Photophysical and Photochemical Behavior of 11-*cis*-Retinal and Its Schiff Base in a Micelle

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The photophysical and photochemical behaviors of 11-*cis*-retinal and its Schiff base have been studied in a micelle made from Triton X-100. Both microsecond laser flash and steady-state techniques were employed. The photoisomerization yield (~0.19) and the triplet quantum yield (~0.13) were determined in the case of 11-*cis*-retinal. In the case of the 11-cis Schiff base, a long-lived (240 s) non-excited-state transient was seen and very little isomerization appears to have occurred. The relative location of the 11-*cis*-retinal and its Schiff base in the micelle could be determined. The retinal was found to be in a polar region, Stern layer, of the micelle while the 11-cis Schiff base was found to be located in a dry, hydrocarbon-core region of the micelle. The nonionic micellar environment did not favor in any manner the photoisomerization of the 11-*cis*-retinal or the 11-cis Schiff base compared to any other solvents (polar or nonpolar).

Introduction

The initial photochemical step in the visual sequence involving the pigment rhodopsin is believed to be the 11-cis to all-trans isomerization of the retinyl chromophore.[1,2] The quantum yield of this photoisomerization was reported to be ~0.63.[3] Our recent comprehensive study[4] of the mechanism of isomerization of the 11-*cis*-retinal Schiff base (11-cis SB) clearly showed that the quantum yield of photoisomerization (ϕ_{PI}) was significantly increased going from nonpolar solvent ($\phi_{PI} \leq 0.01$–0.04) to polar solvents ($\phi_{PI} = 0.20$–0.34). the ϕ_{PI} is also high in a H-bonded complex (0.31).

A non-excited-state transient, X, was also found in all solvents with a lifetime varying from milliseconds in polar solvents to 120 s in hexane.[4] The nature of this transient was not unambiguous, but we proposed X to be the result of a single bond conformational change.[4] On the basis of the facts that for the protonated 11-*cis*-retinal *n*-butylamine Schiff base, the ϕ_{PI} was high in any solvent[4] and the lifetime of isomerization was fast (<8 ps)[5] and comparable to rhodopsin, we proposed that a hydrophobic environment and a protein were not of critical importance in the primary step but that the protein was very probably more important in later steps of the energy transduction process. We also proposed a detailed mechanism for the primary step in vision.[4]

Micelles have been used as models to mimic biological membrane environments for host molecules. Thus, we present here a photophysical and photochemical study of 11-*cis*-retinal and its Schiff base in a nonionic micellar environment (Triton X-100). We hope that this work gives more information about the behavior of the retinyl chromophore in a membrane-like environment.

Experimental Section

11-*cis*-Retinal was obtained from Hoffman–LaRoche. The isomeric purity determined by HPLC techniques was reported previously[6] to be 96%. The *n*-butylamine Schiff base was prepared as described before.[4] After synthesis of the Schiff base it was found[6] by HPLC techniques that 15% of the 11-cis SB had isomerized to all-trans SB. However, this result will not affect in any manner the discussion of our results. Spectrophotometric grade solvents were dried over 3-Å molecular sieves and used without further purification. 11-*cis*-Retinal and its Schiff base were directly dissolved in Triton X-100, followed by dilution with water. The pH of the solution was kept at 8–9 with triethylamine. We also used DTAC and sodium dodecyl sulfate as ionic surfactants, but we observed protonation and hydrolysis of the Schiff Base in a matter of minutes. We, therefore, chose to use Triton X, which is a nonionic micelle. Absorption spectra were recorded on a Cary 15 or a Hewlett-Packard 8540A spectrophotometer.

TABLE I: Absorption Spectral Data for 11-*cis*-Retinal, Its Schiff Base, and All-Trans Retinal

compd	solvent	λ_{max}, nm	ϵ, L M^{-1} cm^{-1}
11-*cis*-retinal	hexane	365	26 360[a]
	MeOH	380	24 940[a]
	Triton X-100	380	
11-*cis*-retinal	hexane	347	32 300[b]
n-butylamine	MeOH	358	26 300[b]
Schiff base	Triton X-100	360	
all-trans retinal	MeOH	385	42 884[a]

[a] From ref 13. [b] From ref 4.

The laser flash experiments were carried out with the 355-nm third harmonic of a Q-switched Nd:YAG laser (11-ns pulse width). The kinetic absorption spectrometer used to detect optical density changes (ΔOD) after excitation has been described previously.[8] The shortest time at which signals could be detected with this setup was ~0.4 μs. Degassing of the samples was performed by bubbling nitrogen gas through each solution; in addition, oxygen was bubbled in separate experiments to examine the triplet excited states. Triplet quantum yields (ϕ_T) were determined by a comparison method.[9] Benzophenone in benzene ($\Delta\epsilon_{TT}^{532} = 7630$, $\phi_T = 1.0$) was used as a reference (actinometer). The intensity of the laser radiation incident on the sample cell was controlled by the use of wire mesh screens. The common energies used were as low as 0.5 to 1 mJ to ensure the absence of nonlinear effects.

Quantum yields of photoisomerization (ϕ_{PI}) were determined by steady-state irradiation experiments, carried out at 355 nm. The excitation source consisted of a 150-W short-arc Xenon lamp and Aminco monochromator (dispersion of 6.5 nm/mm). Quantum yields were determined by a comparison method with a photochromic fulgide [(*E*)-α-(2,5-dimethyl-3-furylethylidene)(isopropylidene)succinic anhydride] as the chemical actinometer ($\phi = 0.20$ and $\Delta\epsilon_{SS}^{494} = 8200$).[4,11,16]

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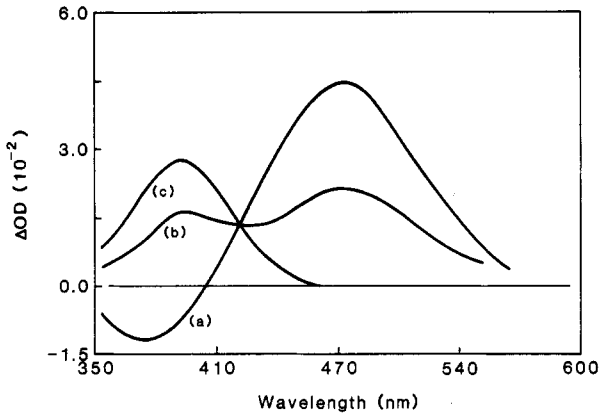

Figure 1. Transient absorption spectra (ΔOD) of 11-*cis*-retinal in Triton X-100, nitrogen degassed, recorded at (a) 3 μs, (b) 16 μs, and (c) 40 μs after 355-nm laser flash excitation where decay of the triplet is complete. When oxygen is bubbled through the sample, the ΔOD spectrum is identical with spectrum c and does not evolve up to 400 μs.

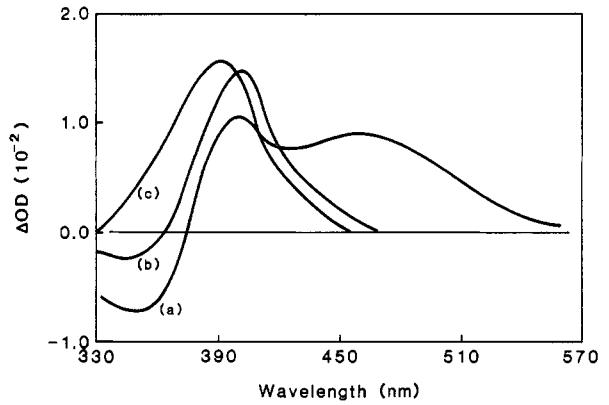

Figure 2. Transient absorption spectra of 11-*cis*-retinal Schiff base in Triton X-100, nitrogen degassed, recorded at (a) 1 μs and (b) 40 μs after 355-nm laser flash excitation and (c) 10 min after irradiation (steady-state experiment). When oxygen is bubbled through the sample the ΔOD spectrum is identical with spectrum b, where decay of the triplet is complete and does not evolve up to 400 μs.

Results and Discussion

DTAC and sodium dodecyl sulfate as ionic surfactants result in protonation and rapid hydrolyis of the Schiff base. The latter is also true for the protonated Schiff base in a Triton X-100 micelle. The absorption spectral data for the 11-*cis*-retinal and its Schiff base in micelle are summarized in Table I. Methanol and hexane data are also given as a matter of comparison. We may note that the wavelength maxima in the micellar environment are comparable to the ones in methanol. Laser flash experiments of 11-*cis*-retinal and its Schiff base in Triton X-100 were carried out with an 11-ns pulse at 355 nm from a Nd:YAG laser both with nitrogen and oxygen bubbling. Figure 1 shows the difference spectrum (ΔOD) observed 3, 16, and 40 μs after 355-nm laser excitation of the 11-*cis*-retinal in Triton X-100 with nitrogen degassing. We observed a transient with a maximum of absorption at 470 nm that decayed to the base line. The (ground-state) absorption at 380 nm was seen to increase/recover from a negative ΔOD to a positive ΔOD). The kinetics at 380 and 470 nm were both of first order with identical lifetimes of 17 μs. When oxygen was bubbled through the sample, the band at 470 nm was totally quenched and the remaining ΔOD spectrum immediately after the pulse (~0.4 μs) showed a maximum of absorption at 390 nm with no evolution of OD up to 400 μs. The shape of the latter absorption spectrum is identical with the ΔOD spectrum observed in nitrogen-degassed solution after complete evolution (~40 μs after the pulse, Figure 1c).

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TABLE II: Triplet Quantum Yield and Triplet Lifetime of 11-*cis*-Retinal

solvent	λ_{max}^{T-T}, nm	ϵ_{max}^{T-T}, L M^{-1} cm^{-1}	ϕ_T	τ_T, μs
hexane[a]	445	62000	0.51	7.2
methanol[a]	450	27000	0.11	15
Triton X-100	470		0.19[b]	17

[a] From ref 8. [b] Calculated with ϵ_{max}^{T-T} = 27000 L M^{-1} cm^{-1}.

Figure 2 shows the difference spectrum (ΔOD) 3 and 40 μs after the pulse for the 11-cis Schiff base in Triton X-100 with nitrogen degassing. We observed a band maximizing at 470 nm that decayed by first-order kinetics with a lifetime of 17 μs. The (ground-state) absorption increase/recovery at 360 nm was observed to follow first-order kinetics to a positive ΔOD with a lifetime of 17 μs. An additional transient was observed maximizing at 400 nm rising with a lifetime of 17 μs (first-order kinetics). When oxygen was bubbled through the sample,[12] the species at 470 nm was totally quenched and the remaining ΔOD spectrum showed a transient, immediately (~0.4 μs) after the pulse, maximizing at 400 nm, which was stable up to 400 μs. The shape and intensity of the absorption spectrum of this transient was identical with the absorption spectrum of the fully evolved OD spectrum (~40 μs) of the nitrogen-degassed solution, Figure 2b.

In *both* cases of the 11-*cis*-retinal and its Schiff base, we assign the transient maximizing at 470 nm to be a triplet–triplet (T–T) absorption based on the oxygen-quenching experiment. Also the T–T absorption of the *retinals* has been observed[9] and occurs in the 450–480-nm region depending on the solvent. Our inability to observe any short lifetime decay of a retinal triplet in the presence of oxygen occurs because of instrumental limitation (~0.4 μs lower limit as noted previously). No triplet transients have been found by direct excitation of the retinal *Schiff base* (i.e., $\phi_T < 0.01$).[4] Since the T–T absorption maximum and the lifetime are identical in both experiments and correspond to those of a retinal, *we believe that the T–T absorption spectrum seen in the Schiff base case is in fact the T–T absorption spectrum of its hydrolysis product, i.e., the 11-cis-retinal.* We could estimate that 10% of the 11-cis SB was hydrolyzed when making the micelle. It seems apparent that the free surfactant catalyzes the hydrolysis of the Schiff base during the incorporation of the molecule in the micelle. We did not observe further hydrolysis for up to 1 h after the making of the micelle, which is consistent with the rate of hydrolysis (100 min) found[14] for the retinal Schiff base at pH 8–9.

The simultaneous presence of the 11-*cis*-retinal and the 11-cis SB within the micelle makes us reconsider the ground-state absorption spectrum of the 11-cis *SB*; see Table I. Thus, the real maximum of absorption of the Schiff base in the micelle could in fact be somewhat blue-shifted from that observed and thus would be more comparable with a nonpolar environment (hexane). However, the lifetime of the triplet of the *retinal* in the micelle (see Table II) is comparable with the lifetime of the triplet of the retinal in methanol. This suggests to us that the 11-*cis*-retinal is located in a polar environment, i.e., the Stern layer of the micelle. We measured the triplet quantum yield (ϕ_T) of the 11-*cis*-retinal in Triton X-100 by the comparison method using the ϵ_{TT} in methanol given by Bensasson and Land.[9] We found a value of 0.19, which is comparable with the ϕ_T found in methanol (0.11), whereas that found in hexane was 0.51;[9] this again suggests that 11-*cis*-retinal is located in a polar region of the micelle.

The oxygen experiment gives further insight in the photophysical path of 11-*cis*-retinal and 11-cis SB. The intensities of the maxima of absorption of the photoproducts in nitrogen-degassed solution (after complete decay of the triplet, i.e., ~40 μs) and in oxygen-saturated solution (immediately after the pulse, i.e., ~0.4 μs) are identical. Bensasson and Land[9] also did not find any oxygen influence on the photochemistry of 11-*cis*-retinal in methanol and, as shown above, we did not either. Therefore, it seems that, within experimental precision, the quantum yield of isomerization via the relaxed triplet state is negligible in methanol or in Triton-X. The slow (17 μs) buildup of absorption found at

TABLE III: Quantum Yield of Photoisomerization of 11-*cis*-Retinal and Its Schiff Base and Lifetime of Transient X

compd	solvent	ϕ_{PI}[a]	τ_X, s
11-*cis*-retinal	hexane	0.23	
	methanol	0.27[b]	
	Triton X-100	0.13[b]	
11-*cis* SB	hexane	≤0.01[c]	12[c]
	hexane/H_2O[d]	≤0.01	50
	methanol	0.19[c]	[e]
	Triton X-100	≤0.01[f]	240

[a] All of the quantum yields have been calculated with the quantitative steady-state experimental data. [b] Calculated with $\Delta\epsilon_{MeOH}^{383}$ (11-cis and all-trans) = 18 000 L M^{-1} cm^{-1} (ref 13). [c] From ref 4. [d] Hexane solution saturated with water (0.011 g/100 g hexane). [e] From ref 4. Lifetime between 0.4 ms and 1 s. [f] Estimated; see text.

the ground-state absorption maximum (380 nm for 11-*cis*-retinal and 360 nm[17] for 11-cis SB) is mainly due to the recovery of the 11-*cis*-retinal ground-state absorption as the relaxed triplet decays. Therefore, we believe that the photoproducts of 11-*cis*-retinal originate from the excited singlet state, a vibronically excited triplet state, or T_2 as previously seen[18,19] in methanol, and from the singlet excited state SB as seen in the case of 11-cis SB[4] in solution.

The ΔOD spectrum of 11-*cis*-retinal, Figure 1, clearly shows that cis–trans isomerization has occurred as previously found in other solvents.[9] However, the ΔOD spectrum of the 11-cis SB up to 400 μs is not compatible with only a cis–trans isomerization since there is a negative ΔOD in the region of 340–360 nm (see Figure 2b). We, therefore, suspected the presence of a non-excited-state transient X as previously seen by us for the unprotonated 11-cis SB in any solvent.[4] We thus performed a steady-state experiment in order to monitor the decay of the transient X. After steady-state irradiation of the micelle solution, we observed a ΔOD spectrum identical with the one obtained in the laser flash experiment after entire decay of the triplet, i.e., a depletion at 350 nm and a positive maximum at 400 nm (see figure 2b). Over time (seconds), the negative signal at 350 nm was seen to recover with the same lifetime of 240 s as the decay of the positive absorption maximum at 400 nm. The final ΔOD spectrum after complete evolution was found to have a maximum of absorption at 390 nm, Figure 2c. This maximum is red-shifted with respect to a ΔOD spectrum for an expected 11-cis to all-trans isomerization of the Schiff base; however, it corresponds well to the maximum of the spectrum for an 11-cis to all-trans isomerization of the *retinal* in a Triton X-100 micelle, Figure 1c. Therefore, we believe the photoproduct with a maximum at 390 nm is the all-trans retinal resulting from the photoisomerization of the 11-*cis*-retinal hydrolysis product of the 11-cis SB.

Table III summarizes all the calculated quantum yields of photoisomerization (ϕ_{PI}) for 11-*cis*-retinal and its Schiff base in hexane and methanol and for 11-*cis*-retinal in Triton X-100. We can see that the ϕ_{PI} for the 11-*cis*-retinal in the micelle is lower by at least a factor of 2 than the ϕ_{PI} in methanol or in hexane. We did not calculate the ϕ_{PI} for the 11-cis *SB* in Triton X-100 since the final stable photoproduct (also see below) is almost entirely all-trans retinal from 11-*cis*-retinal as a hydrolysis product of 11-cis SB, as shown by the wavelength maximum (390 nm) and the shape of the absorption spectrum. We, therefore, believe the ϕ_{PI} for the 11-cis SB to all-trans SB in the micelle is very small, that is, probably comparable to the one in hexane, i.e., ≤0.01.

The main photoproduct of the 11-cis SB in the micelle is the transient X as previously found in hexane solution.[4] We performed another experiment in order to determine how hydrophobic the environment surrounding the 11-cis SB really is. Thus, we prepared a solution of 11-cis SB in hexane saturated with water (0.011 g/100 g of hexane, ~4 mM).[15] After irradiation, we observed the usual spectrum of the X transient, which slowly decayed. The lifetime of X was found to be 50 s (recall that in pure hexane, the lifetime is 120 s). The lifetime of the transient X has been found to decrease with the polarity of the solvent.[4] In the case of the micelle, we find a very long lifetime of 240 s. This result strongly indicates that the 11-cis SB is in a dry hydrocarbon-core region of the micelle. The difference in lifetime in the nonpolar environments hexane (C_6) and hydrocarbon core ($\sim C_{30}$) of the micelle most likely originates from a difference in viscosity of the media. On the other hand, as indicated by its triplet lifetime and its triplet quantum yield, 11-*cis*-retinal is believed to be dominantly in the polar, hydrophylic Stern layer.

Finally, we can say that, compared to other solvent systems, the nonionic hydrophobic micellar environment does not favor in any manner the photoisomerization of the 11-cis Schiff base. A parallel result is true for 11-*cis*-retinal since the quantum yield of isomerization is decreased by at least twofold compared to polar or nonpolar solvents, although 11-*cis*-retinal appears to be located in the more polar environment of the micelle.

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Registry No. 11-*cis*-Retinal, 564-87-4; 11-*cis*-retinal butylamine Schiff base, 52647-48-0.

(17) This value (360 nm) is the *observed* maximum for the ground-state depletion. Recall that in this region (360–380 nm) the ground-state absorption of 11-*cis*-retinal and its Schiff base overlap.

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