

CAROTENOID-TO-BACTERIOCHLOROPHYLL SINGLET ENERGY TRANSFER IN CAROTENOID-INCORPORATED B850 LIGHT-HARVESTING COMPLEXES OF *Rhodobacter sphaeroides* R-26.1

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(Received 30 March 1992; Accepted 30 July 1992)

Abstract—Four carotenoids, 3,4,7,8-tetrahydrospheroidene, 3,4,5,6-tetrahydrospheroidene, 3,4-dihydrospheroidene and spheroidene, have been incorporated into the B850 light-harvesting complex of the carotenoidless mutant, photosynthetic bacterium, *Rhodobacter sphaeroides* R-26.1. The extent of π -electron conjugation in these molecules increases from 7 to 10 carbon-carbon double bonds. Carotenoid-to-bacteriochlorophyll singlet state energy transfer efficiencies were measured using steady-state fluorescence excitation spectroscopy to be $54 \pm 2\%$, $66 \pm 4\%$, $71 \pm 6\%$ and $56 \pm 3\%$ for the carotenoid series. These results are discussed with respect to the position of the energy levels and the magnitude of spectral overlap between the S_1 (2^1A_g) state emission from the isolated carotenoids and the bacteriochlorophyll absorption of the native complex. These studies provide a systematic approach to exploring the effect of excited state energies, spectral overlap and excited state lifetimes on the efficiencies of carotenoid-to-bacteriochlorophyll singlet energy transfer in photosynthetic systems.

INTRODUCTION

One of the important roles carotenoids play in photosynthesis is that of light-harvesting pigment.^{1,2} Carotenoids absorb light energy in the region of the visible spectrum where chlorophyll and bacteriochlorophyll (BChl)† are not efficient absorbers and then transfer this energy for use in driving the primary photochemical electron transfer events in the reaction centers. In photosynthetic bacterial light-harvesting complexes, the efficiency of carotenoid-to-BChl singlet energy transfer is highly variable among preparations from different bacterial species.^{3–10} To explain this, researchers have invoked differences in several factors including: the donor-acceptor distance and geometry, the chemical and stereochemical structure of the carotenoid, the energies of the donor and acceptor excited electronic states and spectral overlap.

Based on extensive electronic spectroscopic studies of C_{40} skeleton carotenoids and their shorter polyene model systems, carotenoids are known to possess three spectroscopically important low-energy electronic states.^{1,2} The first is the ground singlet state, denoted S_0 or alternatively, 1^1A_g , using the notation for the irreducible representations of the idealized C_{2h} point group. Strictly speaking, carotenoids do not possess this high degree of symmetry. However, because many of these molecules retain the spectral characteristics of shorter polyenes that do have C_{2h} symmetry, it is customary to use the same electronic state notation.¹¹ The next state of importance is the first excited singlet state denoted S_1 or 2^1A_g . This is the state most often implicated as the donor state in singlet energy transfer. Direct excitation by one-photon ab-

sorption into this state is forbidden by symmetry, but some carotenoids, particularly those having π -electron chain lengths less than nine carbon-carbon double bonds, display fluorescence from this state.^{12–18} The final state of interest is the second excited singlet state denoted S_2 or 1^1B_u . The $1^1A_g \rightarrow 1^1B_u$ transition has a very high oscillator strength and is responsible for the major visible absorption profile of carotenoids. Fluorescence from this state has also been observed.^{12–15,17–21} It is most pronounced from carotenoids having eight or more carbon-carbon double bonds. Also, it is generally assumed that internal conversion from the 1^1B_u state to the 2^1A_g state is extremely rapid,^{1,2} occurring in 0.2 and 0.34 ps for β -carotene and spheroidene, respectively.^{21,22} Nevertheless, evidence has recently been presented, which suggests that carotenoid-to-BChl energy transfer may occur on a time-scale short enough to compete with $1^1B_u \rightarrow 2^1A_g$ internal conversion.²³ This raises the distinct possibility that both the S_1 and S_2 states of the carotenoid may transfer singlet energy directly to BChl.^{13,18,23}

There are two important excited states of BChl, which can act as potential acceptors of the excitation energy.^{1,2} These are the states associated with the so-called BChl Q_x and Q_y transitions. In the B850 complex of *Rhodobacter sphaeroides* R-26.1 the Q_x transition appears near 590 nm ($16\,900\text{ cm}^{-1}$) whereas the Q_y transition is near 850 nm ($11\,800\text{ cm}^{-1}$). Both the excited states associated with these transitions have been implicated as acceptors of carotenoid singlet energy in the transfer process.^{4,16}

To address the controlling features of carotenoid-to-BChl energy transfer, we have utilized fluorescence excitation spectroscopy in tandem with absorption spectroscopy to investigate the energy transfer efficiencies of four carotenoids, 3,4,7,8-tetrahydrospheroidene, 3,4,5,6-tetrahydrospheroidene, 3,4-dihydrospheroidene and spheroidene (Fig. 1) in-

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†Abbreviations: BChl, bacteriochlorophyll; LDAO, *N,N*-dimethyldodecylamine-*N*-oxide; LDS, lithium dodecylsulfate.

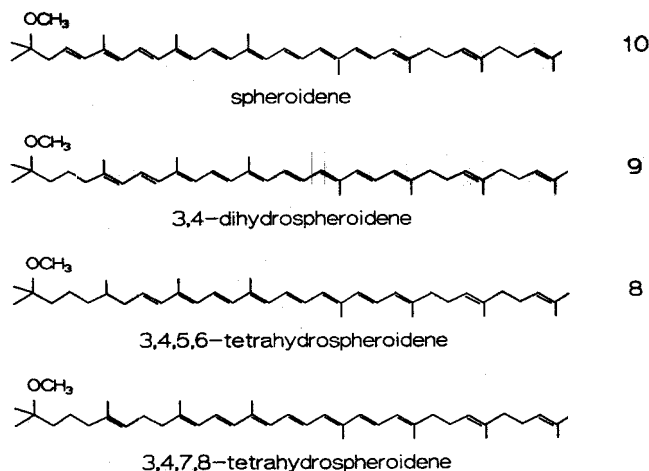


Figure 1. The four carotenoids used in this study. The numbers on the right indicate the number of conjugated carbon-carbon double bonds.

incorporated into the B850 light-harvesting complex from the carotenoidless mutant, photosynthetic bacterium, *Rb. sphaeroides* R-26.1. These molecules have variable extents of π -electron conjugation ranging from 7 to 10 carbon-carbon double bonds. *In vitro* these four carotenoid molecules exhibit a systematic crossover from $2^1A_g \rightarrow 1^1A_g$ to $1^1B_u \rightarrow 1^1A_g$ fluorescence with increasing chain length.¹² These solution fluorescence studies have revealed the positions of the excited 2^1A_g and 1^1B_u states relative to the BChl Q_x and Q_y transitions.¹² Hence, upon incorporation of these four carotenoids into the B850 light-harvesting complex, it is possible to study systematically the effects of the position of the energy levels of the carotenoids relative to those of the BChl and of spectral overlap on the efficiency of carotenoid-to-BChl singlet energy transfer.

MATERIALS AND METHODS

Cells of the *Rb. sphaeroides* R-26.1 bacteria were grown as previously described.²⁴ The B850 complex was prepared as follows: The *Rb. sphaeroides* R-26.1 cells were diluted with 15 mM Tris buffer, pH 8.0, in order to obtain an absorbance of 40–50/cm at 860 nm. A very small amount of $MgCl_2$ and DNAase was added to the cells. DNAase was used to break down DNA, and $MgCl_2$ was used as a cofactor of DNAase. The cells were then passed through a French pressure cell three times at 20 000 psi. During the press procedure both broken and unbroken cells were kept on ice in the dark. The broken cells were then centrifuged in a Sorvall SS34 rotor at 15 000 rpm (27 000 g) at 4–15°C for 10 min. The supernatant was decanted into a flask and kept on ice. The pellet-free supernatant was centrifuged at 50 000 rpm (250 000 g) in a 55.2 Ti rotor at 4°C for 90 min. The yellow supernatant was discarded. At this stage the pellet had a bright shiny color and was gently suspended in 15 mM Tris buffer, containing 150 mM NaCl, pH 8.0. The final absorbance was 40–50/cm at 860 nm. While stirring in low light, *N,N*-dimethyldodecylamine-*N*-oxide (LDAO) was added dropwise to a final concentration of 0.6% vol/vol. The mixture was stirred for 30 min at room temperature in the dark, then centrifuged at 250 000 g for 90 min in the 55.2 Ti rotor at 4°C. The supernatant contained mostly reaction centers, and the pellet contained the B850 light-harvesting complex. The latter was purified using a discontinuous sucrose density gradient consisting of 0.3 M, 0.6 M and 1.2 M sucrose in 20 mM Tris buffer, 0.1% LDS, pH 8.0. The pellet was added to the top of the 0.3 M solution and then centrifuged at 150 000 g in a 55.2 Ti rotor at 4°C for 18 h. The purified B850 light-harvesting complex was located in the 1.2 M sucrose layer.

Spheroidene and 3,4-dihydrospheroidene were extracted from the whole cells of *Rb. sphaeroides* wild-type strain 2.4.1 and *Rhodobacter capsulatus* MT1131 as previously described.¹² Prior to incorporation into the B850 complex these carotenoids were purified by thin layer chromatography. The synthesis and purification of 3,4,5,6-tetrahydrospheroidene and 3,4,7,8-tetrahydrospheroidene have been previously described.^{25,26}

The incorporation of the carotenoids into the light-harvesting complex was carried out as follows: The light-harvesting complex was dialyzed against 15 mM Tris buffer, pH 8.0, overnight. Then, 10% sodium deoxycholate (5 β -cholan-24-oic acid-3 α , 12 α -diol) (wt/vol) in 15 mM Tris buffer, pH 8.0, was added to obtain a final solution of 2% of sodium deoxycholate. A 20-fold molar excess of carotenoids over BChl was added in petroleum ether to the light-harvesting complex. The petroleum ether was evaporated with a stream of nitrogen. The mixture was then sonicated for 30–45 min at 4°C in the dark. The solution was then dialyzed against 0.02% sodium deoxycholate, 15 mM Tris buffer, pH 8, overnight. A sucrose density gradient using 2 M, 1.5 M and 0.75 M in 15 mM Tris buffer, pH 8.0, and spinning at 150 000 g in a 55.2 Ti rotor at 4°C for 18 h eliminated the excess carotenoids. The light-harvesting complex was found at the interface of the 1.5 M and 2 M sucrose solutions. An absorbance of 0.4–0.45/cm was used for the fluorescence experiments.

Fluorescence spectroscopy was performed on a Perkin-Elmer MPF-66 spectrometer using the following conditions: excitation slit, 5 nm; emission slit, 15 nm; $\lambda_{\text{emission}}$, 850 nm; response time, 2 s; sweep speed, 120 nm/min; 780 nm cutoff filter used.

RESULTS AND DISCUSSION

Figure 2 shows the absorption and fluorescence excitation spectra of the four carotenoids, 3,4,7,8-tetrahydrospheroidene, 3,4,5,6-tetrahydrospheroidene, 3,4-dihydrospheroidene and spheroidene, incorporated into the B850 light-harvesting complex from *Rb. sphaeroides* R-26.1. The fluorescence excitation spectra were normalized to the BChl absorption band near 590 nm and the efficiencies of energy transfer were calculated from the ratio of the intensity of the fluorescence excitation spectra to that of the carotenoid absorption. In order to minimize the error resulting from background BChl absorption, the values were obtained by averaging the efficiencies over comparable parts of the spectra; *viz.* the range spanned by the two red-most carotenoid spectral peaks. This was done for at least three separate samples of each complex. The efficiencies for the four incorporated carotenoids were $54 \pm 2\%$ for 3,4,7,8-tetrahydrospheroidene, $66 \pm 4\%$ for 3,4,5,6-tetrahydrospheroidene, $71 \pm 6\%$ for 3,4-dihydrospheroidene and $56 \pm 3\%$ for spheroidene, where the uncertainties represent the standard deviations of the variation in efficiencies over the carotenoid spectral features. These data are summarized in Table 1.

There are two primary electronic interactions that have been cited in the literature as mechanisms for singlet energy transfer between photosynthetic pigments *in vivo*. These are the coulombic and exchange interactions. A special case of the coulombic interaction is the Förster mechanism²⁷ whereby the electronic transition dipoles are proportional to the oscillator strengths for the donor and acceptor radiative transitions. Energy transfer *via* the coulomb mechanism generally requires large transition dipoles (allowed transitions) for both the donor and acceptor molecules and has an r^{-6} donor/acceptor distance dependence. The exchange interaction promotes a simultaneous transfer of two electrons between the donor and acceptor species²⁸ and therefore must operate over shorter distances than the coulombic interaction. The ex-

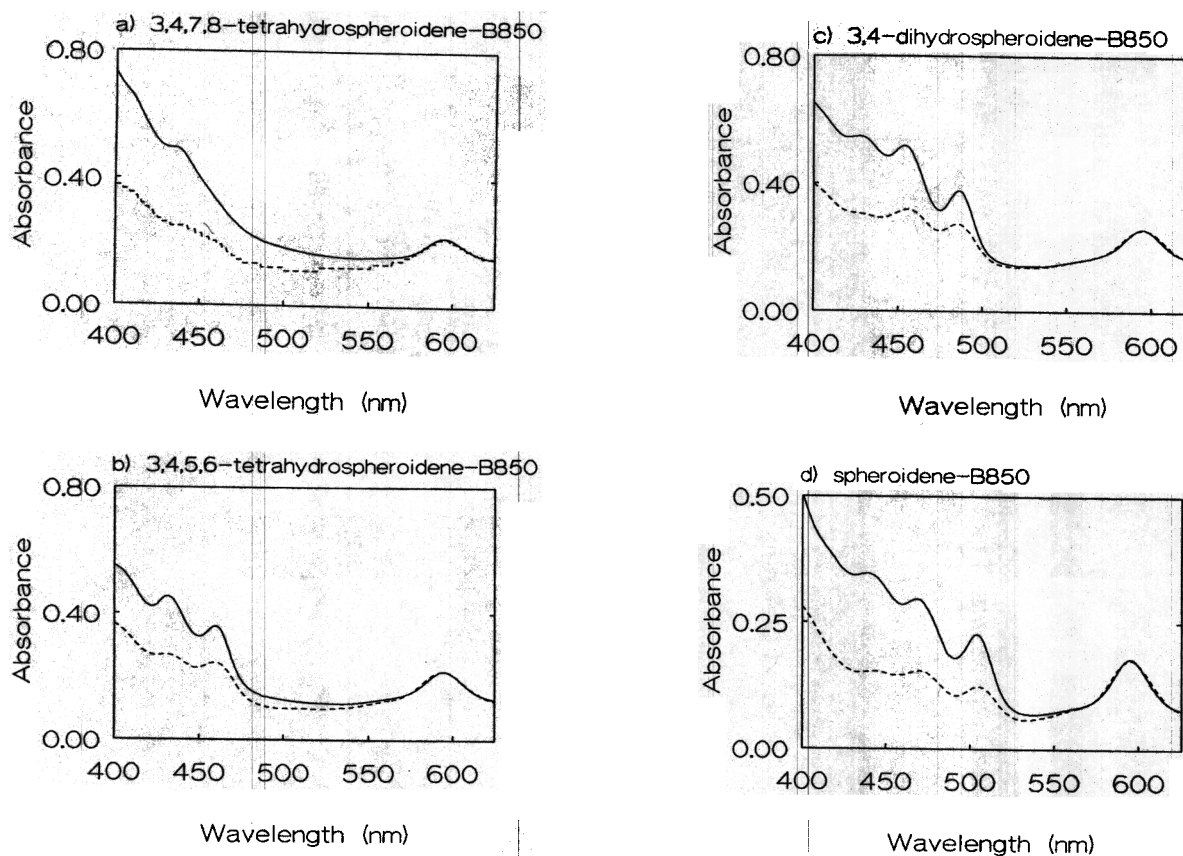


Figure 2. Absorption (solid line) and fluorescence excitation (dashed line) spectra for: (a) 3,4,7,8-tetrahydro-spheroidene; (b) 3,4,5,6-tetrahydro-spheroidene; (c) 3,4-dihydro-spheroidene; and (d) spheroidene incorporated into the B850 complex from *Rhodobacter sphaeroides* R-26. The left y-axis corresponds to the absorption (solid line) trace. The fluorescence excitation curves (in arbitrary units) were normalized to the Q_x transition at 590 nm.

change mechanism does not require large transition dipoles to be present on the donor and acceptor molecules. Thus, it is frequently invoked to account for triplet state energy transfer for which returning the donor molecule to its ground state is a spin-forbidden process. Also, it is the most likely mechanism for carotenoid-to-BChl energy transfer if the energy donor state is the 2^1A_g state of the carotenoid, regardless of whether the accepting state is associated with the BChl Q_x or Q_y transitions. This is because of the extremely small dipole moment associated with the symmetry-forbidden $1^1A_g \rightarrow 2^1A_g$ transition in the carotenoid. If the donor state is the 1^1B_u state of the carotenoid, then either coulomb or exchange interactions may be active in promoting energy transfer to BChl.

According to the exchange mechanism, the rate constant for energy transfer²⁹ is given by

$$k_{ET} = K \exp\left(\frac{-2r}{L}\right) J_{\text{exchange}} \quad (1)$$

where K depends on the specific orbitals involved, r describes the donor-acceptor distance relative to their van der Waals radii, L , and J_{exchange} is the overlap integral given by

$$J_{\text{exchange}} = \frac{\int_{-\infty}^{\infty} F_d(\nu) \epsilon_a(\nu) \nu^{-4} d\nu}{\int_{-\infty}^{\infty} F_d(\nu) d\nu \int_{-\infty}^{\infty} \epsilon_a(\nu) d\nu} \quad (2)$$

Table 1. The energy transfer efficiencies, ϵ_{ET} , spectral origins and relative overlap integrals for the four carotenoids, 3,4,7,8-tetrahydro-spheroidene, 3,4,5,6-tetrahydro-spheroidene, 3,4-dihydro-spheroidene and spheroidene incorporated into the B850 light-harvesting complex from *Rhodobacter sphaeroides* R-26.1

Carotenoid	No. conjugated -C=C- bonds	ϵ_{ET} (%)	2^1A_g state spectral origin (cm^{-1})	Relative value of J_{exchange}
3,4,7,8-Tetrahydro-spheroidene	7	54 ± 2	18 400	0.15
3,4,5,6-Tetrahydro-spheroidene	8	66 ± 4	16 700	0.58
3,4-Dihydro-spheroidene	9	71 ± 6	15 300	0.91
Spheroidene	10	56 ± 3	14 000	1.0

The calculated value of J_{exchange} for the spheroidene-B850 complex is $2.45 \times 10^{-31} \text{ s}^5$.

$F_d(\nu)$ is the emission spectral lineshape function of the donor, $\epsilon_a(\nu)$ describes the absorption spectral lineshape for the acceptor and ν is the spectral frequency. The efficiency of energy transfer, ϵ_{ET} , is related to the rate constant for energy transfer, k_{ET} , by the expression

$$\epsilon_{ET} = \frac{k_{ET}}{k_{ET} + \sum k_i} \quad (3)$$

where $\sum k_i$ represents the sum of the rate constants for all the processes that compete with energy transfer. The extremely small fluorescence efficiencies (10^{-3} – 10^{-5}) from the 2^1A_g states of the carotenoids indicate that this sum is most likely dominated by the rate constant for radiationless internal conversion, k_{ic} , to the ground state.² The 2^1A_g lifetimes of several carotenoids have been measured to be in the range 5.2 ps to 25.4 ps.^{23,30,32} The 2^1A_g lifetime of spheroidene is 9.1 ps.²³

Figure 3 displays the position of the spectral origins of the carotenoid excited states overlaid with the absorption spectrum of the B850 complex. The spectral origins of the 1^1B_u states were assigned from the lowest energy spectral features in the solution absorption spectra. Using the well-resolved optical spectra of model polyenes¹³ as a guide, the 2^1A_g spectral origins were assigned to the highest energy inflection in the emission lineshapes *in vitro*.¹² In order to see the connection between the value of the overlap integral, $J_{exchange}$, and the measured efficiencies, ϵ_{ET} , of the carotenoid-to-BChl energy transfer, the magnitude of the spectral overlap was calculated for each of the four carotenoid-incorporated samples in the following manner: First, the absorption spectrum of the B850 complex and the *in vitro* 2^1A_g emission spectrum from 3,4,7,8-tetrahydrospheroidene (seven carbon-carbon double bonds) were converted from wavelength to energy units (Fig. 4). The $J_{exchange}$ value was then numerically evaluated by summing the products of the intensities of the spectral traces weighted by their corresponding ν^{-4} frequency terms and normalized by the product of the fluorescence and absorption integrals. This yielded the value for the overlap integral of the 3,4,7,8-tetrahydrospheroidene-B850 system. The 2^1A_g spectral curve was then shifted to lower energy by the energy difference between the spectral origins of 3,4,7,8-tetrahydrospheroidene (seven carbon-carbon double bonds) and 3,4,5,6-tetrahydrospheroidene (eight carbon-carbon double bonds) (Fig. 4), and the calculation repeated. This gave the value for the overlap integral of the 3,4,5,6-tetrahydrospheroidene-B850 system. Similar shifts in the 2^1A_g emission profile gave values for the overlap integrals for 3,4-dihydrospheroidene and spheroidene. The relative values of these overlap integrals are summarized in Table 1. It is important to note that the fluorescence spectrum of the 3,4,7,8-tetrahydrospheroidene was used to calculate the overlap integrals for all four carotenoid-B850 systems. Ideally, the actual S_1 emission curves for each of the four carotenoids should be used. However, the present approach is justified by the following arguments: (1) The 3,4,7,8-tetrahydrospheroidene 2^1A_g emission lineshape was very similar (within the signal-to-noise) in width and in vibronic structure to the emission traces of the 3,4,5,6-tetrahydrospheroidene and 3,4-dihydrospheroidene molecules. (2) The experimental lineshape of 3,4,7,8-tetrahydrospheroidene had the best signal-to-noise

ratio for 2^1A_g emission of all of the carotenoids. (3) So far we have been unable to detect 2^1A_g emission from spheroidene in solution, and therefore had to use the 3,4,7,8-tetrahydrospheroidene fluorescence lineshape for the analysis. The assignment of the spectral origin for spheroidene was done by extrapolation from the spectral origins of the other molecules.^{12,18} Also, in this analysis, no attempt was made to adjust the spectral origins of the carotenoid emissions for any dispersive shifts in the spectra brought about by binding the carotenoid into the protein matrix. These shifts are known to be proportional to the dipole strength of the transition.³² For the symmetry-forbidden $1^1A_g \rightarrow 2^1A_g$ transition the solvent shifts are likely to be very small, at most a few hundred cm^{-1} .^{18,32}

The values of the relative overlap integrals are given in Table 1. As the 2^1A_g emission profile is shifted to lower energy from the spectral origins of the 3,4,7,8-tetrahydrospheroidene (7 carbon-carbon double bonds) to spheroidene (10 carbon-carbon double bonds) the overlap integral increases in magnitude. This is directly attributable to enhanced overlap with the strong Q_y transition of the BChl (Fig. 4). This result is paralleled by changes in the energy transfer efficiencies of the B850 complex incorporated with 3,4,7,8-tetrahydrospheroidene, 3,4,5,6-tetrahydrospheroidene and 3,4-dihydrospheroidene. For these three cases the energy transfer efficiencies increase with the extent of π -electron conjugation and suggest a correlation between the efficiencies and spectral overlap. The energy transfer efficiency of the spheroidene-B850 complex, on the other hand, decreases in spite of the corresponding increase in the overlap.

There are two possible explanations for the failure of the spheroidene system to follow the trend in spectral overlap. The first explanation is that our estimation of the spectral origin of the spheroidene 2^1A_g energy is too high. If the energy of the 2^1A_g state of spheroidene were lower, its fluorescence would have less overlap with the Q_y of the BChl, giving a lower energy transfer efficiency. This is unlikely because the spheroidene-containing B800–850 complex from *Rb. sphaeroides* shows nearly 100% efficient energy transfer.^{8,10} This would not be the case if spheroidene had poor overlap with the 800 and 850 nm Q_y transitions in this spectral region. The second and more plausible explanation is that increasing the number of carbon-carbon double bonds to 10 (for spheroidene) lowers the energy of the 2^1A_g state, leading to an enhancement in the rate of $S_1 \rightarrow S_0$ internal conversion. For the carotenoid-B850 complexes studied here, and particularly in the case of the spheroidene-B850 complex, radiationless decay to S_0 successfully competes with energy transfer. Otherwise, the measured energy transfer efficiencies would be 100%.

Figure 5 shows a series of plots of energy transfer efficiency versus $J_{exchange}$ based on the equation given above for ϵ_{ET} and assuming various values of k_{ic} . The figure was derived in the following manner: The efficiency of energy transfer was assumed to be governed by the equation

$$\epsilon_{ET} = \frac{K' \times J_{exchange}}{K' \times J_{exchange} + k_{ic}} \quad (4)$$

where K' is an electronic factor that depends on the orbitals involved and on the donor-acceptor distance. K' is assumed

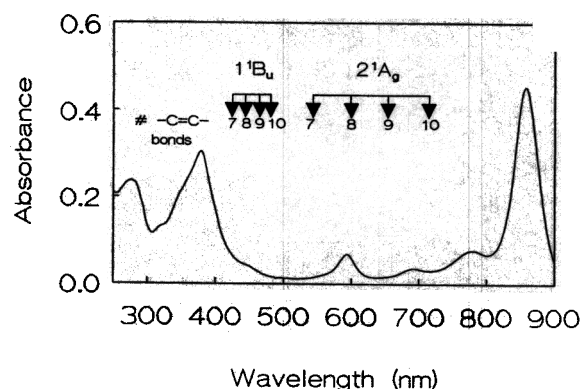


Figure 3. The positions of the carotenoid spectral origins deduced from previous studies¹² relative to the B850 BChl absorption spectral features.

to be identical for the four carotenoid–B850 complexes studied here. K' was calculated from the energy transfer efficiency (0.56) for the spheroidene–B850 complex, the measured value (1/9.1 ps) for the 2^1A_g internal conversion rate constant, k_{ic} ,²³ and the spheroidene, 2^1A_g emission–B850 absorption, spectral overlap integral ($2.45 \times 10^{-31} \text{ s}^5$), giving $K' = 5.71 \times 10^{41} \text{ s}^{-6}$. Using this value of K' , a series of curves were generated that describe the effect of spectral overlap and internal conversion rates on the energy transfer efficiency. Overlaid with these plots are four points giving the measured efficiencies and spectral overlaps of the (a) spheroidene-, (b) 3,4-dihydro-spheroidene-, (c) 3,4,5,6-tetrahydro-spheroidene- and (d) 3,4,7,8-tetrahydro-spheroidene-incorporated B850 complexes.

A five-fold change in k_{ic} is consistent with the trend in the measured energy transfer efficiencies as illustrated in Figure 5. Larger than five-fold changes also could explain the data, given the uncertainties in overlap integrals and the steep dependence of energy transfer efficiency for small overlaps. This magnitude change in the internal conversion rate for this series of carotenoids is readily accounted for by the “energy gap law,”³³ which predicts $k_{ic} = \text{constant} \times \exp(-\gamma\Delta E/\hbar\omega_M)$ where ΔE is the $S_1 - S_0$ energy difference, $\hbar\omega_M$ is the energy of the high frequency “acceptor” modes and where γ can be related to the relative displacement of the potential surfaces in the two electronic states. For aromatics, the dominant acceptor modes usually are the C–H stretching vibrations ($\hbar\omega_M \sim 3000 \text{ cm}^{-1}$). For linear polyenes, the C–C stretching vibrations $\hbar\omega_M \sim 1300\text{--}1600 \text{ cm}^{-1}$ may be more appropriate.³¹ According to the energy gap model, the internal conversion rate of spheroidene ($\Delta E \sim 14\,000 \text{ cm}^{-1}$) will be five times faster than the rate of 3,4,5,6-tetrahydro-spheroidene ($\Delta E \sim 18\,400 \text{ cm}^{-1}$) when $\hbar\omega_M/\gamma = 2700 \text{ cm}^{-1}$. This would require $0.5 < \gamma < 0.6$, well within the range of cited γ (0.5–1.5) for radiationless decay processes in aromatics.

The efficiencies of energy transfer for the four complexes can be rationalized from changes in spectral overlaps and the increase in the rate of 2^1A_g to 1^1A_g internal conversion with increasing conjugation. The efficiencies will increase with increasing overlap integral up to the point at which $2^1A_g \rightarrow 1^1A_g$ internal conversion becomes dominant. This is the case for the spheroidene–B850 complex where the overlap integral is the largest in the series, but a diminishing of the efficiency

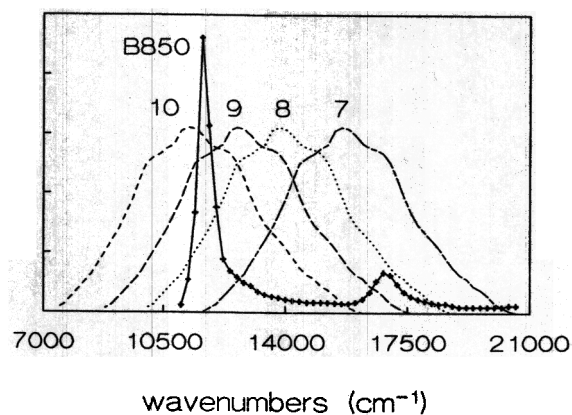


Figure 4. The carotenoid 2^1A_g emission trace from 3,4,7,8-tetrahydro-spheroidene (7 denotes seven carbon–carbon double bonds for this carotenoid) shifted to correspond to the spectral origins of 3,4,5,6-tetrahydro-spheroidene (8), 3,4-dihydro-spheroidene (9) and spheroidene (10). These are overlaid with the B850 absorption spectral lineshape and plotted on an energy (cm^{-1}) scale.

of energy transfer occurs owing to the rapid, 9.1 ps, 2^1A_g lifetime of spheroidene.

This analysis does not take into account that singlet energy transfer to BChl via the 1^1B_u state of the carotenoid also is plausible.^{13,18,23} Evaluation of the overlap integrals between the 1^1B_u emission and the BChl absorption shows that the overlap is considerably less sensitive to the extent of carotenoid conjugation than for the case of 2^1A_g –BChl energy transfer. This derives from the fact that the $1^1B_u \rightarrow 1^1A_g$ fluorescence spectra are relatively insensitive to the length of conjugation. Also, similar 1^1B_u energies suggest similar internal conversion rates, which would lead to smaller changes in energy transfer efficiencies than those measured here. Although a significant amount of 1^1B_u state-mediated energy transfer cannot account for the trend in the energy transfer

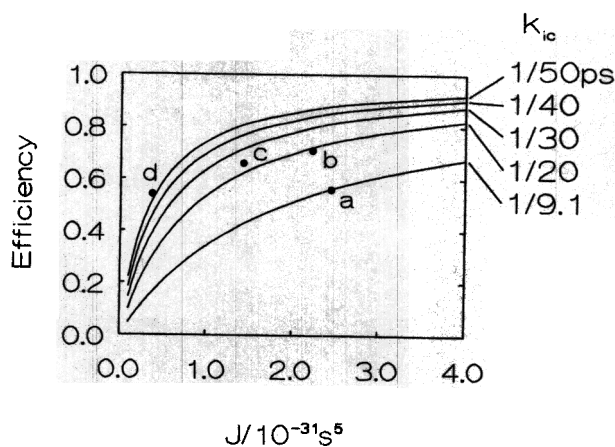


Figure 5. The effect of spectral overlap, J_{exchange} (denoted J in the figure), on the efficiency of singlet energy transfer from the 2^1A_g carotenoid electronic state to the BChl acceptor. Overlaid on the plot are four points that refer to the measured efficiencies and spectral overlaps of the (a) spheroidene-, (b) 3,4-dihydro-spheroidene-, (c) 3,4,5,6-tetrahydro-spheroidene- and (d) 3,4,7,8-tetrahydro-spheroidene-incorporated B850 complexes.

efficiencies, one cannot rule out the possibility that some carotenoids in these complexes may be transferring singlet energy *via* this route.

Finally, in these experiments, it is not possible to be completely sure that all of the carotenoids are bound to the protein in sites that result in singlet energy transfer. If carotenoids were bound in the protein but not capable of transferring the singlet energy (*e.g.*, are uncoupled from the BChl), the observed efficiencies would be systematically low. However, because the incorporation of all four carotenoids was handled identically, any such effects should be present for all four complexes and lead to a uniform reduction in their transfer efficiencies. Assuming that similar binding positions and orientations of the four different carotenoids exist, the trend in the data should remain the same. Experiments are underway to explore the structural and stoichiometric relationships between the carotenoids and BChl molecules in these complexes.

Acknowledgements—This work was supported by grants to H.A.F. from the National Institutes of Health (GM-30353) and the University of Connecticut Research Foundation, and to J.L. from the Netherlands Foundation of Chemical Research (SON), which is financed by the Netherlands Organization for the Advancement of Pure Research (NWO).

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