

Effect of Molecular Structures and Solvents on the Excited State Dynamics of the S₂ State of Carotenoids Analyzed by the Femtosecond Up-Conversion Method^{†,‡}

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Carotenoids have dual functions in photosynthesis: light harvesting and photoprotection against singlet oxygen by quenching of the triplet state.^{1,2} This differentiation is realized mainly by differences in energy levels and relaxation dynamics. Carotenoids are, in general, derivatives of polyenes with the C_{2h} point symmetry group, and thus the transition to the lowest singlet excited (S₁) state is optically forbidden, whereas transition to the second singlet excited (S₂) state allowed. Many derivatives are, however, not exactly C_{2h}, nor is the transition exactly forbidden. The relaxation from the S₂ state of carotenoids violates Kasha's rule and Ermolaev's rule;³ emissions from both the S₂ and S₁ states are observed, and a triplet state is rarely formed. The steady-state spectroscopies^{4,5} suggest that the relaxation from the S₂ state follows the energy gap law of internal conversion.⁶ Moreover, the relaxation processes depend on the molecular structure: when the symmetry of the conjugated double-bond is maintained, the main emission originates from the S₂ state, and when the symmetry is broken (e.g., by a keto carbonyl group), the S₁ emission becomes stronger,^{4,5,7} even though the energy gap (ΔE_{21}) is almost the same.³ Because the S₂ energy levels, and thus ΔE_{21} , are mainly determined by the solvent polarizability,^{8,9} one of the reasons for these phenomena is a fast internal conversion through vibronic coupling. Thus, we measured fluorescence lifetimes of the S₂ state of β -carotene and its analogues to analyze the excited state dynamics with the fs up-conversion apparatus. The results of this study indicate that the relaxation processes were affected by both the molecular structure and the solvent properties.

β -Carotene (**1**) purchased from Wako Pure Chemicals was purified by crystallization. Two analogues (β -apo-8'-carotenal (**2**) and ethyl- β -apo-8'-carotenoate (**3**)) purchased from Fluka were purified by silica gel and KC18-thin-layer chromatography.

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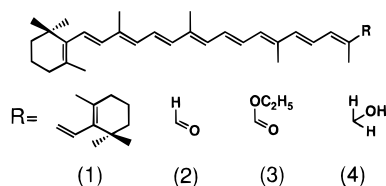
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† Abbreviations used: FWHM, full width at half maximum.

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β -Apo-8'-carotenol (**4**) was obtained by reduction of β -apo-8'-carotenal with NaBH₄ and purified. The all-trans forms of respective compounds were used.



Fluorescence lifetimes were measured with the fs up-conversion system^{10,11} at 22 °C. The light source was a Ti-Sapphire laser (780 nm, 76 MHz, 180-fs pulse width (FWHM), Figure 1), and its second harmonics (390 nm) were used to excite the samples. The sum frequency of the excitation pulse and fluorescence was obtained using a BBO crystal. All measurements were carried out at the magic angle (54.7°). Samples were dissolved with organic solvents in a 1-mm light path cell. Fluorescence lifetimes were estimated using the convolution calculation,¹¹ and a time-resolution was 20 fs after convolution. The energy gap, ΔE_{21} , was estimated using the difference between the 0–0 absorption band of the S₂ state and the 0–0 emission band of the S₁ state.⁵ This was done because the 0–0 absorption band of S₁ state was hardly detected by steady-state spectroscopy. Instead, the S₁ emission was clearly observed in solvents with high polarizability and almost insensitive to solvent polarizability.⁴ Due to this treatment, there is an ambiguity for ΔE_{21} with a magnitude of 300–400 cm⁻¹.

The S₂ lifetime of β -carotene (**1**) in *n*-hexane was measured and determined to be 180 ± 10 fs at 540 nm which, within experimental error, is consistent with previous studies.^{10–12} The S₂ lifetime of β -apo-8'-carotenol (**4**) was 199 ± 4 fs, a little longer than that of β -carotene. The S₂ lifetime changed in accordance with the molecular structure: 165 ± 8 fs for ethyl- β -apo-8'-carotenoate (**3**) and 119 ± 13 fs for β -apo-8'-carotenal (**2**). The inverse of the observed lifetimes is expressed in a logarithmic scale as a function of the energy gap, ΔE_{21} (Figure 2, filled circles), and these four points produced nearly a straight line.

A polar solvent induced changes in the S₂ lifetimes. In the case of β -carotene, the solvent effect was negligible. On the other hand, the S₂ decay time of other analogues was accelerated in methanol: 149 ± 7, 127 ± 6, and 96 ± 13 fs for β -apo-8'-carotenol, ethyl- β -apo-8'-carotenoate, and β -apo-8'-carotenal (Figure 1), respectively. The 96 fs lifetime of β -apo-8'-carotenal was the shortest measured so far. The lifetimes correlated to the energy gap of the β -carotene analogues (Figure 2, open circles) but did not correlate to the energy gap of β -carotene (Figure 2, arrow). A solute–solvent interaction might contribute to the relaxation dynamics.

The energy gap law of internal conversion is expressed as follow:¹³

$$k_{ic} = c \exp(-\gamma \Delta E / h\omega_M)$$

where k_{ic} is a rate constant for internal conversion; c is a pre-exponential factor related to the electronic coupling matrix element; γ is related to displacement of potential surface in individual states; ΔE is the energy gap; and $h\omega_M$ is a high frequency acceptor mode. For the last term, the C=C stretching mode is known to be the main factor in polyenes.^{9,14}

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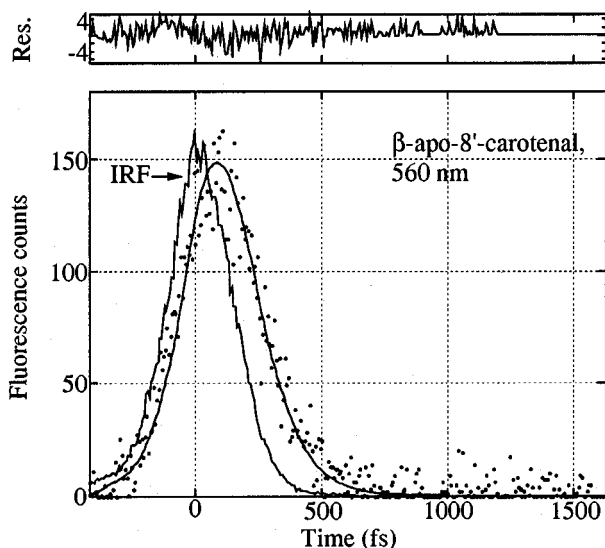


Figure 1. Fluorescence decay curve from the S_2 state of β -apo-8'-carotenal in methanol at 22 °C. The excitation wavelength was 390 nm (180 fs pulse width, 76 MHz), and fluorescence at 560 nm was monitored. The instrumental response function was shown as IRF. Each data point with a time interval of 6.6 fs is represented by dots, and the calculated best-fit curve is overlaid on the observed data points. The weighted residuals (Res) were plotted in the upper window.

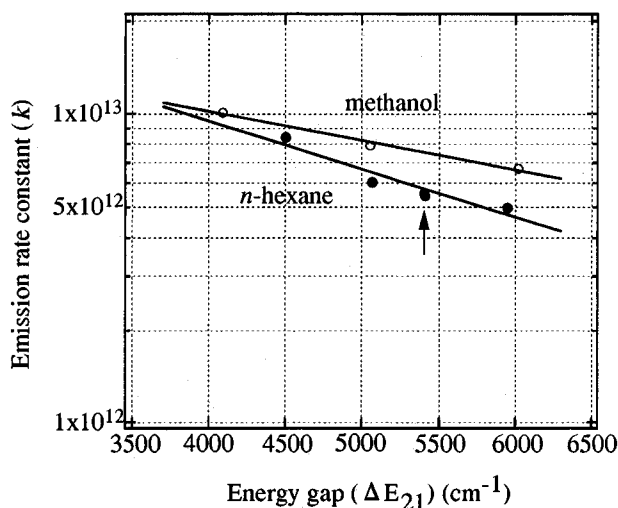


Figure 2. Emission rate constants from the S_2 state of β -carotene and analogues as a function of energy gap, ΔE_{21} . The inverses of the observed S_2 lifetimes are shown in a logarithmic scale as a function of the energy gap. Lines are the regression lines for individual experimental series in n -hexane (filled circles) and methanol (open circles). The arrow indicates the overlapped points for the case of β -carotene. Respective points correspond to β -apo-8'-carotenal, ethyl- β -apo-8'-carotenoate, β -carotene, and β -apo-8'-carotenol from left to right.

The observed S_2 lifetimes of β -carotene and its analogues were governed mainly by internal conversion. Since $\ln(k_{ic})$ is proportional to the energy gap, ΔE_{21} , it is concluded that the internal conversion process from the S_2 state of the above compounds is governed by the energy gap law in both solvent systems (Figure 2), even though there is no conclusive evidence regarding c for the S_2 to S_1 transition (cf. ref 13). This is the first direct evidence for this relaxation process, and it is consistent with results obtained using steady-state measurements.^{4,5}

A regression analysis, however, produced different slopes for the two solvent systems: the relative value in n -hexane was 65% larger than that in methanol. Thus the solvent effect should

be seriously considered. In a nonpolar solvent, i.e., n -hexane, the solute-solvent interaction is limited to the hydrocarbon groups, thus the dispersive effect is the main determinant for the electronic state. That is, the relaxation dynamics are determined mainly by ΔE_{21} . Symmetry of the molecular structure and of the π -electron system appears to be less significant to relaxation in n -hexane. This was also applicable to the relaxation of β -carotene in methanol. There is no specific interaction site between β -carotene and the polar groups of methanol, thus a dispersive effect of the solvent is the primary determinant of the S_2 state. The ΔE_{21} of β -carotene in methanol was essentially the same as that in n -hexane (Figure 2), thus the same decay constants in the two solvents is reasonable.

On the other hand, in a polar solvent, relaxation processes reflect the characteristics of molecules. It is known that the relaxation from the S_2 state of carotenoids is, in general, fast in polar solvents,¹⁵ consistent with the current study. The solvent effect was negligible, however, for β -carotene. In contrast, the three β -carotene analogues had a significant decrease in S_2 lifetimes, and this decrease correlated with the degree of the asymmetric characteristics of the carbonyl group conjugating to the double bond of polyenes. This correlation can be explained by a change in the interaction between solvents and the polar groups of the solutes; the three analogues contain oxygen and/or hydrogen atoms with which polar solvents can interact through a hydrogen bond, and the solvent molecules have a specific orientation in the ground state. This orientation might influence the excited state of the solutes. The S_2 lifetimes of the above analogues were in the range of 100 fs, thus it is unlikely that reorganization of solvent molecules occurred during such a short time. The polarized characteristics of the solute in the ground state may hold in the excited state.

There are two factors responsible for the slope, i.e., ω_M and γ . In the case of carotenoids or polyenes, it is known that the C=C stretching mode is the primary mode for relaxation.^{9,14} The resonance Raman studies¹⁶ indicate that a keto-carbonyl or ester-carbonyl group makes the C=C stretching vibration mode in the S_1 state more sensitive to its surroundings. In the case of β -apo-8'-carotenal,¹⁶ the frequency in the S_1 state shifted from 1757 cm^{-1} in n -hexane to 1713 cm^{-1} in chloroform, and the bandwidth broadened from 44 to 84 cm^{-1} . The solvent-induced frequency shift, however, is not large enough to explain the observed difference in the slope. Thus, the change in slope might be due to a difference in γ . The γ is closely related to the displacement of potential surface in the S_1 and S_2 states. The potential surface of the S_1 state is more affected by the solvent than that of the S_2 state.¹⁷ Therefore, we postulate that the strong solute-solvent interaction shifts the potential surface of the S_1 state along the configuration coordinate to cross with the S_2 potential surfaces in (highly) polar solvents, resulting in a high internal conversion rate between these two states.

In conclusion, we have directly demonstrated that the internal conversion from the S_2 state of carotenoids obeys the energy gap law of internal conversion, and we have also observed the solvent effect on the relaxation of polyenes, i.e., shortening of the S_2 lifetimes in polar solvents. The solvent-induced change in potential surface of polyene requires more precise examination.

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