with some difference in the ratio of amplitudes of the double-exponential process for absorption changes at 500 nm.

Discussion

Generally the electronic states of linear polyenes, e.g., retinal, and its Schiff bases are similar to those for an idealized *all-trans* polyene with C_{2h} symmetry. The two lowest lying π, π^* absorptions are assigned to the optically allowed transition to the $^1B^{+*}_u$ state and to the forbidden transition to the $^1A^{-*}_g$ state.[15b,26] The relative position of these two states depends on the polarity of the medium and protonation of the Schiff base of retinal.[15b] It was also suggested[17,27] that significant mixing between these two states can occur. The effect of protonation of the SB causes the $^1B^{+*}_u$ state to be the lowest of the two.[15b]

The transient spectrum observed immediately after excitation by a 350 fs laser pulse is a $S_1 \rightarrow S_n$ transition. The photoproducts formed in a few picoseconds could be a triplet state of *all-trans* RPSB (or its isomer), an excited state of the isomer of *all-trans* RPSB, a "hot" ground state of *all-trans*, or its isomer. However, the quantum yield of RPSB triplet state formation was reported to be very low[25] in contrast to the retinal, where the triplet state yield is about 0.7.[27] The product of photoisomerization (9-*cis*, 11-*cis*, or 13-*cis*) has an absorption maximum in the 430−450 nm wavelength region and does not contribute to the absorption in the 460−750 nm region. The quantum yield of photoisomerization of RPSB is typically lower than 0.2 and depends on the solvent.[7,8] Taking into account the fact that the shape of the transient spectra does not change during its lifetime (Figure 2 and Figure 6), one can suggest that no other intermediates are formed with absorption in the 500−750 nm range. An additional argument to support this statement is the similarity of the excited-state absorption of SB retinal

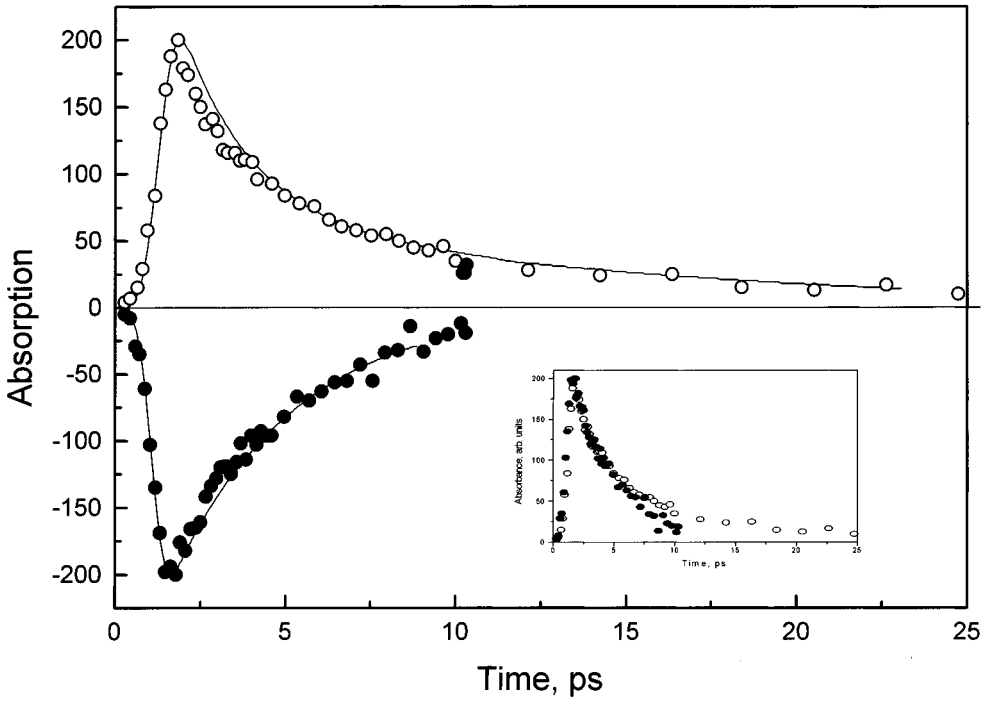

Figure 5. Kinetics of the transient absorption changes of 13-*cis* RPSB in methanol at 500 (open circles) and 650 nm (solid circles). The solid lines are best fit with parameters shown in Table 2. Inset shows the comparison of normalized kinetics at 650 and 500 nm.

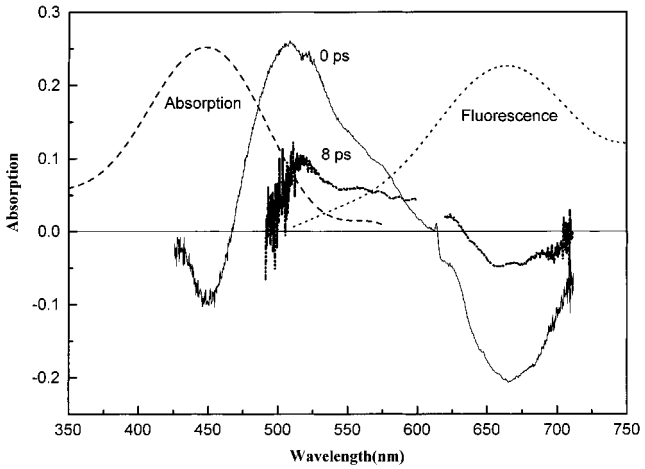

Figure 6. Transient spectrum of *all-trans* RPSB in methanol obtained at time zero and 8 ps after excitation with a 0.3 ps laser pulse at 380 nm and ground-state absorption and emission spectra of RPSB.

(which does not have a stimulated emission band in the 500−750 nm wavelength region, Figure 8) with that of RPSB (Figure 6).

The main results of the present paper can be summarized as follows: (1) Different dynamics are observed for the stimulated emission and the $S_1 \rightarrow S_n$ absorption for RPSB. (2) The excited-state dynamics of RPSB are almost independent of the solvent used (methanol, acetonitrile, or 1-butanol). (3) The fluorescence quantum yield and stimulated emission lifetime were found to have a strong temperature dependence. (4) An activation energy of ~600 cm^{-1} was estimated for the photoisomerization process in methanol.

The above results suggest that the emission originates from part of the excited-state surface, where the Franck−Condon coupling with the ground state is strong. The wave packet movement along the torsional coordinate decreases the Franck−Condon coupling and results in the red-shift of the fluorescence spectrum. At the same time the excited-state population remains high. The different dynamics for the stimulated emission and the excited state absorption were also observed previously for triphenylmethane dyes (TMD).[18,19] The data for the TMD photoisomerization were interpreted in terms of a barrierless photoisomerization. The nonradiative decay of TMD is thought to result from a near crossing of the ground- and excited-state potential surfaces.[18−22] A strong viscosity dependence in the range from 0.5 to 10 cP was found for TMD with negative electronic activation energy for the photoisomerization process. It was explained by the changes of the thermal distribution of the molecules in the ground state.

For the time-resolved fluorescence measurements of RPSB[13,14] no delay of the fluorescence was observed in the red wing of the fluorescence band. Two components of lifetimes 90 fs and 3.5 ps were observed in the kinetic profiles of the fluorescence with different contributions depending on the probe wavelength. Our observation for the decay of stimulated emission is in general agreement with previously reported.[13] We found that the stimulated emission band decays with a lifetime of 2−3 ps, depending on the sample. The fast 90 fs component could not be observed due to the limited time resolution of our instrument. The absorption decay at 520 nm wavelength was found to be biexponential with lifetimes of 2−3 and 10−12 ps. The relative weight of each component is about 50% at this wavelength. On the basis of our data and the fluorescence results of Kandori et al.,[13] we concluded that isomerization dynamics in the excited-state manifold are responsible for 2−3 ps component and that 10−12 ps is the lifetime of radiationless transition of the excited state in the 90° torsional twist. If one assumes that a barrier height of 600 cm^{-1} is present along the photoisomerization coordinate and the lifetime of fluorescence decay is 2−3 ps at room temperature, then the preexponential factor in eq 3 is equal to $(6.6-10) \times 10^{12}$ s^{-1}. This is compatible to the relaxation rate of the excited state of retinal in bR (2×10^{12} s^{-1})[4] and in rhodopsin (5×10^{12} s^{-1}),[3] where the barrier height may be significantly smaller.

The origin of the fast component of 90−200 fs found in the fluorescence decay[13] is less clear. It was suggested by Kandori et al.[13] that distribution of the fluorescence lifetime may be due to the inhomogeneous distribution of the RPSB molecules in the ground state. It is also possible that the fast component corresponds to the effective mixing of the $^1B^{+*}{}_u$ and $^1A^{-*}{}_g$ states. Another possibility for the double-exponential fluorescence decay at the blue side of the fluorescence band could be due to the presence of the *c-cis* and *s-trans* rotamers[28] as was suggested for diphenylbutadiene.[29] If the absorption spectra of

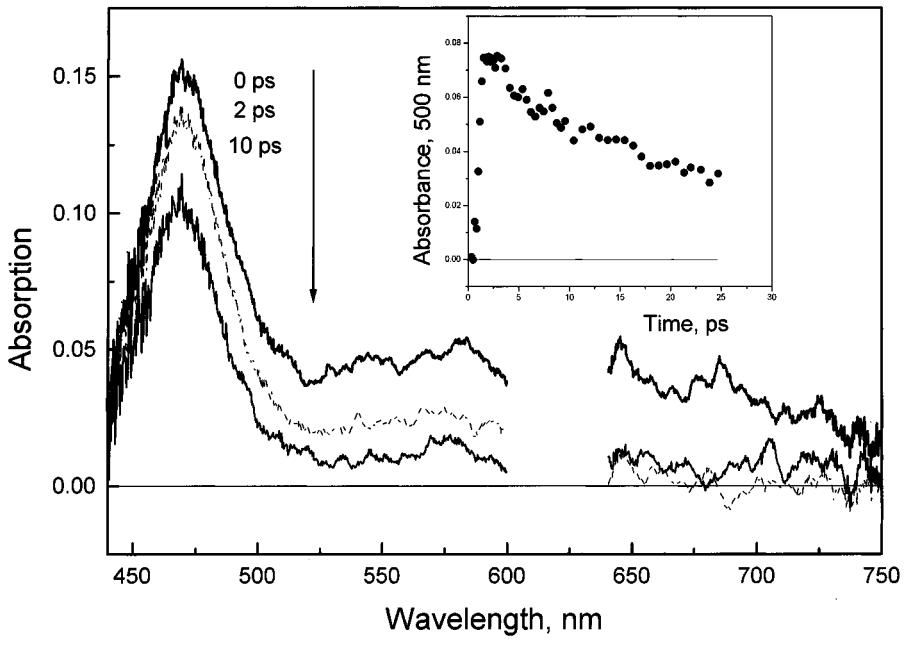

Figure 7. Transient spectrum of *all-trans* SB in methanol obtained at different times after excitation with a 0.3 ps laser pulse at 380 nm. Inset shows kinetics of absorption changes at 465 nm.

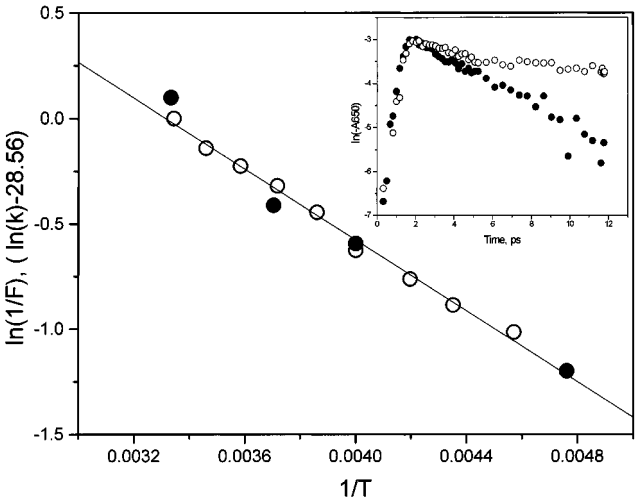

Figure 8. Plot of log inverse lifetime of stimulated emission decay of RPSB in methanol measured at different temperatures vs inverse temperature. The solid line represents the best linear fit, corresponding to the activation energy for photoisomerization process in the excited state. Inset: $\log(-\Delta A_{650}(t))$ measured in methanol at 300 K (closed circles) and 210 K (open circles).

the two rotamers are close to each other, but fluorescence spectra are slightly different, it would explain the observed wavelength-dependent fluorescence dynamics of retinal in solution. A similar behavior was observed for fluorescence dynamics of bR.[30] It is interesting to point out that the preexponential factor in eq 3, 6.6×10^{12} s^{-1}, and the fast component rate of 10^{13} s^{-1} are very close.

The similar results observed in methanol and 1-butanol for *all-trans* RPSB suggest that the viscosity barrier in the excited state is small (Table 2). It could be attributed to the cylindrical shape of retinal molecules, which could lead to a photoisomerization with small volume changes.

It is interesting to compare the photoisomerization process of RPSB with that of retinal in the protein of bacteriorhodopsin (bR). In protein, bR retinal has the $^1B^{+*}{}_u$ state located significantly lower than the $^1A^{-*}{}_g$ state (3000 cm^{-1}).[31] It has been suggested that photoisomerization in bR is barrierless and occurs on a time scale of 0.5 ps[4] (much faster than it is found for RPSB in all solvents studied). The preexponential factors for the wave packet propagation along the isomerization coordinate are very close in solution (6.6×10^{12} s^{-1}, in bR, and rhodopsin 5×10^{12} s^{-1}) assuming a zero activation energy barrier in bR. In these two cases the largest difference may be observed in the rate of the nonradiative transition to the ground-state surface of retinal. In solution, the rate of the excited-state decay is 0.083×10^{12} s^{-1} (slow component). If the decay from the 90° twist is a rate-limiting step of the decay of the excited state of retinal in bR, then this rate constant is much higher (at least 2×10^{12} s^{-1}). This may be a result of different relative position of the excited-state minimum and the ground-state maximum and a larger energy gap between these two surfaces. Moreover, the isomerization in bR is very selective around the C13═C14 double bond and the quantum yield of photoisomerization is about 0.6. In solution the isomerization of RPSB is much slower and is not selective around just one double bond of retinal, and the quantum yield of photoisomerization is at least 2−3 times smaller.

There are at least two reasons for the different rates of photoisomerization and quantum yields for RPSB in solution and in the protein. The first is an electronic factor: a barrier present in the excited state of RPSB in solution which reduces the initial rate of photoisomerization by a factor of 20 (3 ps in solution against 200 fs in bR). The second is a steric factor responsible for the optimal relative position of excited-state minimum and ground-state maximum around a photoisomerization angle of 90° as well as adjusting the energy gap between these two potential surfaces. As a result of protein catalysis as it is indicated by the data of the present paper and by results of dynamics of site-directed mutagenesis in bR,[10] isomerization around C13═C14 bond is catalyzed by the presence of charged groups of amino acid residues. This acceleration of the photoisomerization process around the double bond increases quantum yield and makes the photoisomerization more selective as compared to photoisomerization of RPSB in solution.

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References and Notes

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