Solvent Dependence of the Ultrafast S_2-S_1 Internal Conversion Rate of β-Carotene

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The solvent dependence of the steady-state and time-resolved fluorescence emission from the S_2 (1^1B_u) state of *all-trans*-β-carotene was investigated in a range of polar and nonpolar solvents and in hexane/carbon disulfide mixtures. The steady-state absorption and fluorescence emission maxima in both polar and nonpolar solvents showed parallel shifts with increasing solvent polarizability, indicating that the emitting state is the S_2 (1^1B_u) state. The lifetime of the S_2 state was determined using the fluorescence upconversion technique, and lifetimes varying from 120 fs in quinoline to 177 fs in hexane were found. An intramolecular relaxation process occurring on a similar time scale was observed as a broadening in the reconstructed time-dependent emission spectra in hexane and as emission wavelength-dependent lifetimes in all the solvents studied. Correlations were observed between the S_2 lifetimes, the π^* solvatochromic parameter, and the ratio of the absorption fine structure. A correlation between the rate of internal conversion from the S_2 state and the previously reported S_1 (2^1A_g) state Raman shifts of the C═C stretching mode supports the contention that the S_2 and S_1 states are vibronically coupled. The implications of these results for light harvesting in photosynthesis are also discussed.

I. Introduction

Carotenoids are a widespread class of natural pigments with a number of important functions in biological systems.[1] In photosynthesis, carotenoids play essential roles in photoprotection and light harvesting, and they are also important in the assembly and maintenance of the structure of some carotenoprotein complexes.[2] Carotenoids can protect an organism from reactive oxygen species such as singlet molecular oxygen (O_2 $^1\Delta_g$) in two ways. The first line of defense is to prevent the formation of these species in the first place. This is achieved by rapidly quenching the photosensitizing triplet state of (bacterio)chlorophylls, resulting in the formation of the low-energy (and harmless) carotenoid triplet state.[3] The second opportunity to confer photoprotection is to efficiently quench these species, should they be formed.[4]

In their light-harvesting role, carotenoids absorb light in the blue-green region and transfer energy to the (bacterio)chlorophylls with up to 100% efficiency.[2] Exactly how nature achieves this, and the role of each of the two (and possibly more) low-lying excited singlet states of carotenoids from which energy transfer could occur, is an important issue and one that requires further investigation.[5] Similarly, because reverse energy transfer from (bacterio)chlorophylls to the lowest excited singlet state of some carotenoids is energetically feasible, it has been proposed that this process could also be a protective mechanism that antenna complexes use to dissipate excess energy.[6] The lifetimes and energetics of the two lowest excited singlet states of carotenoids are therefore important parameters in determining their involvement in these energy-transfer processes.

The second excited singlet state, the S_2 state (1^1B_u, assuming C_{2h} symmetry), is readily located by its intense absorption in the visible region. However, the one-photon transition to the lowest excited singlet state S_1 (2^1A_g) from the ground state (1^1A_g) is symmetry forbidden, and only extremely weak shoulders in the red tail of the S_2 absorption have been observed.[7] Fluorescence has been detected from a number of carotenoids, and even though the emission is extremely weak, it has allowed the determination of some carotenoid singlet-state energies.[5] Emission from either the S_1, the S_2, or both states can be observed, depending on the number of conjugated double bonds (N), the solvent, and the nature of any substituents. The estimation of the energy level of the S_1 state of longer carotenoids ($N \geq 10$), however, has proved particularly difficult, and as comparison to the emission spectra from model polyenes has shown, the origin has frequently been misassigned.[8]

By making use of the energy gap law for radiationless transitions,[9] the S_1 energy of a carotenoid can be predicted if the S_1 lifetime has been accurately measured, usually by transient absorption methods. Plots of the natural logarithm of the deactivation rate against the S_1-S_0 energy gaps that have been experimentally determined show a good correlation, permitting extrapolations to smaller energy gaps.[10,11] For *all-trans*-β-carotene ($N = 11$), which has a lifetime of 10 ± 2 ps in a range of solvents,[12−14] this approach predicts the energy of the S_1 state to be 14 100 cm^{-1} (709 nm),[15] in excellent agreement with recent emission measurements.[16,17] Since these most recent results place the energy of the lowest excited singlet state of *all-trans*-β-carotene below that of chlorophyll *a*, energy transfer from this state must now be considered unfavorable. This being the case, the accurate determination of the lifetime of the S_2 state, which relaxes to the S_1 state by ultrafast internal conversion, takes on a new significance.

Until recently, it has only been possible to estimate the S_2 excited-state lifetimes of carotenoids. The lifetimes have been indirectly obtained either from the fluorescence quantum yields and the radiative rates calculated from the integrated absorption spectra or from analysis of the steady-state Raman and fluorescence profiles, or they have been directly measured by

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the transient absorption method. The S_2 lifetimes of *all-trans*-β-carotene estimated by these methods are 200 ± 100 fs in hexane,[18] or 170 fs in isopentane (at 170 K),[19] or 250 fs in ethanol and 200 fs in carbon disulfide[14] (at room temperature). With the development of femtosecond Ti:sapphire lasers, time-resolved fluorescence upconversion measurements[20] with blue-green excitation and high repetition rates have become possible. For *all-trans*-β-carotene S_2 lifetimes of 195 ± 10 fs in *n*-hexane[21] or 180 ± 10 fs in *n*-hexane or methanol[22] have been reported. Taken together, these results tend to suggest that the S_2 lifetime of β-carotene is essentially solvent independent, although fluorescence upconversion measurements indicate that this is not the case for spheroidene.[23]

To determine the involvement of each of the excited singlet states in the light-harvesting process, it is necessary to know what the lifetimes would be in the protein environment of photosynthetic systems in the absence of any energy transfer. One way to do this is to first determine whether the singlet lifetimes are dependent on the environment and then, if they are, measure the lifetimes in simple solvent "models" of the protein environment. The observation of singlet-state lifetimes in vivo that are significantly shorter than in the model solvents would then suggest the involvement of that singlet state in light harvesting. Since there has only been one such study (of the light-harvesting complexes LH2 and LH1 of *Rhodobacter sphaeroides*) where the S_2 lifetimes have been determined to be shorter in vivo than in carbon disulfide and a few other solvents,[23] we have begun an investigation of the involvement of the two excited singlet states of carotenoids in a number of photosynthetic antenna complexes.[24]

As part of this study, we initially wanted to determine the environmental dependence of the S_2 lifetimes of carotenoids and learn something about the factors controlling the ultrafast internal conversion from the excited S_2 to S_1 state. We have therefore employed the fluorescence upconversion technique to make a systematic study of the relaxation kinetics of the S_2 state of *all-trans*-β-carotene in a wide range of polar and nonpolar solvents and some solvent mixtures. We report here that the S_2 lifetime is indeed solvent dependent but does not show a simple correlation with the solvent polarizability (and therefore the S_2-S_1 energy gap). Instead, we find a reasonably linear correlation with the π^* scale of solvatochromism, a parameter incorporating both the solvent polarity and polarizability.

II. Experimental Section

Materials. The solvents used were carbon disulfide (99.9%), dichloromethane (>99.8%), acetone (>99.8%), tetrahydrofuran (>99.5%), and *n*-hexane (99%) from Merck and di-*n*-propyl ether (99.5%), cyclohexane (99+%), toluene (99+%), and benzonitrile (99+%) from Aldrich. Diethyl ether (>99.5%) and benzyl alcohol (>99.5%) were from Riedel-de Haën, 1,2-dichloroethane (>99.5%) was from Fluka, ethanol (99.5%) was from Kemetyl, and quinoline (>98%) was from Kistner.

The solvents were purified by column chromatography with neutral activated Al_2O_3 (Merck, type I), with the following exceptions: acetone was used as received, carbon disulfide was refluxed and distilled from phosphorus pentoxide, quinoline was refluxed and distilled from zinc under reduced pressure, and dichloromethane and dichloroethane were neutralized by addition of anhydrous potassium carbonate.[25] All solvents were additionally stored over molecular sieves.

all-trans-β,β-Carotene was obtained from Sigma and was purified by flash column chromatography on neutral Al_2O_3 (Merck, type I) with 7% acetone in petroleum ether as eluent and then recrystallized from benzene−methanol. 15-*cis*-β,β-Carotene (94%) was a gift from Hoffman La Roche and was used as received.

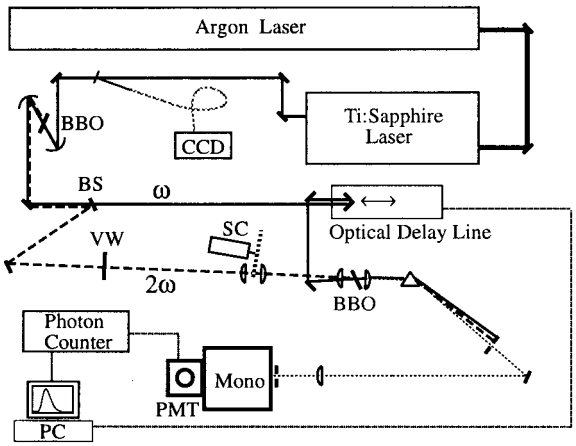

Figure 1. Ultrafast fluorescence upconversion setup. CCD = spectrograph with charge-coupled device detection, BS = dichroic beam splitter, VW = variable waveplate, SC = rotating sample cell. Details are described in the text.

Spectral Measurements. The absorption spectra of the carotenoids in 1 nm steps were determined with a Beckman DU-70 spectrophotometer. For time-resolved measurements the absorbance of β-carotene was up to 3 per mm at the laser excitation wavelength (except in ethanol where it was only 0.2 per mm because of poor solubility), corresponding to a concentration of up to 300 μM. The absorption spectra recorded after the laser measurements indicated that usually only a small amount of bleaching or isomerization had occurred and that the photoproducts absorbed in the UV.

Steady-state emission spectra were recorded with magic angle polarization with a calibrated and corrected SPEX Fluorolog 112 spectrofluorometer. The slit widths used gave a band-pass of 11 and 5.4 nm in the excitation and emission monochromators, respectively. The background Raman signal from the solvent was subtracted by running a solvent blank, and the second derivatives were examined after Savitzky-Golay smoothing. Fluorescence yields were determined by exciting a standard, fluorescein (Lambda Physik) in 0.1 M NaOH (ϕ_f = 0.90) at the same wavelength, and the usual corrections were made for the number of absorbed photons and for changes in the solvent refractive index.[26] Repeat measurements in hexane, ethanol, and carbon disulfide indicate that it is difficult to make accurate measurements of such low fluorescence yields. All solutions were air-saturated, and the experimental temperature was 20 °C.

Upconversion Measurements. The samples were excited at an 82 MHz repetition rate using a mode-locked Ti:sapphire laser (Spectra Physics Tsunami) pumped by an argon ion laser. The IR laser pulses typically had a spectral width of 22 nm fwhm, indicating that the pulse duration was ⩾60 fs, assuming near transform-limited pulses. The output was focused into a 1 mm BBO type I crystal and recollimated, using spherical concave mirrors (f = 102 mm), in an off-axis arrangement. The energy of the frequency-doubled light pulses was intentionally kept low at the sample (<0.25 nJ), to minimize photodestruction of the carotenoids.

The layout of the upconversion spectrometer is shown in Figure 1. The polarization of the pump beam, separated from the gating fundamental beam with a thin dichroic filter, was set with a variable waveplate (a Berek's polarization compensa-

tor, New Focus) and focused by a lens into the sample contained within a rotating cell. The fluorescence was collected and focused with lenses into a 0.5 mm type I BBO crystal mounted on a rotation stage. The near-IR gate beam was focused into the crystal with the same lens in a near-collinear arrangement (ca. 3° off-axis), after traversing a variable delay stage. The upconverted light was collected and dispersed with a UV fused silica lens and prism, spatially filtered with an iris, and folded with a UV-enhanced mirror. The sum frequency beam was focused through a UV filter onto the slits (0.5 mm, 4 nm bandpass) of a 0.1 m monochromator (ISA H10 UV, *f*/3.5), calibrated at 15 wavelengths (using 0.2 mm slits) using low-pressure mercury and cadmium spectral line lamps. The signal was detected with a Peltier element cooled, low-noise photon-counting photomultiplier (Hamamatsu R4220P, dark noise was <2 cps) and counted with a gated photon counter (Stanford Research Systems SR400).

The cross-correlation signal was measured with the same optical path by changing the monochromator wavelength and by rotating the crystal and prism, and it was attenuated with neutral density filters. The cross-correlation signals were close to Gaussian in shape with a solvent-dependent fwhm varying from >140 to 250 fs for hexane and 1 mm of CS_2, respectively. The cell path length was thus reduced to 0.2 mm for the more dispersive solvents to reduce the pulse broadening. A step size of 10 fs was used with an integration period of 1 s per step, and typically 3−5 scans were averaged per wavelength. Since we found that 15-*cis*-β-carotene isomerized rapidly to the trans form during the measurements and that *all-trans*-β-carotene was destroyed very rapidly in the chlorinated solvents, only single scans were made at a few wavelengths near the fluorescence maxima before changing the sample. Transient bleaching of β-carotene in a thin cell of benzyl alcohol was also observed, apparently a consequence of the higher viscosity of this solvent.

Fluorescence decays were typically recorded at 10−12 wavelengths spanning nearly the entire region of the steady-state emission spectra with parallel polarization, since the anisotropy in each solvent was determined to be close to 0.4 and non-decaying over the S_2 lifetime, within experimental error. The shortest fluorescence wavelength that could be measured with 485 nm excitation, the wavelength used for most of the measurements, was 510 nm. Excitation wavelength-dependent rise or decay times were not observed. To enable the entire emission region to be measured for spectral reconstruction, excitation into the 0−1 vibronic level of *all-trans*-β-carotene in *n*-hexane was made at 450 nm. Single-exponential decays were used to fit these transient fluorescence signals (measured from 472 to 608 nm), and the integrated intensities at each wavelength were normalized to the steady-state emission intensities. To reconstruct the spectra, the fits were also corrected for the shift in time zero.

Fluorescence transients were usually fitted with a single-exponential decay, or a biexponential rise and decay, with a floating background and were convoluted with response functions, using the SPECTRA program. Only in a few cases was there an improvement in the fit upon inclusion of a rise time, but the χ^2 and residuals indicated that the rise time of $\leq$15 fs was not warranted. Examination of the residuals at longer times sometimes indicated the need for a second decay component with longer lifetimes and small amplitude (<1%). This incomplete (or slow) recovery of the baseline may be emission from the S_1 state, and its origin is being investigated further in shorter chain carotenoids and polyenes. The accuracy in the lifetimes at a given wavelength is estimated to be better than 10 fs (20 fs for the samples in which rapid bleaching or isomerization was observed), on the basis of fits made using different instrument response functions and from repeat measurements, some made with unpurified β-carotene and solvents.

III. Results

Solvation Effects on the Spectra. It is well-known that the fully allowed absorption transition of carotenoids is solvent dependent and shifts almost linearly with the refractive index term of the polarizability, $R(n) = (n^2 - 1)/(n^2 + 2)$, indicating that dispersive interactions are dominant.[27−29] This is the case for both nonpolar and polar solvents, although the slopes and intercepts differ.[29] A similarly detailed study has not yet been made of the weak fluorescence emission spectra in both nonpolar and polar solvents. The emission maxima of carotenoids are expected to show either almost no shift with solvent or spectral shifts which parallel that of the absorption, when the emission is from the forbidden S_1 state or the allowed S_2 state, respectively. The maxima of the absorption and fluorescence spectra of *all-trans*-β-carotene in some polar and nonpolar solvents (listed in Table 1) are shown plotted against the polarizability function $R(n)$ in Figure 2. The maxima were plotted instead of the origins because the fluorescence origin appears only as a shoulder in the region where the correction for the Raman band of the solvent is greatest, and it could not therefore be reliably located from the second derivatives.

The slopes and intercepts determined for the shift in the absorption maxima in nonpolar and polar solvents are in very good agreement with those previously reported,[29] although we have calculated $R(n)$ for the hexane/carbon disulfide mixtures on a molar basis instead of the previously used volume basis. The spectral shift of the absorption and fluorescence maxima are parallel (within experimental error) in both nonpolar and polar solvents if benzonitrile and quinoline are omitted from the fits. For these two solvents smaller Stokes shifts were observed. However, since it is particularly difficult to make the correction for the overlapping background emission from these solvents, we believe that errors in this procedure are probably responsible for the deviation from the fit (about 7 nm).

In addition to examining the spectral shifts, we also examined the profile and intensity of the absorption and emission spectra to determine whether they too changed with the solvent. The absorption spectrum of *all-trans*-β-carotene in all solvents is rather broad (fwhm $\sim 4000 \pm 80$ cm^{-1}), and only two discrete vibronic bands, the origin and the more intense 0−1 vibronic component, are seen. The 0−1 vibronic spacings determined from the second derivatives of the absorption spectra are close to 1500 cm^{-1}, indicating that the symmetric C═C double-bond stretch is dominant.[30] The emission spectra, however, are significantly narrower (2800 ± 300 cm^{-1}) than the absorption spectra, and because they show only a single peak in all solvents, they are not mirror symmetric with respect to the absorption spectra.

A solvent-dependent feature that is however apparent is the change in the absorption fine structure, defined as the ratio of the 0−0 to 0−1 peak height measured from the interpeak valley (% III/II).[31] The fine structure ratios (listed in Table 1) are clearly smaller in polar solvents, and they also show a decrease with increasing solvent polarizability. By overlaying the normalized absorption spectra plotted on the wavenumber scale, it is clear that the decrease in % III/II is not the result of a change in the relative intensities of the vibronic bands (from the baseline), with the exception of carbon disulfide and its mixtures. In this solvent, the relative increase in the 0−0 band

TABLE 1: Solvent Dependence of the Photophysical Properties[a] of the S_2 State of *all-trans*-β-Carotene

solvent	no.	ν_{abs}	ν_{em}	ΔE_{21}	% III/II	f	k_r	τ	ϕ_{fc}	ϕ_{fe}
hexane	1	22 220	19 080	6450	39	2.55	9.59	177	1.70	1.0
cyclohexane	2	21 980	18 760	6190	36	2.41	9.54	171	1.63	1.2
25% CS_2[b]	3	21 810	18 690	6050	25	2.28	9.32	156	1.45	1.2
50% CS_2[b]	4	21 320	18 320	5640	17	2.11	8.96	140	1.27	1.1
75% CS_2[b]	5	20 990	17 920	5260	17	2.00	8.51	132	1.15	1.2
CS_2	6	20 700	17 510	4920	20	1.93	8.02	129	1.08	1.5
propyl ether	7					2.54		168		
ether	8	22 200	18 980	6390	35	2.61	9.32	163	1.52	1.0
toluene	9	21 570	18 450	5800	29	2.23	9.24	144	1.36	1.6
ethanol	10	22 120	18 980	6360	29	2.59	9.22	144	1.33	1.4
tetrahydrofuran	11	21 830	18 730	6080	28	2.46	9.30	143	1.33	
acetone	12	22 030	18 900	6250	25	2.59	9.06	134	1.23	0.9
dichloroethane	13					2.37		132		
dichloromethane	14	21 620	18 480	5870	18	2.42	8.93	125	1.17	1.0
benzyl alcohol	15	21 280	18 180	5530	13	2.13	8.64	125	1.10	
benzonitrile	16	21 320	18 480	5640	22	2.16	9.05	122	1.12	1.6
quinoline	17	21 010	18 120	5360	13	1.93	9.05	120	1.14	

[a] Photophysical parameters (estimated errors) are as follows: ν_{abs}, absorption maximum ($\pm$100 cm^{-1}); ν_{em}, fluorescence emission maximum ($\pm$250 cm^{-1}); ΔE_{21}, energy gap between the S_2 and S_1 states ($\pm$300 cm^{-1}), assuming the energy of the S_1 state is 14 200 cm^{-1}; % III/II, absorption fine structure ratio ($\pm$5%); f, oscillator strength calculated using eq 1 and normalized to the least-squares fit of the integrated absorption term plotted against the solvent polarizability ($\pm$0.15); k_r, radiative rate (10^8 s^{-1}) calculated using eq 2 and normalized to the least-squares fit of the integrated absorption term plotted against the solvent polarizability ($\pm 0.6 \times 10^8$ s^{-1}); τ, lifetime of the S_2 state ($\pm$10 fs, except solvents 13−15, $\pm$20 fs); ϕ_{fc}, fluorescence quantum yield (10^{-4}) calculated using the radiative rate and lifetime ($\pm 0.2 \times 10^{-4}$); ϕ_{fe}, fluorescence quantum yield (10^{-4}) experimentally determined ($\pm 0.5 \times 10^{-4}$). [b] Volume % of carbon disulfide in hexane.

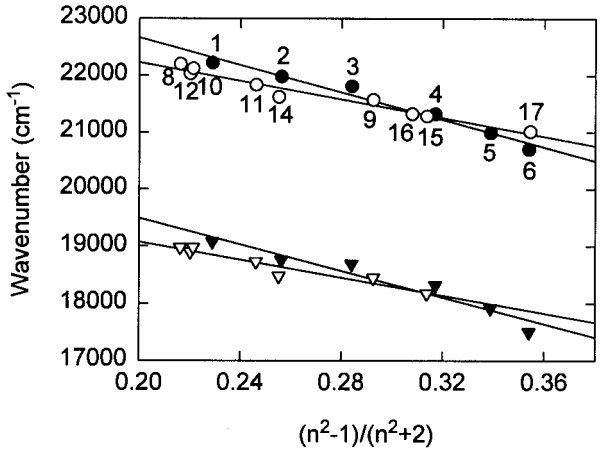

Figure 2. Maxima of the steady-state absorption (circles) and fluorescence emission (triangles) spectra of *all-trans*-β-carotene plotted against the solvent polarizability parameter $R(n)$. The numbers refer to the solvents listed in Table 1, and open and closed symbols are used for polar and nonpolar solvents, respectively. The fits are as follows: closed circles, $\nu_{abs} = 25\,080-12\,060\ R(n)$; open circles, $\nu_{abs} = 23\,870-8181\ R(n)$; closed triangles, $\nu_{em} = 21\,810-11\,560\ R(n)$; and open triangles, $\nu_{em} = 20\,640-7787\ R(n)$. The fluorescence emission maxima for benzonitrile (solvent 16) and quinoline (solvent 17) were excluded from the latter fit.

intensity appears to be the result of a decrease in the spacing between the absorption peaks.

The oscillator strength of *all-trans*-β-carotene was determined for 10 solvents and mixtures from the integrated absorption of 3.8 μM solutions (assuming an absorption coefficient ϵ of 134 300 M^{-1} cm^{-1} in cyclohexane[31]) using the relation

$$f = 4.319 \times 10^{-9} \int \epsilon \, d\bar{\nu} \qquad (1)$$

The integration was made to a high-energy limit varying from 27 030 to 25 000 cm^{-1} for hexane to carbon disulfide. The oscillator strength of the $S_0 \rightarrow S_2$ transition was calculated for all the solvents from the parameters of a least-squares fit of the integrated absorption term plotted against the refractive index function $R(n)$, since good linearity is observed for carotenoids.[32] The normalized oscillator strengths calculated in this manner are in good agreement with the literature values.[28,33]

The radiative rates were also calculated in a similar manner using the Strickler−Berg relationship[34]

$$k_r = 2.880 \times 10^{-9} n^2 \frac{\int I(\bar{\nu})\, d\bar{\nu}}{\int \bar{\nu}^{-3} I(\bar{\nu})\, d\bar{\nu}} \int \frac{\epsilon(\bar{\nu})}{\bar{\nu}}\, d\bar{\nu} \qquad (2)$$

where $I(\bar{\nu})$ is the fluorescence intensity, and the integrated absorption terms for each solvent were again normalized to the refractive index function. The calculated radiative lifetimes (Table 1) vary only slightly with solvent, increasing from 1.04 ns in hexane to 1.25 ns in carbon disulfide, because the decrease in the integrated absorption on going from hexane to carbon disulfide is mostly compensated by the increase in the refractive index term. Our calculated radiative rates and experimentally determined fluorescence yields, which are in the range (0.9−1.6) $\times 10^{-4}$, in good agreement with the results of Shreve et al.,[14] can be used to predict that the lifetime of the S_2 state lies in the range 100−190 fs.

Time-Resolved Emission. One of the advantages of the fluorescence upconversion technique is that spectral information can be achieved with a one-color excitation laser pulse. By recording emission decays at different wavelengths, the time-dependent fluorescence emission spectrum can be reconstructed, as shown for *all-trans*-β-carotene in *n*-hexane in Figure 3a. These transient spectra are very similar to the steady-state emission spectrum excited at the same wavelength, and no "hot band" emission is seen at shorter wavelengths during the rise of the exciting pulse. On normalizing the time-dependent emission spectra, some small changes are however seen between two time extremes (expanded ~100-fold compared to Figure 3a) and the steady-state emission in the inset of Figure 3b. The spectra are narrower upon initial formation, and there is a time-dependent increase in the relative intensity of the longest wavelength emission, resulting in spectral broadening with a time constant of ~300 fs. Probably as a result of this asymmetric broadening, there is also a small red shift (about 6 nm) in the maximum.

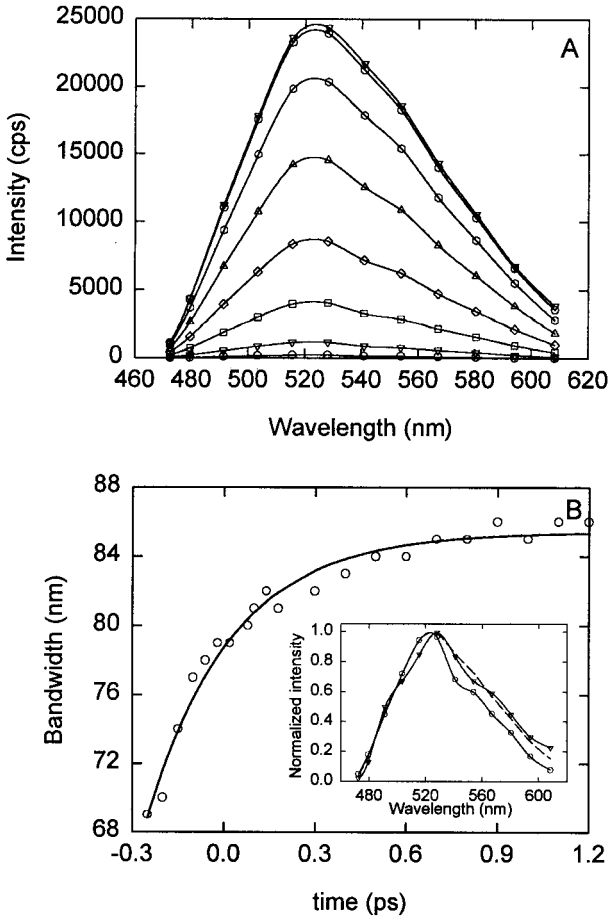

Figure 3. Reconstructed time-resolved emission spectra of *all-trans-*β-carotene in *n*-hexane (A). The times shown are (in order of increasing intensity) −200, −150, −100, −60, −20, 20, 60, and 100 fs. The emission wavelength-dependent lifetimes (in order of increasing wavelength) are 151, 167, 176, 177, 175, 177, 176, 174, 180, 181, 184, and 185 fs. Plot B shows a single-exponential fit (the lifetime is 280 fs) to the increase in the bandwidth (full width at half-maximum) with time. The inset shows the normalized time-dependent emission spectra at −200 fs (circles) and 800 fs (triangles), along with the steady-state emission spectrum (dashed line), which is essentially identical to the spectra at 60 and 100 fs in plot A.

The decay of the S_2 state of *all-trans-*β-carotene was measured in a range of solvents, and the lifetime was determined to be solvent dependent. The average S_2-state lifetimes in the region of the emission maxima are listed in Table 1. The longest lifetime we obtained was 177 fs in hexane, in excellent agreement with another fluorescence upconversion measurement,[22] and the shortest lifetime was ∼120 fs in the polar solvents benzonitrile and quinoline. The lifetimes we determined in ethanol and carbon disulfide are, however, significantly shorter than those previously obtained by the femtosecond transient absorption technique.[14] A difference in the S_2 lifetime determined by the two methods is in agreement with recent results reported for spheroidene in pentane.[23]

In addition to the solvent dependence, the decay lifetimes also show an emission wavelength dependence in all solvents, with the shortest lifetime being found at the shortest wavelength and slightly longer decays (a total range of ∼20 fs) being found in the red region. This is consistent with the relative increase in the red-edge emission and broadening observed in the time-dependent fluorescence spectra in hexane (Figure 3). The fluorescence decay kinetics for hexane, ethanol, and quinoline at wavelengths near the emission maxima are shown in Figure 4. We found no evidence for an emission wavelength-dependent

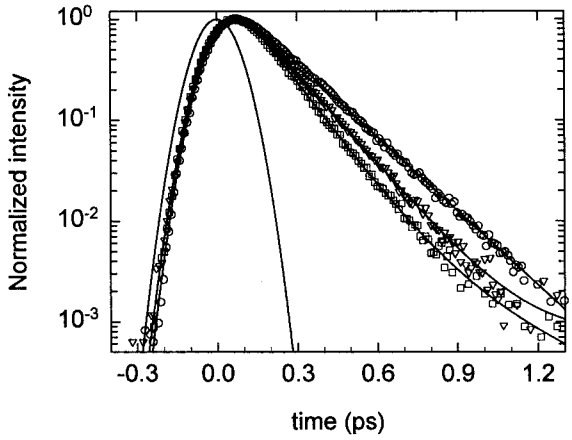

Figure 4. Examples of the subpicosecond fluorescence decay kinetics obtained for *all-trans-*β-carotene in *n*-hexane (circles), ethanol (triangles), and quinoline (squares) following excitation at 485 nm. The Gaussian response function shown is 170 fs, and the fits are normalized (the actual intensities are 13 300, 6100, and 7700 cps). The lifetimes of the fits shown and the emission wavelengths are as follows: *n*-hexane, 178 fs [567 nm]; ethanol, 144 fs, >10 ps (0.06%) [543 nm]; and quinoline, 122 fs, 370 fs (0.8%) [567 nm].

rise time in any solvent, which would indicate a dynamic Stokes shift. The S_2 decays of 15-*cis-*β-carotene in hexane, carbon disulfide, and acetone were also measured at wavelengths near the emission maxima, and the lifetimes were found to be identical to the all-trans isomer, within experimental error.

In an attempt to determine the origin of the solvent dependence, the lifetimes of the S_2 state of *all-trans-*β-carotene were plotted against a range of bulk and molecular solvent properties. In Figure 5a the lifetimes are plotted against the refractive index function $R(n)$ for both nonpolar (filled circles) and polar (open circles) solvents. The correlation of the lifetimes with $R(n)$ in nonpolar solvents is quite good, but in contrast to the behavior observed for the absorption and emission spectral shifts (Figure 2), the correlation in polar solvents is rather poor. For a given polarizability, it is clear that the lifetime is shorter in polar solvents than in nonpolar solvents. Improved fits are obtained for polar and nonpolar solvents, again treated separately, by plotting in Figure 5b the lifetimes against a polarity function $R(\epsilon) = (\epsilon - 1)/(\epsilon + 2)$, where ϵ is the dielectric constant of the solvent. In this plot, toluene was included in the fit of nonpolar solvents because it has a small dielectric constant.

These plots indicate that there is a general correlation with both the polarity and polarizability of the solvent and suggest that a solvent parameter combining both of these factors should be considered. One such parameter, incorporating polarizability and polarity with about equal weight (in nonaromatic solvents), is the empirical π^* scale of solvation.[35] The S_2 lifetimes plotted against the π^* parameter are shown in Figure 5c, and it is apparent that a good fit is obtained for both polar and nonpolar solvents treated together, if carbon disulfide and its mixtures in *n*-hexane are excluded (solvents 3−6). The vertical range bars included in Figure 5c indicate the spread of lifetimes in the direction of the least-squares fit and thus, in some cases, depend on the range of emission wavelengths measured. For dipropyl ether we have used the π^* value of diisopropyl ether, although this value is believed to be lower than it should be because of steric shielding of the ether group.[35] The π^* values for the two alcohols[36] must also be considered less certain, since these solvents were not included in the recent reexamination and extension of the π^* scale employing primarily one indicator (4-nitroanisole).[35]

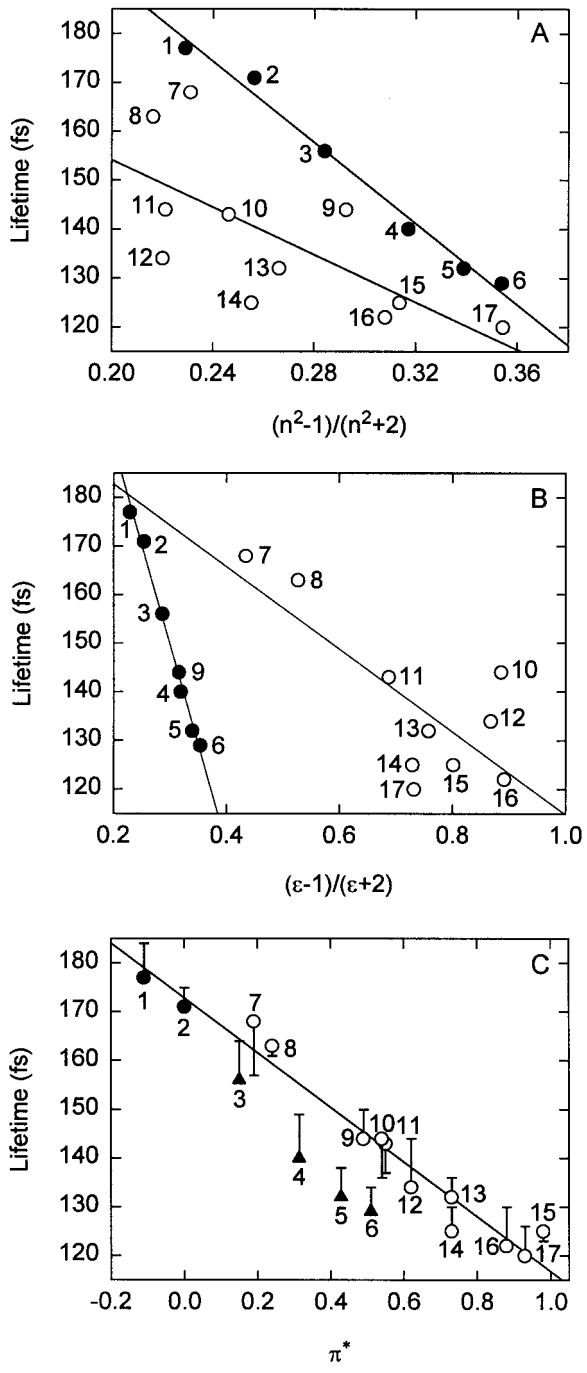

Figure 5. Plots of the S_2 lifetimes (±10 fs, except solvents 13−15, ±20 fs) of *all-trans*-β-carotene against the solvent polarizability function (A), polarity function (B), and π^* solvatochromic parameter (C). The numbers refer to the solvents listed in Table 1, and open and closed symbols are used for polar and nonpolar solvents, respectively. Carbon disulfide and its mixtures in hexane (solvents 3−6) are excluded from the fit shown in plot C, and the range bars indicate the spread of the lifetimes in the direction of the best fit.

IV. Discussion

The Energy Gap. Our time-resolved fluorescence upconversion measurements demonstrate that the lifetime of the S_2 state of β-carotene is clearly solvent dependent. It is reasonable to assume that the decrease in the lifetime of the S_2 state with solvent indicates an increase in the rate of internal conversion to the S_1 state, because the rate constants for all other pathways are almost certainly orders of magnitude smaller.[3,5] Thus, according to the energy gap law,[9] an exponential increase in the rate of this radiationless transition can be expected if there is a decrease in the energy gap ΔE_{21} between the S_2 and S_1 states.

The energy of the S_2 state can readily be obtained from the absorption and fluorescence spectra which, as shown in Figure 2, show parallel shifts with increasing polarizability in both polar and nonpolar solvents. The energy level of the S_1 state of carotenes, on the other hand, is believed to be essentially solvent independent, and only very small shifts have previously been observed (200 cm^{-1} for mini-7, a β-carotene derivative with seven conjugated double bonds, on going from hexane to carbon disulfide).[18] Assuming the energy of the S_1 state is 14 200 cm^{-1},[16] the S_2-S_1 energy gap ΔE_{21} is calculated to lie in the range 6500−4900 cm^{-1} (Table 1). The decrease in ΔE_{21} with increasing solvent polarizability is therefore rather small.

For the S_1 state of carotenoids with different conjugation lengths ($N = 5-9$) and a large energy gap span of (22 700−15 300 cm^{-1}), a "simplified" form of the energy gap law (with all parameters other than the energy gap kept constant) is indeed followed.[15] However, it is not clear whether this form of the energy gap law can be applied to the case of the radiationless transition from the S_2 to the S_1 state, since the energy gap is much smaller. A correlation between the fluorescence yields of the S_2 state of the mini-β-carotenes with eight and nine conjugated double bonds with the energy gap ΔE_{21} has been observed in a few nonpolar solvents.[16] Similarly, correlations between the ratio of the S_2 to S_1 fluorescence yields or intensities and the energy gap have also been reported.[16,37] However, these results on their own should not be interpreted as supporting evidence for a simple energy gap law relationship, because the radiative rate of the S_1 state also depends on the solvent and energy gap.[16,17]

For β-carotene in nonpolar solvents, the experimentally determined S_2 lifetimes do show a reasonable correlation (Figure 5a) with the refractive index function $R(n)$. However, plotting the natural logarithm of the deactivation rate against the energy gap (Table 1) results in a significantly worse correlation. This is apparently the result of smaller than expected energy gaps and/or deactivation rates in carbon disulfide and the higher molar ratio mixtures of this solvent in hexane. A smaller deactivation rate for carbon disulfide is not consistent, however, with the behavior observed in the plot of the S_2 lifetimes against the π^* scale (Figure 5c), assuming the π^* value is not solute dependent in carbon disulfide.

It has long been established that the absorption bands of carotenoids display particularly large red shifts in carbon disulfide.[27] The anomalous red shift in carbon disulfide, and also in carbon tetrachloride and some polar solvents, that is additional to that expected from an alkane extrapolation has been called the *excess* red shift.[38] This can be observed in Figure 2 (and in other polarizability plots that have been published[29,38]) as deviations of the absorption (and fluorescence) maxima of *all-trans*-β-carotene in carbon disulfide and its hexane mixtures (solvents 4−6) from an extrapolation of the maxima determined in the two alkanes (solvents 1 and 2). To account for this additional shift, an interaction between these solvents and the carotenoid affecting the microscopic polarizability around the solute was presumed.[38] This could be the result of close packing of these small molecules around the conjugated backbone,[27] and multipolar interactions may additionally be important.[29]

Regardless of there being any correlation of the S_2 deactivation rate with energy gap in "noninteracting", nonpolar solvents, clearly no correlation exists for polar solvents. This is apparent

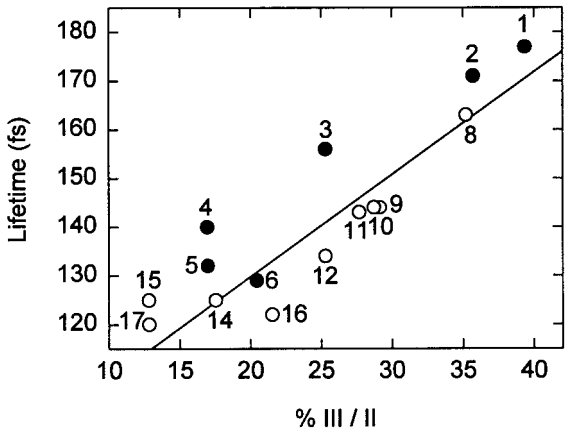

Figure 6. Plot of the S_2 lifetimes ($\pm$10 fs, except solvents 13−15, $\pm$20 fs) of *all-trans*-β-carotene against the absorption fine structure ratio ($\pm$5%). The numbers refer to the solvents listed in Table 1, and the hexane−carbon disulfide mixtures (solvents 3−5) were excluded from the fit.

by comparing the lifetimes determined in ethanol, ether, acetone, and tetrahydrofuran with hexane and cyclohexane. In these solvents the S_2-S_1 energy gaps are all in the same range, and therefore similar lifetimes are expected if ΔE_{21} was the only factor controlling the rate of internal conversion. The plots of Figure 5 indicate that the permanent dipoles of the solvent must also play a role in enhancing the radiationless deactivation rate. For polar solvents, it has been shown by Nagae et al.[29] that although the energy of the S_2 state decreases linearly with increasing polarizability, the intercept and slope are smaller than for nonpolar solvents. The stabilization of the highly polarizable S_2 state by the electric field generated by fluctuations of the permanent solvent dipoles in a long cavity was predicted to result in a reduction of the dispersive interaction, mainly through interactions with multipoles of the carotenoid.[29] As a consequence, only small *excess* red shifts can be expected for polar solvents with high polarizabilities (such as benzonitrile, benzyl alcohol, and quinoline). The fact that the S_2 lifetime is shortest in these solvents indicates that the *excess* red shift is not therefore a good measure of the solvent interactions that result in reduced S_2 lifetimes.

Fine Structure Ratio. In addition to the polarizability-dependent spectral shifts, we also noticed fairly large differences in the vibronic fine structure of the absorption spectra of *all-trans*-β-carotene with solvent. We can attribute this decrease in the fine structure ratio to a change in shape or broadening of the individual vibronic bands, because the relative intensities of the vibronic bands remain constant. Any band broadening must occur, however, with little or no overall broadening of the absorption profile at half-maximum. The change in fine structure ratio has some relevance, because it shows some correlation with the π^* solvatochromic parameter and the S_2 lifetime (Figure 6), if the hexane/carbon disulfide mixtures are excluded. For this solvent mixture, the interactions of the two components with β-carotene are apparently different, resulting in a smaller fine structure ratio for the 1:1 and 1:3 (by volume) hexane/carbon disulfide mixtures (solvents 4 and 5) than for pure carbon disulfide (solvent 6). We may conclude that the changes in the fine structure ratio are a crude, but potentially useful, indicator of the same intermolecular interactions that are responsible for changes in the deactivation rate from the S_2 state.

Relaxation Processes. In contrast to the absorption spectra, the steady-state fluorescence emission spectra of *all-trans*-β-carotene are narrower and rather featureless in all solvents. A normalized plot of the modified fluorescence, $I(\bar{\nu})/\bar{\nu}^3$, and absorption $\epsilon(\bar{\nu})/(\bar{\nu})$, spectra and its mirror image reflected about the crossing point in cyclohexane indicates that it is mainly low-energy emission that is lost. The absence of mirror symmetry could be the result of the vibrational to electronic relaxation rates being of comparable magnitude.[39] However, we have found no evidence of an excitation (or emission) wavelength-dependent rise time, and we estimate the rise times to be $<$20 fs. Furthermore, we do not observe excitation wavelength-dependent decay lifetimes (for excitation from 400 to 485 nm) or any "hot band" emission, which we might expect to see if the vibrational relaxation time was slower than the S_2 population relaxation time.[39] Instead, an ultrafast relaxation to a geometry with fewer conformers than initially populated[40] and/or weaker intermolecular interactions with the solvent, which is considered to be static within the short lifetime of the excited state,[19,41] could account for the narrowing of the fluorescence emission spectra.

Emission wavelength-dependent decay times were however observed in all solvents, with the shortest lifetime being found at the shortest wavelength and slightly longer decays being found to the red. Fitting these decays to the same single-exponential decay time resulted in significantly worse fits, even if a second decay component was used. The time-dependent emission spectra (Figure 3) therefore show some broadening and increasing asymmetry to the red with time, and perhaps as a consequence, they also show a small red shift. These changes are indicative of an intramolecular relaxation process occurring on a comparable time scale to the decay of the S_2 state. A single-exponential fit (Figure 3b) indicates the broadening occurs with a lifetime of about 0.3 ps, although given the low spectral resolution, the increase in the bandwidth need not be single exponential. With the observation of similar emission wavelength-dependent lifetime behavior for spheroidene in some solvents,[23] such a relaxation process may prove to be intrinsic to the carotenoid backbone.

Vibronic Coupling. The first direct evidence for an excited singlet state (2^1A_g) lying lower in energy than the $1B_u^+$ state was obtained by Hudson and Kohler for the polyene *all-trans*-1,8-diphenyloctatetraene.[42,43] It was shown that this strictly one-photon forbidden transition for polyenes with C_{2h} symmetry becomes partially allowed because there is a breakdown of the symmetry and mixing with most likely the $1B_u^+$ state.[43,44] The high-resolution measurements made on *all-trans*-octatetraene in a series of linear alkane crystals at 4.2 K are particularly illustrative of how even the smallest environmental perturbations result in a lifting of the symmetry selection rule.[45] Further evidence of a distortion from ideal C_{2h} symmetry comes from electrooptical absorption measurements of polyenes and carotenoids in solvents and polymer films, since small but significant changes in the dipole moment ($\Delta\mu = 1-5$ D, with the exception of *all-trans*-retinal), in addition to the large changes in polarizability, have been observed.[46−48]

For carotenoids, some out-of-plane distortion (bending or twisting of the chain) is probably always found and is either inherent to the flexible methyl-substituted polyene chain,[49] particularly when substituted with rings that are twisted out of the plane,[44] or is a consequence of dynamic interactions with the solvent. The methyl groups also introduce an in-plane distortion in the form of some S-shaped bending of the backbone of *all-trans*-carotenoids, and this can be seen as a weak absorption (forbidden for C_{2h} symmetry) in the cis-band region of both the observed and theoretical absorption spectra of spheroidene and its shorter conjugated derivatives.[49] For *cis*-

carotenoids, the intensity of this additional band, which is found in the UV about 140 nm from the longest wavelength absorption maximum in hexane, depends on the number and position of the cis double bonds and reaches a maximum for the central isomer (15-cis for β-carotene).[31] The introduction of an in-plane bend in the center of the conjugated backbone of β-carotene does not apparently result in a reduction in the S_2 lifetime for this sterically unhindered isomer.

A consequence of a breakdown of the C_{2h} symmetry, thereby allowing mixing of the strongly allowed S_2 state with the S_1 state, is an increase in the radiative strength of the S_1 state of polyenes.[43] The S_1 radiative lifetimes of *all-trans*-β-carotene, calculated using the experimentally measured fluorescence quantum yields and the lifetime of the S_1 state, have recently been reported in hexane, toluene, and carbon disulfide.[16,17] These enable an estimation of the matrix element V_{12} (vibronic or environmentally induced) coupling the S_1 and S_2 states to be made, if it is assumed that all the radiative strength of the forbidden S_1 state is obtained by mixing with the S_2 state.[43]

$$V_{12}^2 = k_r(S_1)(\Delta E_{21})^2/k_r(S_2) \quad (3)$$

With radiative lifetimes of 2.5−0.5 μs,[16,17] matrix elements in the range 110−250 cm^{-1} can be determined. These values are comparable to the matrix elements determined for *all-trans*-1,8-diphenyloctatetraene: 90,[50] 215,[43] or 500 cm^{-1}.[51]

The oscillator strength of the S_1 state can be calculated using the relationship[43]

$$f(S_1) = f(S_2)(V_{12}/\Delta E_{21})^2 \quad (4)$$

since the energy gaps and the S_2 oscillator strengths are known (listed in Table 1). Using the matrix elements calculated above, S_1-state oscillator strengths of (1.0−5.0) × 10^{-3} are obtained. Although these oscillator strengths, corresponding to absorption coefficients of only 50−250 M^{-1} cm^{-1}, are much smaller than those previously reported for *all-trans*-1,8-diphenyloctatetraene (0.05−0.15),[43] they are comparable to those of neurosporene.[7] This indicates that the S_1 absorption for β-carotene could be observable, although attempts to detect this transition in solvents have not so far been successful.[17]

The breakdown of the strict mirror symmetry and the coupling of the S_2 and S_1 excited states also has consequences for the vibrational frequencies of polyenes.[52] Vibronic coupling between the ground and S_1 excited states of polyenes and carotenoids is known to be particularly strong for the C═C double-bond symmetric stretching mode, and large changes in the vibrational frequency of this mode have been observed in both high-resolution fluorescence and in picosecond time-resolved resonance Raman measurements. In the ground state this mode is found in the 1500−1600 cm^{-1} region, whereas in the S_1 state it is higher than 1700 cm^{-1},[52] with the exception of a Schiff base of dodecapentaenal where this mode decreases upon excitation.[53] For polyenes and carotenoids without a center of symmetry, depressed S_1 and almost unchanged S_0 C═C stretch frequencies have been attributed to an additional vibronic coupling to a higher electronic state, which is believed to be the S_2 state.[52,54]

The strength of vibronic coupling is inversely proportional to the energy gap and proportional to the square of the vibronic coupling element for the electronic states involved.[52] Since only the S_2 energy changes significantly with solvent, a change in the frequency of the C═C stretching mode in the S_1 state with solvent implies a vibronic coupling of the S_1 and S_2 states.[54] In symmetric carotenoids such as *all-trans*-β-carotene, the vibronic coupling element for these states should be zero, and thus no solvent dependence is expected. However, for this carotene and also for two β-apocarotenal derivatives, decreases in the S_1 Raman frequencies of the Franck−Condon-active C═C stretching mode with decreasing S_2 energy (increasing solvent polarizability) were in fact observed.[54] In contrast, the S_0 frequencies showed only a slight solvent dependence.

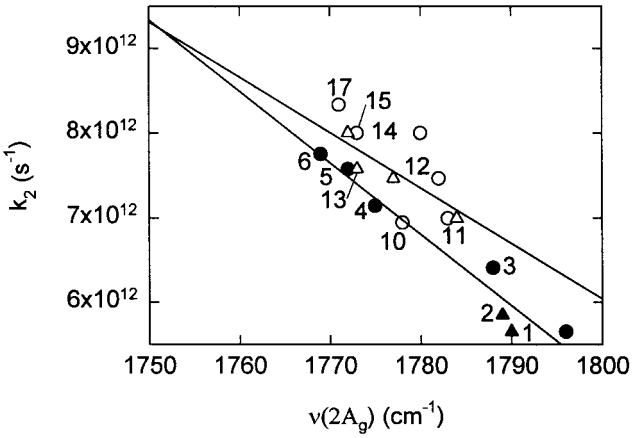

Figure 7. Plot of the rate of deactivation from the S_2 state of *all-trans*-β-carotene against the C═C double-bond symmetric stretching mode in the S_1 state, measured by time-resolved resonance Raman spectroscopy. The numbers refer to the solvents listed in Table 1, and open and closed symbols are used for polar and nonpolar solvents, respectively. The triangles show data from ref 54, and the circles show data from ref 55. Solvents common to both studies are hexane (solvent 1), tetrahydrofuran (solvent 11), acetone (solvent 12), and dichloromethane (solvent 14).

If the reduction in the S_2 lifetime is indeed the result of a distortion from C_{2h} symmetry and vibronic coupling of the S_2 and S_1 states, the shift in the Raman bands with solvent should show a correlation with the S_2 lifetime. A general correlation between the S_2 lifetimes we determined for *all-trans*-β-carotene and the Raman peaks determined by Noguchi et al.[54] as well as by Kuki[55] can be seen in Figure 7, although the variation in the Raman shifts of some of the solvents common to both studies is quite large. The correlation is improved when the nonpolar and polar solvents are treated separately. It should be noted that this plot cannot be used to predict S_2 lifetimes for other carotenoids for which S_1 Raman shifts have been reported. This is apparent for 15-*cis*-β-carotene, which has the same S_2 lifetime as the all-trans isomer, but a S_1 Raman shift of 1760 cm^{-1} in benzene, 17 cm^{-1} less than for *all-trans*-β-carotene.[56]

Intermediate State. It is important to consider whether the behavior that we observe results from the presence of an intermediate B_u^- state lying between the $1B_u^+$ (S_2) and $2A_g^-$ (S_1) states, since some peaks observed in the fluorescence spectra of β-apo-8′-carotenal have been attributed to this state.[57] The gas-phase vertical energies of the five lowest singlet states of polyenes of different conjugation length calculated by Tavan and Schulten[58] were used in support of this assignment. However, these calculations also predict that the gas-phase vertical energy of the B_u^- state has 1.5 times the energy of the $2A_g^-$ state (and 3 times the lowest triplet-state energy). Applying this relationship to β-carotene in solution suggests that the B_u^- state will lie close to, but above, that of the $1B_u^+$ state for all solvent polarizabilities.

From our experimental results we cannot rule out the presence of an intermediate state lying below and close in energy to the $1B_u^+$ state. In the more polarizable solvents, better fits were often obtained when a second decay component with a longer

lifetime was used. Unfortunately, since the amplitude of this component was often very small, reliable lifetimes could not be obtained. However, the absence of any dramatic solvent or excitation wavelength effects on the S_2 lifetime does not suggest the presence of an intermediate electronic state, assuming it has forbidden character. Furthermore, the existence of a second allowed state absorbing in the visible region is unlikely on the basis of the reasonable agreement between the experimental lifetimes and those calculated with the fluorescence yields and radiative rates.[14] We therefore consider it more likely that the longer lifetime component is emission from the S_1 state.

Implications for Light Harvesting. Until recently, it was generally assumed that the S_2 state was too short-lived to participate in singlet−singlet energy transfer to (bacterio)-chlorophylls, and thus the energy must be transferred (with up to 100% efficiency) from the longer-lived forbidden S_1 state. On the other hand, if energy transfer via the S_1 state is energetically unfavorable, as is now believed to be the case for β-carotene to chlorophyll *a*, the S_2 state must play a role in light harvesting.[59] Additional direct evidence of at least the partial involvement of the S_2 state of carotenoids in light harvesting comes from some kinetic measurements of antenna complexes of purple bacteria.[23,60,61] For there to be a reasonable yield of energy transfer from the S_2 state, however, the rate of energy transfer must be sufficiently high to compete with the ultrafast intrinsic relaxation times. Theoretically this seems possible, since excitation transfer times from the S_2 state of neurosporene to bacteriochlorophyll *a* have been calculated for a Coulombic coupling mechanism to be as short as 26 fs.[62]

To quantify the role of each of the two carotenoid excited singlet states in light harvesting, the in vivo singlet lifetimes have to be accurately measured with sufficient time resolution. In addition, the singlet lifetimes intrinsic to the protein environment should also be determined, ideally in a carotenoprotein complex identical to the antenna protein, but with no energy-transfer pathway. In the absence of such a protein complex, a "model" protein environment which takes account of any effects, such as changes in the energy levels and distortions in the structure arising from the interactions of the carotenoid with the protein, that result in enhanced deactivation rates is required. The choice of a simple solvent "model" for the protein can most easily be made by examination of the carotenoid absorption spectrum. A comparison of the absorption maximum of spheroidene, for example, in vivo in the LH2 complex of the purple bacterium *Rhodopseudomonas sphaeroides* with the maxima observed in organic solvents, indicates that the protein environment is of quite high polarizability.[28,63] The observation of significantly shorter S_2 lifetimes for spheroidene (and for β-carotene, in this work) in polarizable solvents[23] suggests then that such a protein environment will be less than optimal for the efficient energy transfer from the S_2 state. However, our results also show that changes in the polarizability and the S_2 energy level are not the only factors affecting the lifetime of the S_2 state, and an additional parameter that should be examined when choosing a model solvent is the fine structure ratio.

The observation of a correlation between the S_2 lifetime and the C═C symmetric stretching mode of *all-trans-*β-carotene in the S_1 state suggests an alternative method to determine the effect of carotenoid−protein interactions on the singlet lifetimes. Time-resolved resonance Raman measurements have been reported for spinach chloroplasts, and the in vivo C═C symmetric stretching mode attributed to β-carotene was observed at a much lower wavenumber (1753 cm^{-1}) than for the extracted carotenoids in tetrahydrofuran (1793 cm^{-1}).[64] If the in vivo Raman signal arises predominantly from *all-trans-*β-carotene, it is valid to extrapolate our plot (Figure 7) of the Raman shifts versus the deactivation rate. Extrapolation of the fits to both nonpolar and polar solvents indicates an S_2 lifetime in the protein $\leqslant$110 fs, only a little shorter than the lifetimes determined in quinoline or benzonitrile.

V. Summary and Conclusions

The lifetime of the S_2 state of *all-trans-*β-carotene in simple organic solvents has been measured using the fluorescence upconversion technique, and solvent-dependent lifetimes in the range 120−180 fs have been found. Although the S_2 lifetimes are slightly dependent on the emission wavelength, they do not depend on the excitation wavelength and no rise times were resolved, indicating the vibrational relaxation is ultrafast and $<$20 fs. The reconstructed time-dependent emission spectra in hexane show no evidence of hot band emission and are similar in appearance to the steady-state emission spectra. Evidence of an intramolecular relaxation process occurring on the 0.3 ps time scale comes from the observation of an asymmetric increase in the bandwidth of the time-dependent spectra, consistent with the observation of longer S_2 lifetimes at the lowest energy emission wavelengths.

The S_2 lifetime is affected by both the solvent polarizability and the polarity, and a correlation with the π^* solvatochromic parameter is observed when carbon disulfide is considered separately. The energy of the S_2 state, on the other hand, decreases linearly with increasing solvent polarizability, indicating the increase in the deactivation rate of the S_2 state cannot be explained by a change in the energy gap between the S_2 and S_1 states alone, assuming the S_1 energy is near constant. Vibronic coupling of the S_2 and S_1 states, as a result of the breakdown of strict C_{2h} symmetry, is assumed to be important. Direct evidence for the vibronic coupling of the S_1 state of *all-trans-*β-carotene with a higher lying singlet state comes from picosecond resonance Raman measurements of the highest wavenumber Franck−Condon-active C═C stretching mode of the S_1 state.[54,55] From measurements of the solvent dependence of this Raman-active mode it has been concluded that it is the S_2 state that is vibronically coupled to the S_1 state,[54] and this is supported by the observation of a correlation between the S_2 lifetimes and the Raman shifts.

The correlation between the S_2 lifetime and the ratio of the absorption fine structure indicates that the fine structure ratio is potentially a very useful measure of the same intermolecular interactions that led to the enhanced radiationless deactivation of the S_2 state. Both the fine structure ratio and the position of the absorption maxima should therefore be examined when choosing a simple solvent model for the protein environment of photosynthetic light-harvesting complexes. Time-resolved resonance Raman measurements of the symmetric C═C stretching mode on the picosecond time scale could potentially also offer complementary information about the nature of the in vivo interactions between the carotenoid and the protein environment affecting the S_2 deactivation rate.

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