Direct Determination of a Lifetime of the S_2 State of β-Carotene by Femtosecond Time-Resolved Fluorescence Spectroscopy

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Femtosecond time-resolved fluorescence spectroscopy (up-conversion) was applied to directly determine the lifetime of the S_2 state of β-carotene in *n*-hexane at room temperature. Upon excitation with a 425-nm pulse, the lifetime was measured to be 195 ± 10 fs throughout the whole emission wavelength, and the emission anisotropy ratio at 539 nm was 0.39 ± 0.02 throughout the emission time. Absence of a dynamic Stokes shift suggests that intramolecular relaxation occur within 50 fs, followed by internal conversion from the S_2 to S_1 state in 195 fs.

Carotenoids have important physiological functions in photosynthesis. They work as light-harvesting pigments due to a large oscillator strength in the visible region and efficient singlet energy transfer to chlorophylls. Further, they work as quenchers of chlorophyll triplet state and of singlet molecular oxygen due to their low-lying triplet state.[1]

Carotenoids are, in general, derivatives of polyenes belonging to the C_{2h} point group.[2] Two energetically low-lying singlet states are expected; one is closely related to the 2^1A_g (S_1) state, which is dipole forbidden from the ground state by parity, and the other is related to the 1^1B_u (S_2) state, which is responsible for a strong visible absorption (Figure 1).[3] Thus, the following photophysical processes are expected:

$$S_0 \xrightarrow{h\nu} S_2 \xrightarrow{k_{21}} S_1 \xrightarrow{k_{10}} S_0$$

Optical excitation to the S_2 state induces an internal conversion to the S_1 state, followed by relaxation to the ground state. Singlet energy transfer to chlorophylls is a competitive relaxation process from both states,[4] and hence the accurate estimation of rate constants is very important. A lifetime of the S_1 state ($1/k_{10}$) was measured by ground-state recovery experiments, ~10 ps for β-carotene[5] (molecular structure is shown below and steady-state spectra in Figure 1), whereas that of the S_2 state ($1/k_{21}$) was estimated by the subpicosecond transient absorption of β-carotene.[4b] Shreve et al.[4b] measured kinetics including two relaxation processes and detected a lifetime of the S_2 state to be 200 or 250 fs in ethanol or CS_2, respectively, and a lifetime of the S_1 state, 9.5 or 11 ps, respectively. However, a direct measurement of the lifetime of S_2 state in the femtosecond time regime is necessary to characterize the excited-state dynamics.

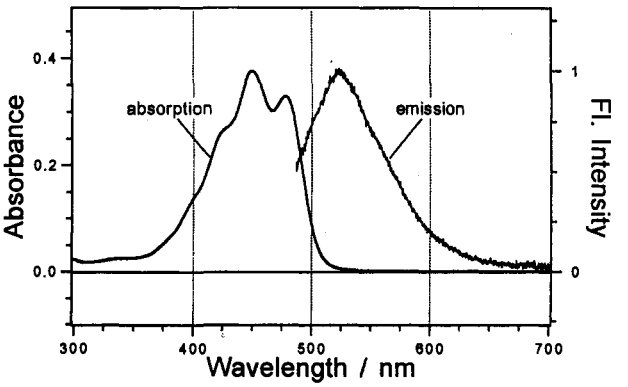


Figure 1. Absorption and corrected fluorescence spectra of β-carotene in *n*-hexane at room temperature. The sample concentration was either 2.5×10^{-5} or 1.3×10^{-6} M for absorption or emission measurements, respectively. The emission spectrum was obtained by excitation at 430 nm.

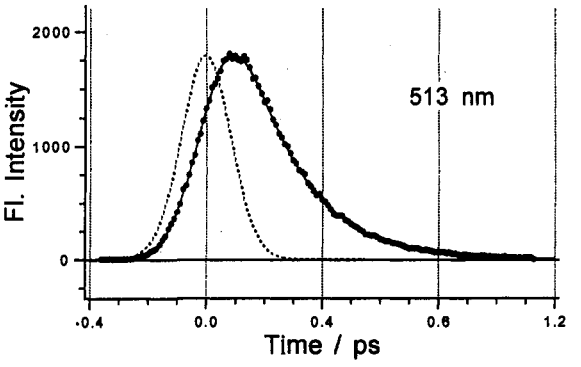
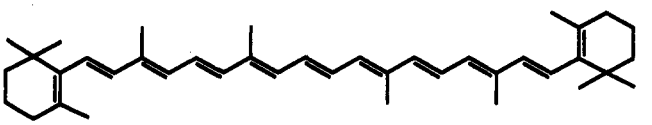


Figure 2. Typical fluorescence decay kinetics (at 513 nm) of β-carotene in *n*-hexane at room temperature. The instrumental response function (IRF) is shown by a broken line. The polarization angle between the excitation and the gate pulses was set to 54.7°. Solid circles indicate the observed photon counts with 10-fs intervals. The solid line is the best-fit curve, obtained by convolution with the present IRF and a single exponential decay of 195-fs lifetime. The χ-square was adopted as an index of the best fit, and the simulation was performed in 5-fs intervals. The χ-square was less than 0.5 for the best fit.



We adopted the fluorescence up-conversion technique[6] to determine directly a lifetime of the S_2 state of β-carotene. In our system, a combination of sum frequency generation and single-photon counting detection ensured a high sensitivity for fluorescence in the femtosecond time regime.[7] The second harmonics (425 nm) of a mode-locked Ti:sapphire laser (Spectra Physics, Tsunami) was used to excite a sample, and the fluorescence and a fundamental gate pulse (850 nm) were focused onto a phase-matched thin BBO crystal to generate ultraviolet sum frequency light. The instrumental response function, obtained by the cross-correlation measurement between the excitation and the gate pulses, had a Gaussian shape of 200 fs (fwhm, Figure 2, broken line).

β-carotene was purchased from Wako Chemicals, and its *all-trans* form was purified by high-performance liquid chroma-

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Table 1. Lifetimes of the S_2 State of β-Carotene Obtained by Femtosecond Fluorescence Measurements[a]

probing wavelength (nm)	lifetime (fs)	probing wavelength (nm)	lifetime (fs)
488	195 ± 10	567	200 ± 10
513	195 ± 5	595	200 ± 10
539	195 ± 5	624	190 ± 30

[a] Isotropic fluorescence was monitored at each wavelength.

tography.[8] β-Carotene dissolved in *n*-hexane was kept under N_2-saturated conditions during measurements at room temperature. The steady-state fluorescence spectrum (Figure 1) showed that the fluorescence predominantly came from the S_2 state,[8,9] and this was partly due to a highly symmetric structure of the conjugated double bond system in β-carotene.[2,9] Vibrational structure was appreciable; peak locations were estimated at 488, 521, and 561 nm by deconvolution of the spectrum. Based on the emission spectrum, we chose six wavelengths (Table 1) and applied the femtosecond up-conversion. For each measurement, a fresh β-carotene/*n*-hexane solution (2.5×10^{-5} M) was prepared and put in a 1-mm cell. The excitation energy was 80 mW with a 0.1-mm diameter (repetition rate, 82 MHz), thus about 0.6% of the molecules were excited per pulse. No spectral change of the sample absorption was found after measurements.

Upon excitation of β-carotene in *n*-hexane with a 425-nm and a 200-fs pulse, the fluorescence was observed only in the femtosecond regime at all monitoring wavelengths. Typical kinetics at 513 nm (monitoring wavelength, 320 ± 1 nm) are shown in Figure 2, where the deconvolution gave a lifetime of 195 ± 5 fs. It is noted that the fluorescence appeared without any delay and decayed to almost zero within 1 ps. The kinetic feature was essentially wavelength-independent; at six monitoring wavelengths, the lifetimes of the S_2 state of β-carotene were in a range of 190–200 fs (Table 1). Fluorescence anisotropy was measured at 539 nm (Figure 3); it gave a high value, $r = 0.39 \pm 0.02$, throughout the emission time, indicating that the fluorescence directly originated from the S_2 state.[10] The fluorescence spectrum estimated by integration of measured photon counts was essentially similar to the steady-state spectrum, also indicating the origin of emission to be the S_2 state. The fluorescence quantum yield was estimated to be 1.7×10^{-4} by the integrated oscillator strength for the S_2 state ($f = 2.66$), which was based on the extinction coefficient;[11] this yield was obtained by direct measurement of the S_2 lifetime and was almost the same as that in previous reports.[10,12]

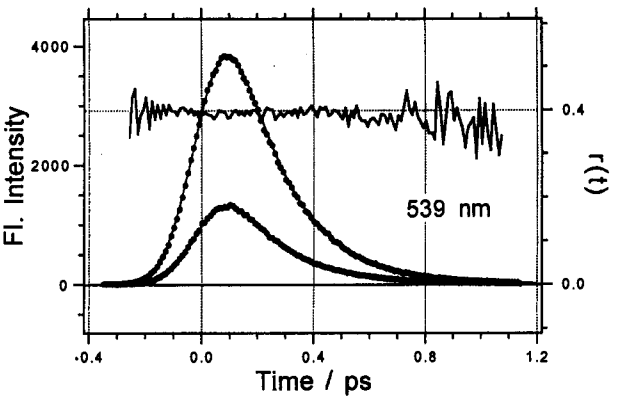

Figure 3. Anisotropic fluorescence decays (filled circle) and anisotropy change (solid line) of β-carotene in *n*-hexane measured at 539 nm. Filled circles indicate the observed photon counts (upper, vertical polarization, and lower, horizontal polarization). The smooth solid curves are the best-fit curves with lifetimes of 190 fs for both decays.

The 425-nm excitation yields the 0–2 vibrational transition of the S_2 state with ~2500 cm^{-1} excess energy. A dynamic Stokes shift due to an intramolecular vibrational relaxation process(es) is expected, as is often observed by ultrafast spectroscopic techniques.[13] However, the observed kinetics gave a single lifetime for the whole emission spectrum (Table 1). The absence of dynamic Stokes shift suggests that the intramolecular relaxation in the S_2 state occurs faster than the present time-resolution, and we estimated it shorter than 50 fs. This value agrees with the prediction by Watanabe et al.[14] The time-resolved spectroscopy with ~10-fs resolution could indicate the dynamic Stokes shift, probably as well as the oscillatory signal due to a coherent C–C stretching in the time domain.

Our measurement of the lifetime of the S_2 state of β-carotene in *n*-hexane agrees well with that obtained by transient absorption spectroscopy (200 fs in ethanol and 250 fs in CS_2).[4b] These facts imply that the fast internal conversion rate is an intrinsic property of β-carotene, probably due to the structure of excited state, and is less affected by the solvent environment. In the case of carotenoids containing a keto carbonyl group, a much faster internal conversion rate is expected.[8,9]

In conclusion, an up-conversion technique gave a highly accurate lifetime of the S_2 state of β-carotene in *n*-hexane and at the same time suggested the intramolecular relaxation time shorter than 50 fs. This leads to a better understanding of the excited-state dynamics of carotenoids.

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