Ultrafast Electronic Relaxation and Hydrogen-Bond-Formation/Dissociation Dynamics of Photoexcited All-*trans* Retinal in Protic Solvents

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Received: October 5, 1999; In Final Form: December 9, 1999

The ultrafast electronic relaxation and the hydrogen-bond-formation/dissociation dynamics of photoexcited all-*trans* retinal in 1-butanol/cyclohexane mixed solvents have been studied by femtosecond time-resolved visible absorption spectroscopy. Four transient absorption bands, which can be assigned to the S_3, S_2, S_1, and T_1 states, were observed in neat cyclohexane. The shapes and the dynamics of these absorption bands agree very well with those reported previously for all-*trans* retinal in hexane. In contrast, only three transient absorption bands, which can be assigned to the S_3, S_2, and T_1 states, were identified in the mixed solvents. The band assigned to the S_2 state showed a time-dependent peak shift, which is attributed to solvent reorganization on a picosecond time scale. A kinetic analysis of the three transient absorption bands has led to the conclusion that no state-ordering change of the (n, π^*) and (π, π^*) states takes place in the excited singlet manifold upon hydrogen-bond formation. The 1-butanol concentration dependence of the absorption spectra shows that the free and hydrogen-bonded species coexist in the S_3 and T_1 states, but that all of the retinal molecules are hydrogen-bonded in the S_2 state. These observations indicate that an ultrafast hydrogen-bond-formation reaction takes place during or just after the $S_3 \rightarrow S_2$ internal conversion and is complete within a time scale much shorter than the S_2 lifetime. Dissociation of the hydrogen bonding is most likely to take place during or after the $S_1 \rightarrow T_1$ intersystem crossing and is complete within a time scale much shorter than the T_1 lifetime. The observed longer lifetime of the hydrogen-bonded S_2 state is consistent with the higher isomerization quantum yield in protic solvents than in aprotic nonpolar solvents.

1. Introduction

The electronic structure and photophysical/photochemical properties of retinal strongly depend on its surrounding environment. In particular, the properties of retinal in protic solvents are quite different from those in aprotic nonpolar solvents because of hydrogen bonding. The fluorescence and photoisomerization quantum yields of all-*trans* retinal generally increase upon hydrogen-bond formation, whereas the triplet quantum yield decreases. The fluorescence quantum yields of all-*trans* retinal in hexane and 3-methylpentane have been reported to be 10^{-4} or less;[1−3] all-*trans* retinal has been considered as "nonfluorescent" in aprotic nonpolar solvents. In contrast, in alcohols and in acid solutions, fluorescence is much stronger and is easily detected.[4,5] The reported values of the triplet quantum yield of all-*trans* retinal in nonpolar solvents range between 0.4 and 0.7.[6−9] We recently obtained a value of 0.74 by femtosecond time-resolved ultraviolet absorption spectroscopy.[10] The triplet quantum yield in alcohol solutions is 0.1 or less.[8,9]

The marked solvent dependence of the photophysics of all-*trans* retinal has been ascribed to a change in the ordering of the closely lying singlet excited states that occurs upon hydrogen-bond formation.[1,2,11,12] In aprotic nonpolar solvents, the S_1 state is thought to be an (n, π^*) state (see below). The observed low fluorescence and high triplet quantum yields in aprotic nonpolar solvents are consistent with the (n, π^*) S_1 state, if the π character of the T_1 state[13] is taken into account. On the same basis, the high fluorescence and low triplet quantum yields in protic solvents have been attributed to the optically allowed S_1 (π, π^*) state, which is believed to become lower in energy than the (n, π^*) state upon hydrogen-bond formation. Takemura et al.[2] were the first to discuss the observed increase of the fluorescence and decrease of the triplet quantum yields in relation to the mixing of the (n, π^*) and (π, π^*) states upon hydrogen-bond formation. Papanikolas et al.[11] studied all-*trans* decatetraenal, a model molecule for all-*trans* retinal, in an ethanol/methanol glass and found that the absorption and fluorescence excitation spectra are different from each other. They ascribed the difference to coexistent fluorescent hydrogen-bonded and nonfluorescent free species. They assumed, a priori, that the increase of the fluorescence quantum yield upon hydrogen-bond formation was due to a state-ordering change along the proton-transfer coordinate. Alex et al. discussed, in a similar way, the increase in the fluorescence quantum yield in relation to a state-ordering change.[12] However, the criterion of "fluorescent" and "nonfluorescent" is always equivocal. The fluorescence quantum yield of all-*trans* retinal in methanol is as small as 4×10^{-3},[9] but nevertheless, its laser-excited fluorescence is visible to the eye. All-*trans* retinal in methanol is fluorescent, but such a small fluorescence quantum yield does not necessarily mean that the S_1 state is optically allowed. We also note the fact that the fluorescent excited state is not always

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10.1021/jp993551o CCC: $19.00
Published on Web 02/23/2000

the S_1 state. Generally speaking, stationary spectroscopies, such as those used by the authors cited above, do not give any direct evidence for a possible change in the state ordering. Time-resolved spectroscopies, in contrast, give direct information about the excited-state dynamics that is essential for elucidating the excited-state electronic structure, including the state ordering.

For the free all-*trans* retinal in aprotic nonpolar solvents, a number of time-resolved spectroscopic studies have been reported.[3,10,14−18] Recent femtosecond time-resolved fluorescence up-conversion spectroscopy has confirmed that the S_3 state is the strongly allowed B_u^+ (π, π^*) state.[3] Our femtosecond time-resolved ultraviolet−visible absorption studies[10] have established the relaxation sequence in the excited singlet manifold, which consists of the three singlet excited states, S_3, S_2, and S_1. The S_2 and S_1 states have been identified, beyond any doubt, as the Ag^- (π, π^*) and (n, π^*) states, respectively. In contrast, very few time-resolved spectroscopic studies have been reported so far for hydrogen-bonded retinal in protic solvents. Dawson and Abrahamson[19] measured the methanol concentration dependence of the T_1 lifetime and determined the equilibrium constant between the free and hydrogen-bonded species of T_1 all-*trans* retinal in methylcyclohexane. We measured the Raman spectra of photoexcited all-*trans* and 9-*cis* retinal in methanol and found that the two spectra were identical to each other and similar to those measured for the molecule in hexane.[20] We assigned these spectra to T_1 all-*trans* retinal. Larson et al.[21] measured the femtosecond time-resolved absorption of all-*trans* retinal in ethanol. Although they did not present any transient spectra, they observed the excited-state absorption from both the triplet and the singlet states, the stimulated-emission gain, and the ground-state bleaching signals. The singlet-excited-state absorption induced by two-photon excitation was also measured. They assigned the S_1 state of all-*trans* retinal in ethanol to the A_g^- (π, π^*) state without any experimental grounds. However, the kinetics model they proposed is arguable, because a state with 1.8 ps lifetime is located energetically above a state with a subpicosecond lifetime.

Thus, the hydrogen-bonding interaction of retinal in its excited electronic states is not well-known, including the possible state-ordering change described above. The equilibrium between the free and the hydrogen-bonded species in the singlet excited states has not been studied at all, nor have the hydrogen-bond-formation and -dissociation dynamics. In the present paper, the femtosecond time-resolved visible absorption spectra of all-*trans* retinal in 1-butanol/cyclohexane mixed solvents are presented in order to study the excited-state properties of the all-*trans* retinal in protic solvents. The electronic structure and state ordering of hydrogen-bonded all-*trans* retinal are discussed. The chemical equilibria and dynamics between the free and hydrogen-bonded species in the excited electronic states are also examined. No evidence has been found in favor of the state-ordering change of the (π, π^*) and (n, π^*) states upon hydrogen-bond formation. The free and hydrogen-bonded species coexist in the S_3 and T_1 states, while no free species exists in the S_2 state.

2. Experimental Section

Materials. Cyclohexane and 1-butanol (both HPLC grade) were purchased from Wako Chemicals Co. and used as received. No precautions were taken to dry these solvents. All of the solvents were aerated. Commercial all-*trans* retinal (Sigma Chemical Co.) was used without further purification.

Spectroscopy. The ultraviolet−visible absorption spectra of all-*trans* retinal in 1-butanol/cyclohexane mixed solvents were recorded at room temperature on a commercial spectrometer (Hitachi, U-3500). The concentration of 1-butanol was varied from 0 to 8.56 mol dm^{-3}, while the concentration of all-*trans* retinal was fixed at 1.7×10^{-5} mol dm^{-3}.

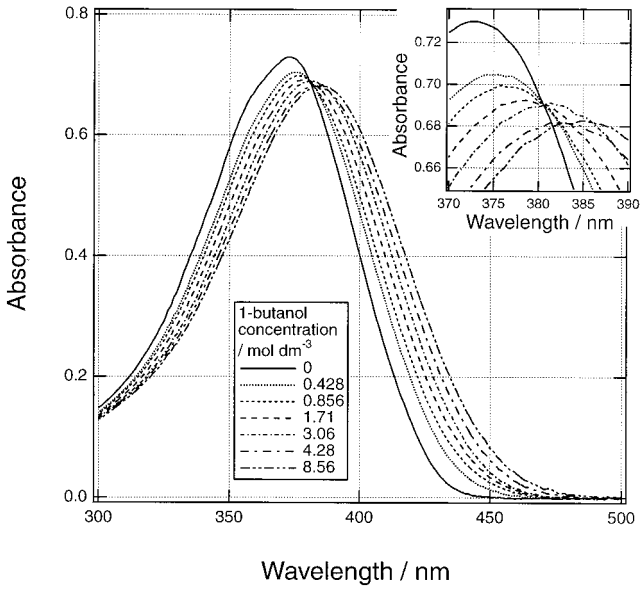

Figure 1. Ground-state absorption spectra of all-*trans* retinal in the mixed solvent 1-butanol/cyclohexane. The 1-butanol concentration, [1-butanol], is indicated in the figure. In the inset, the isosbestic point at 380.6 nm is shown.

The femtosecond time-resolved visible absorption spectra of all-*trans* retinal in the mixed solvents were measured using the femtosecond time-resolved spectroscopic system described elsewhere.[22,23] The pumping conditions used are as follows: center wavelength, 400 nm; pulse energy, 5 μJ; and excitation density, 2×10 J m^{-2}. The pulse energy of the visible wide-band probe pulse was less than 0.1 μJ. A set of time-resolved spectra consisted of 96 time-delay points from −4 to 120 ps. The exposure time of the CCD was 1 s, and the number of the exposures at each time-delay point was 10. The chirp correction was made by using the optical Kerr effect cross-correlation method[23] that we developed previously. The time resolution estimated from the pump−probe cross-correlation time was 0.3 ps. Eleven solutions with different 1-butanol concentrations of 0, 1.78, 3.06, 4.01, 4.76, 5.35, 5.94, 6.69, 7.64, 8.92, and 10.7 mol dm^{-3} were used. The values 0 and 10.7 mol dm^{-3} correspond to neat cyclohexane and 1-butanol, respectively. The molar fractions of 1-butanol are given in the fifth column of Table 1. All of the solutions were prepared with the same retinal concentration (2×10^{-3} mol dm^{-3}) and with the same volume (0.2 dm^3). The temperature of the solution emitted from a jet nozzle was 293 ± 2 K in the vicinity of the focus point of the laser beam.

3. Results and Discussion

Ground-State Spectra. The stationary ultraviolet−visible absorption spectra of all-*trans* retinal in 1-butanol/cyclohexane mixed solvents are shown in Figure 1. In the low-concentration region, where the 1-butanol concentration is between 0 and 3.06 mol dm^{-3}, a clear isosbestic point is found at 380.6 nm. Accordingly, two species must exist in equilibrium in this concentration range. One is definitely the free all-*trans* retinal, and the other is most likely the 1:1 hydrogen-bonded complex of all-*trans* retinal and 1-butanol. In the higher-concentration region, the absorption spectra do not intersect the low-concentration spectra at the isosbestic point. This kind of

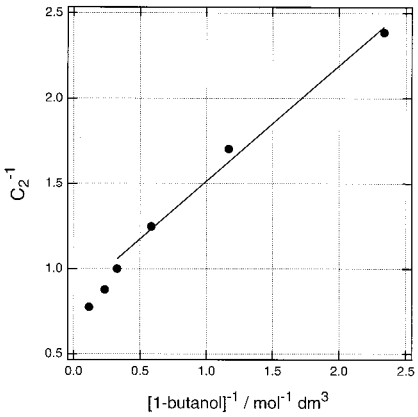

Figure 2. Benesi−Hildebrand plot based on the linear-combination fitting of the absorption spectra in Figure 1 (see text). The data (solid circles) in the low-concentration region were fit to a line to obtain the equilibrium constant.

deviation was also reported by Das and Hug, who used 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), instead of 1-butanol, as the solvent.[4] They suggested that the formation of other species, such as a 1:*n* ($n > 1$) hydrogen-bonded complex or a protonated form, might occur. However, it is also possible to ascribe the superfluous red shift in the higher-concentration region to a solvent environmental effect.[24] In other words, the electronic transition energy of retinal can be influenced to a certain extent by the dielectric constant of the solvent and by intermolecular forces other than the hydrogen-bonding interaction. The absorption spectra in the low-concentration region are well reproduced by a linear combination of the two basis spectra: one is the spectrum in neat cyclohexane, and the other is that in the mixed solvent with a 1-butanol concentration of 3.05 mol dm^{-3}. The first spectrum is ascribed to the free all-*trans* retinal, while the second is ascribed to a mixture of the free and the hydrogen-bonded species. We note that, even at a considerably high 1-butanol concentration such as 3.05 mol dm^{-3}, free all-*trans* retinal coexists with the hydrogen-bonded species because of the weak hydrogen-bonding ability of 1-butanol. The linear-combination fitting procedure yields two coefficients c_1 and c_2 for each mixed-solvent spectrum, where c_1 and c_2 represent the contribution of the two basis spectra at 1-butanol concentrations of 0 and 3.05 mol dm^{-3}, respectively. The isosbestic point guarantees the condition $c_1 + c_2 = 1$. The well-known Benesi−Hildebrand method[25] is modified to give the following equation:

$$c_2^{-1} = \alpha(K^{-1}[\text{1-butanol}]^{-1} + 1) \qquad (1)$$

where α is the ratio of the concentration of the hydrogen-bonded retinal to the total retinal concentration in the mixed solvent at 3.05 mol dm^{-3}. K is the equilibrium constant given by [hydrogen-bonded retinal][free retinal]$^{-1}$[1-butanol]$^{-1}$, where [x] indicates the concentration of species x. The linear relationship between c_2^{-1} and the reciprocal of the 1-butanol concentration (Figure 2) gives the following constants: $\alpha = 0.84$ and $K = 1.2$ dm^3 mol^{-1}. If a stronger hydrogen-bond-forming solvent is used instead of 1-butanol, K becomes larger and α approaches unity. In fact, solvents such as phenol, HFIP, and methanol that form stronger hydrogen bonds give much larger K values of 6 $\times$ 10^3,[2] 4 $\times$ 10^2,[4] and 7 dm^3 mol^{-1},[19] respectively.

Time-Resolved Spectra. Representative femtosecond time-resolved visible absorption spectra of all-*trans* retinal in the mixed solvents are shown in Figure 3 for three 1-butanol concentrations: (a) 0, (b) 1.78, and (c) 10.7 mol dm^{-3}. In these spectra, the positive signals correspond to the photoinduced transient absorptions and the negative signals to ground-state bleaching or stimulated-emission/Raman gains.

The time-resolved absorption spectra in neat cyclohexane (Figure 3a) are nearly identical to those in hexane.[10,27] On the basis of a SVD (singular value decomposition) analysis,[10] we are able to decompose these spectra into four components that can be ascribed to the S_3, S_2, S_1, and T_1 states of the free all-*trans* retinal. The band shapes and dynamics of these SVD-decomposed spectra in cyclohexane are in an excellent agreement with those in hexane that were reported in our previous paper.[10] In the spectra in Figure 3a, the band around 600 nm in the −0.2 ps spectrum corresponds to the S_3 state, which decays within the time resolution of the present experiment. In the SVD analysis, an S_3 lifetime of 30 fs is assumed.[3] The broad absorption feature in the wavelength range of 450−650 nm in the 0.2−0.6 ps spectra is due to the S_2 state. The lifetime of the S_2 state is determined to be 0.7 ps from the SVD analysis. The spectrum at 2 ps corresponds to the S_1 state, whose lifetime is determined to be 34 ps. The intense band at 445 nm in the 40−100 ps spectra is assigned to the T_1 state. Conveniently, we have the four transient absorptions of the free all-*trans* retinal very well separated in the raw time-resolved spectra in Figure 3a.

The analysis of the mixed-solvent spectra is not straightforward because of the failure of the SVD analysis. This failure is caused by the continuous spectral shifts that are involved in the observed time-resolved spectra. A strong absorption band is observed around 550 nm in the mixed-solvent spectra in Figure 3b. The peak of this absorption band shifts continuously from 552 nm at 0.2 ps to 545 nm at 1.0 ps. If such a spectral shift is involved in the time-resolved spectra, SVD gives an intractably large number of singular values and becomes useless. Thus, we need to analyze the time-resolved absorption spectra in Figure 3b,c on a different basis as in the following. First, the spectra in Figure 3b,c are very similar to each other. Only two small differences are notable: the small peak shift of the absorption bands in the first few picoseconds and the intensity difference of the band in the 20−100 ps time range. The peak shift is attributable to the solvent environmental effect similar to that observed for the ground-state absorption. The intensity difference in the 20−100 ps time range is due to the coexistence of the free and hydrogen-bonded species in the T_1 state (see below).

We now concentrate on the spectra in Figure 3b. At −0.2 ps, a broad absorption band is observed in the 550−600 nm wavelength range. This band resembles the corresponding absorption band in Figure 3a and is assigned to the $S_n \leftarrow S_3$ transition. As this S_3 feature decays very quickly, an intense absorption band rises around 550 nm and reaches its maximum intensity at 0.4 ps. The shape of this absorption band is different from that of the band corresponding to the S_3 state, which indicates that a new electronic state is formed after the decay of the S_3 state. We assign the band around 550 nm to the $S_n \leftarrow S_2$ transition. This $S_n \leftarrow S_2$ absorption band is accompanied by negative signals in the wavelength ranges 400−430 and 620−800 nm. In the first wavelength range, ground-state bleaching may contribute to the negative signal, as the longer-wavelength tail of the ground-state absorption exists there. In contrast, the negative signal in the 620−800 nm range is ascribed solely to a stimulated-emission gain. Detection of the stimulated-emission-gain signal in the mixed solvent is consistent with the observation of a much larger fluorescence quantum yield in protic solvents than in aprotic nonpolar solvents. In the time

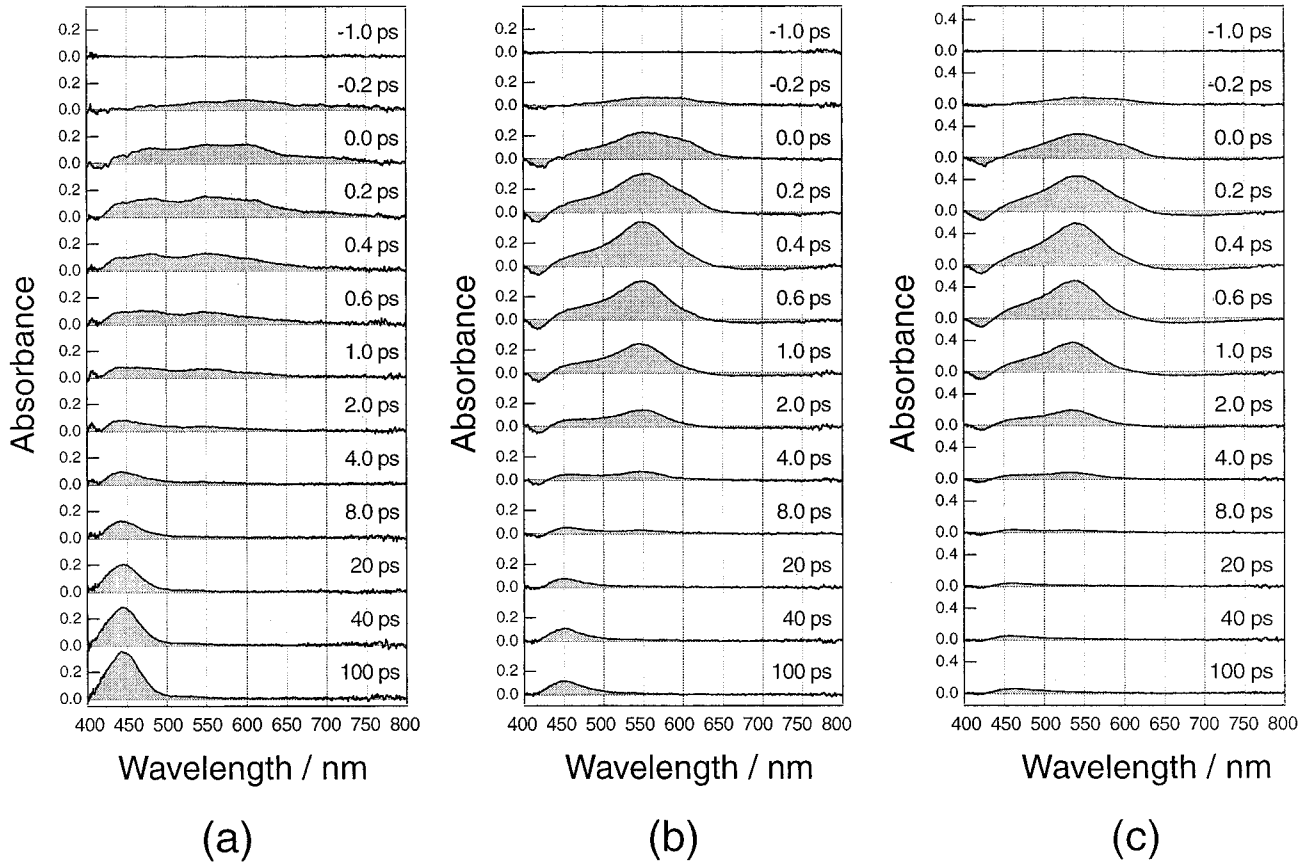

Figure 3. Femtosecond time-resolved absorption spectra of all-*trans* retinal (a) in neat cyclohexane, (b) in the mixed solvent 1-butanol/cyclohexane ([1-butanol] = 1.78 mol dm^{-3}), and (c) in neat 1-butanol ([1-butanol] = 10.7 mol dm^{-3}).

range 20−100 ps, an isolated absorption band is observed around 450 nm, which is slightly red-shifted from the $T_n \leftarrow T_1$ absorption band in neat cyclohexane. This absorption band is certainly assigned to the T_1 state. The red shift of the $T_n \leftarrow T_1$ absorption band that occurs upon hydrogen-bond formation is well-known.[4,8,19] The decrease in the $T_n \leftarrow T_1$ absorbance in the mixed solvent corresponds well to the lower triplet quantum yields found in protic solvents compared to those found in aprotic nonpolar solvents. We also note that no absorption band that can be assigned to the S_1 state is observed in the mixed solvent.

Kinetic Analysis. Because of the severe overlap of the S_3 and S_2 absorption bands, it is not possible to determine accurately the S_3-decay and the S_2-rise time constants for the mixed solvents. However, we can estimate the S_3-decay time constants from the change in absorbance that occurs at the wavelength where the $S_n \leftarrow S_2$ absorption is nearly canceled by the $S_2 \rightarrow S_0$ stimulated-emission gain. As an example, Figure 4 shows such data at 628 nm for the 1-butanol concentration of 1.78 mol dm^{-3}. The single-exponential fitting of these data gives a time constant of 0.2 ps. This time constant corresponds to the S_3 lifetime. Similar fittings for the other concentrations of 1-butanol give the S_3 lifetimes as shown in the second column of Table 1. We note that the S_3 lifetime in the mixed solvents is not very dependent on the 1-butanol concentration and that it is significantly longer than the value (30 fs) in neat cyclohexane.

It is also not possible to determine accurately the S_2 lifetime, in this case because of the continuous shift of the absorption band in the 0.4−1.0 ps time range. In fact, exponential fittings of the absorption intensities at different wavelengths between 400 and 700 nm give time constants in the range between 1.2

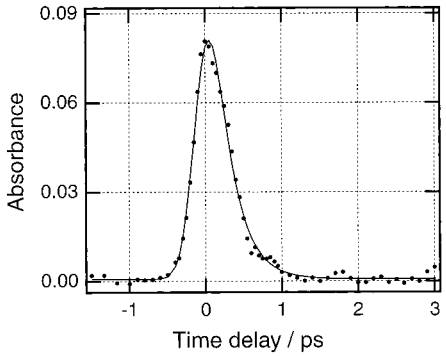

Figure 4. Time-delay dependence of the $S_n \leftarrow S_3$ absorbance at 628 nm, where the $S_n \leftarrow S_2$ absorption is canceled with the $S_2 \rightarrow S_0$ stimulated-emission gain. The single-exponential fitting curve is shown as a solid line. The 1-butanol concentration is 1.78 mol dm^{-3}.

and 2.7 ps. We, therefore, plot the peak intensity of the band at the varying wavelength vs time delay. The result for the 1-butanol concentration of 1.78 mol dm^{-3} is shown in Figure 5. A single-exponential fitting is performed in the time range 1.5−8 ps in order to avoid the S_3 contribution. We obtain an S_2 lifetime of 1.9 ps. The same procedure for different concentrations of 1-butanol gives the lifetimes shown in the third column of Table 1. Exponential fittings of the decay curves of the $S_2 \rightarrow S_0$ gain signals at 679 nm also give the time constants of the S_2 state, and we find that these values coincide with the S_2 lifetimes reported in Table 1 within experimental uncertainty. The S_2 lifetime in the mixed solvents is about 3 times longer than the 0.7 ps value that was obtained in neat cyclohexane. The rise time constants of the T_1 state are determined from the absorbance change at 450 ± 2.5 nm and are listed in the fourth column of Table 1. The T_1 rise time at the 1-butanol concentra-

TABLE 1: 1-Butanol Concentration Dependence of Singlet-Excited-State Lifetimes of All-*trans* Retinal and Molar Fraction of H-Bonded Species in Each Excited State

				molar fraction		
	lifetime (ps)				S_0	S_3
[1-butanol] (mol dm^{-3})	S_3	S_2	S_1	1-butanol	H-bonded retinal	H-bonded retinal
0	0.03	0.7 ± 0.2	34 ± 4	0	0	0
1.78	0.2 ± 0.1	1.9 ± 0.8	21 ± 3	0.19	0.69	0.77
3.06	0.2 ± 0.1	1.7 ± 0.6	24 ± 3	0.32	0.79	0.85
4.01	0.3 ± 0.1	1.6 ± 0.6	22 ± 3	0.42	0.83	0.88
4.76	0.2 ± 0.1	1.6 ± 0.2	19 ± 3	0.49	0.85	0.90
5.35	0.2 ± 0.1	1.6 ± 0.4	20 ± 5	0.54	0.87	0.91
5.94	0.2 ± 0.1	1.6 ± 0.8	25 ± 5	0.60	0.88	0.92
6.69	0.2 ± 0.1	1.6 ± 0.2	21 ± 4	0.66	0.89	0.93
7.64	0.2 ± 0.1	1.6 ± 0.3	21 ± 1	0.75	0.90	0.93
8.92	0.2 ± 0.1	1.6 ± 0.4	21 ± 1	0.86	0.92	0.94
10.7	0.2 ± 0.1	1.6 ± 0.4	22 ± 1	1	0.93	0.95

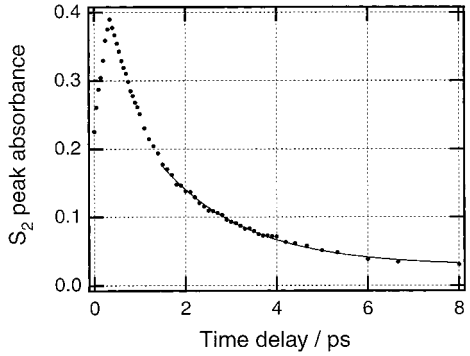

Figure 5. Time-delay dependence of the $S_n \leftarrow S_2$ peak absorbance. The single-exponential fitting curve in the time range 1.5−8 ps is shown as a solid line. The 1-butanol concentration is 1.78 mol dm^{-3}.

tion of 1.78 mol dm^{-3} is 21 ps. The precursor of the T_1 state in the mixed solvent cannot be the S_2 state, because the S_2 lifetime is much shorter than 21 ps. There is no evidence for the participation of a higher triplet state in the decay process of the S_2 state. Thus, it is most straightforward to consider that the S_1 state is the precursor of the T_1 state in the mixed solvent, as in the case of the aprotic nonpolar solvent.

Equilibrium between Free and Hydrogen-Bonded All-*trans* Retinal. In the mixed solvent with a 1-butanol concentration of 1.78 mol dm^{-3}, the free and hydrogen-bonded all-*trans* retinal exist in an equilibrium in the ground state. The lifetime of the S_3 state has been shown to be as short as 0.2 ps. Therefore, it is likely that the free and hydrogen-bonded species coexist also in the S_3 state. The estimated fractions of the hydrogen-bonded species in the S_0 and S_3 states are tabulated in the sixth and seventh columns of Table 1, respectively. Note that the fractions of the hydrogen-bonded S_3 species were estimated from those in the S_0 state and the ratio of the molar absorption coefficients of the hydrogen-bonded and free species at the pumping wavelength. Figure 6a−d shows the time-resolved absorption spectra of all-*trans* retinal at −0.2 ps for 1-butanol concentrations of (a) 0, (b) 1.78, (c) 5.35, and (d) 10.7 mol dm^{-3}. At −0.2 ps, the spectra are dominated by the $S_n \leftarrow S_3$ absorption bands. As shown by the dotted curve in Figure 6b, the mixed-solvent S_3 spectrum with a 1-butanol concentration of 1.78 mol dm^{-3} is reproduced very well with a linear combination of the two spectra in Figure 6a,d. We conclude that the S_3 spectrum in Figure 6b is made up of two distinct species, one of which is the free S_3 all-*trans* retinal whose spectrum is shown in Figure 6a. The other is most probably the 1:1 hydrogen-bonded S_3 all-*trans* species that is produced by the photoexcitation of the 1:1 hydrogen-bonded species in

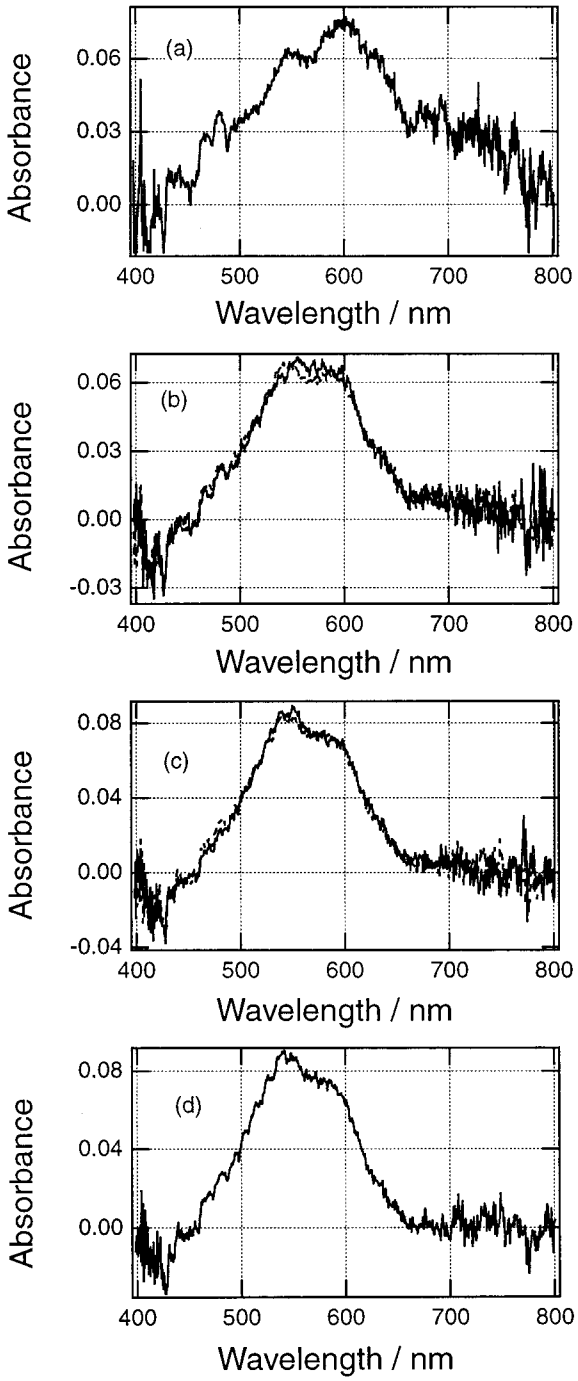

Figure 6. Time-resolved absorption spectra at −0.2 ps for the four 1-butanol concentrations (a) 0, (b) 1.78, (c) 5.35, and (d) 10.7 mol dm^{-3}. In (b) and (c), the calculated spectra [linear combinations of (a) and (d)] are shown as dotted curves.

the S_0 state. The S_3 spectrum with a 1-butanol concentration of 5.35 mol dm^{-3} (Figure 6c) is also reproduced very well with a linear combination of the two spectra in Figure 6a−d. Because the S_3 lifetime is ultrashort, the coexisting free and hydrogen-bonded species are not likely to be in an equilibrium.

A similar two-component fitting analysis was performed for the time-resolved absorption spectra at 0.4 ps, which is dominated by the $S_n \leftarrow S_2$ absorption. The fitting was not successful whenever the S_2 spectrum in neat cyclohexane was used as one of the two components. There is no contribution of the free S_2 species to the spectra in the mixed solvents, indicating that all of the retinal molecules are hydrogen-bonded in the S_2 state at 0.4 ps. All-*trans* retinal appears to be a stronger base in

the S_2 state than in the ground state. The free and hydrogen-bonded species coexist in the S_3 state at −0.2 ps, and therefore, the hydrogen-bond formation must take place during or after the $S_3 \rightarrow S_2$ internal conversion. Because no significant spectral change except for the aforementioned shift is observed for the S_2 spectrum in the time range 0.4−1.0 ps, the hydrogen-bond formation must be completed in a very short time, most probably within a few hundred femtoseconds.

The $S_n \leftarrow S_1$ absorption band is only weakly observed in neat cyclohexane and is not observed at all in the mixed solvents. The two-component fitting analysis is, therefore, not applicable to the S_1 state. However, we do have a piece of information on the hydrogen bonding in the S_1 state, as described below. The lifetime of the free S_1 species in neat cyclohexane has been determined to be 34 ps by SVD analysis. The rise dynamics of the $T_n \leftarrow T_1$ absorption band in the mixed solvents are very well represented by a single-exponential function with a 21 ps time constant and do not contain a component with a rise time constant of 34 ps. Therefore, a free S_1 species with a 34 ps lifetime does not exist in the mixed solvents as the dominant precursor of the T_1 state. All of the retinal molecules in the S_1 state are hydrogen-bonded, or there is a rapid equilibrium between the free and hydrogen-bonded species and the intersystem crossing to the T_1 state predominantly occurs from the hydrogen-bonded S_1 species. In either case, the lifetime of the hydrogen-bonded S_1 state must be 21 ps.

The time-resolved absorption spectra at 100 ps are shown in Figure 7 for 1-butanol concentrations of (a) 0, (b) 1.78, (c) 5.35, and (d) 10.7 mol dm^{-3}. At 100 ps, the $T_n \leftarrow T_1$ absorption dominates the spectrum. As shown by the dotted curves, the mixed-solvent T_1 spectra in Figure 7b,c are reproduced very well with linear combinations of the two neat-solvent T_1 spectra in Figure 7a,d. All of the other seven mixed-solvent T_1 spectra (not shown) are also successfully reproduced in the same manner. We, therefore, conclude that the mixed-solvent T_1 spectra are made up of two distinct T_1 species: one is the free T_1 species, and the other is the 1:1 hydrogen-bonded T_1 species. The appearance of the free T_1 species at 100 ps, therefore, means that hydrogen-bond dissociation takes place during or after the $S_1 \rightarrow T_1$ intersystem crossing. The two-component fitting analysis for the 40−90 ps time range indicates that the same linear-combination coefficients as those used at 100 ps apply. The free and hydrogen-bonded T_1 species are in an equilibrium in the mixed solvents in the time range 40−100 ps. Accordingly, the hydrogen-bond dissociation time is much shorter than 40 ps.

These conclusions are not compatible with those of Das and Hug.[4] By using nanosecond time-resolved visible absorption spectroscopy, they observed a transient absorbance decrease at 445 nm and a corresponding increase at 480 nm for all-*trans* retinal in the HFIP/cyclohexane mixed solvents. They also observed a red shift of the $T_n \leftarrow T_1$ absorption band upon hydrogen-bond formation. On the basis of these observations, they argued that the hydrogen-bond formation and dissociation reactions were slower than S_1 decay and that the equilibration process between the free and hydrogen-bonded species took place in the T_1 state with a time constant of a few tens of nanoseconds. HFIP forms stronger hydrogen bonds than does 1-butanol, and therefore, the replacement of 1-butanol with HFIP will make the hydrogen-bond formation rate faster. The difference in hydrogen-bonding ability does not explain the inconsistency between our conclusion and those of Das and Hug. It is possible, however, that the inconsistency is due to the difference in the concentration of the hydrogen-bond-forming solvent. In their measurements, Das and Hug used HFIP concentrations about 100 times smaller than the 1-butanol concentrations used in the present study. Such low concentrations may result in slower hydrogen-bond formation because of diffusion. It may also be possible that what they called a hydrogen-bonded complex of all-*trans* retinal and HFIP was actually a protonated form of retinal; the proton-transfer reaction might be much slower. A time-resolved infrared spectroscopic study will be effective in solving this problem, as the C=O stretching frequency will clearly tell whether it is hydrogen-bond formation or protonation.

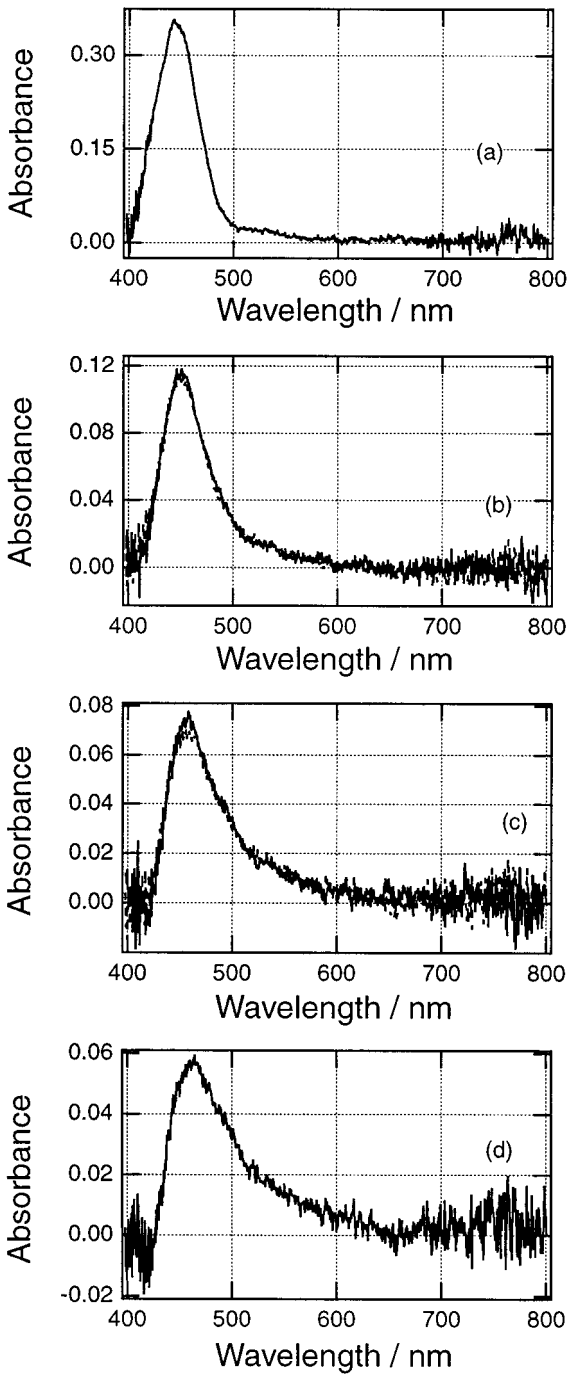

Figure 7. Time-resolved absorption spectra at 100 ps for the four 1-butanol concentrations (a) 0, (b) 1.78, (c) 5.35, and (d) 10.7 mol dm^{-3}. In (b) and (c), the calculated spectra [linear combinations of (a) and (d)] are shown as dotted curves.

State Ordering and Dynamics of the Excited Singlet Manifold. Three singlet lifetimes have been obtained by SVD

analysis for all-*trans* retinal in neat cyclohexane: 30 fs (nominal) for the S_3 state, 0.7 ps for the S_2 state, and 34 ps for the S_1 state. For the mixed solvent with a 1-butanol concentration of 1.78 mol dm^{-3}, three time constants were obtained by a kinetic analysis of the time-resolved spectra: 0.2 ps for the S_3 lifetime, 1.9 ps for the S_2 lifetime, and 21 ps for the S_1 lifetime. The corresponding three time constants in the other mixed solvents and in neat 1-butanol are similar to those at 1.78 mol dm^{-3} and are independent of the 1-butanol concentration within experimental uncertainty (Table 1). It seems that only one unique 1:1 hydrogen-bonded species, whose dynamics are characterized by the three time constants given above, exists in each of the mixed solvents and in 1-butanol. The spectral shift observed on going from Figure 3b to Figure 3c is then ascribed to the solvent environmental effect.[24]

We observe strong induced emissions from the S_2 state in the spectra in Figure 3b. This fact means that the S_2 state is strongly fluorescent in the mixed solvents. In contrast, we do not observe any stimulated emission that can be assigned to an S_1 state with a 21 ps lifetime. The S_1 state is, therefore, not the fluorescing state in the mixed solvents. We conclude that the fluorescence of hydrogen-bonded all-*trans* retinal is emitted not from the S_1 state but from the S_2 state.

We have so far identified the three excited singlet states of hydrogen-bonded all-*trans* retinal: the strongly one-photon-allowed S_3 state with the 0.2 ps lifetime, the strongly one-photon-allowed S_2 state with the 1.9 ps lifetime, and the one-photon-forbidden S_1 state with the 21 ps lifetime. The most straightforward way of assigning these states is to adopt the same assignments as those already established for free all-*trans* retinal, namely, B_u^+ (π, π^*) for S_3, A_g^- (π, π^*) for S_2, and (n, π^*) for S_1. Note that we no longer have a constraint that requires the S_1 state to be one-photon-allowed. It must be pointed out here that the meaning of the symbols A_g and B_u is more equivocal in the hydrogen-bonded species than in the free species, because both the S_2 and the S_3 states are now found to be strongly one-photon-allowed. It seems that mixing of the A_g^- and the B_u^+ (π, π^*) states is induced by hydrogen bonding, as already discussed by Birge et al.[28] In the aprotic nonpolar solvent hexane, the radiative lifetime of the S_3 B_u^+ (π, π^*) state is estimated to be 50 times shorter than that of the S_2 A_g^- (π, π^*) state.[3] In the mixed solvents, in contrast, the S_2 state must be one-photon-allowed as strongly as the S_3 states is; otherwise, the $S_2 \rightarrow S_0$ gain cannot be observed. The ground-state absorption spectra in Figure 1 show that the S_2 and S_3 states in the mixed solvents have absorption cross sections that are comparable to that of the S_3 state in nonpolar solvents. Then, the radiative lifetime of the hydrogen-bonded S_2 state is 20−30 times shorter than that of the free S_2 state. Considering the 3-times-longer lifetime of the hydrogen-bonded S_2 state, we can account well for the 2-order-of-magnitude increase in the fluorescence quantum yield upon hydrogen-bond formation without assuming any state-ordering change. We conclude that the S_2 and S_3 states in hydrogen-bonded all-*trans* retinal are both one-photon-allowed (π, π^*) states and that there is no change of the state ordering between the (π, π^*) S_2 and the (n, π^*) S_1 states upon hydrogen-bond formation.

The longer S_2 lifetime in the mixed solvents is consistent with the increase in the all-*trans* → mono-*cis* isomerization quantum yield upon hydrogen-bond formation. The total quantum yield in ethanol is a few times higher than the yield in aprotic nonpolar solvents.[29] According to the scheme that we proposed,[10] the all-*trans* → mono-*cis* isomerization in nonpolar solvents proceeds from the S_2 state via the perpendicular excited singlet state (π^*). If the $S_2 \rightarrow S_1$ conversion rate remains unchanged upon hydrogen-bond formation, the 3-times-longer S_2 lifetime corresponds well with the quantum-yield increase of a few times.

Finally, the S_3 lifetime in the mixed solvents is about 0.2 ps and is much longer than that of the free S_3 species. Strictly speaking, this lifetime is an average of the lifetimes for the free and hydrogen-bonded S_3 states. The lifetime may also be affected by conversion from the free to the hydrogen-bonded S_3 species. However, analysis of the ground-state absorption spectra based on eq 1 shows that 68% of the retinal molecules are already hydrogen-bonded in the ground state, even at the 1-butanol concentration of 1.78 mol dm^{-3}. The contribution of the free S_3 state must be small. The S_3 lifetime in the mixed solvents is thus regarded as the S_3 lifetime of the hydrogen-bonded all-*trans* retinal. The prolongation of the S_3 and S_2 lifetimes upon hydrogen-bond formation must arise from mixing between the A_g^- and B_u^+ (π, π^*) states. A quantum chemical approach is necessary to elucidate how hydrogen bonding induces the state mixing between the A_g^- and B_u^+ (π, π^*) states of retinal.

4. Conclusions

Femtosecond time-resolved visible absorption spectroscopy of all-*trans* retinal in 1-butanol/cyclohexane mixed solvents was performed. The three excited singlet states identified in the mixed solvents are assigned to the S_3 B_u^+ (π, π^*), S_2 A_g^- (π, π^*), and S_1 (n, π^*) states, respectively. No evidence was found to favor a state-ordering change between the (n, π^*) and (π, π^*) states upon hydrogen-bond formation. The free and hydrogen-bonded species coexist in the S_3 state. On the contrary, all of the retinal molecules are hydrogen-bonded in the S_2 state. In the T_1 state, an equilibrium is achieved between the free and hydrogen-bonded species. The increase in the fluorescence and the isomerization quantum yields and the decrease in the triplet quantum yield upon hydrogen-bond formation have been successfully explained in terms of the prolonged lifetime of the hydrogen-bonded S_2 state. Thus, the primary factor in determining the marked solvent effects of retinal photophysics/photochemistry has been elucidated. The present study gives the first example of subpicosecond hydrogen-bond formation and dissociation reactions.

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