

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

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**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  $\pi$ -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.<sup>1,2</sup> 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.<sup>3–10</sup> 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.<sup>1,2</sup> 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.<sup>11</sup> 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  $\pi$ -electron chain lengths less than nine carbon-carbon double bonds, display fluorescence from this state.<sup>12–18</sup> 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.<sup>12–15,17–21</sup> 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,<sup>1,2</sup> occurring in 0.2 and 0.34 ps for  $\beta$ -carotene and spheroidene, respectively.<sup>21,22</sup> 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.<sup>23</sup> This raises the distinct possibility that both the  $S_1$  and  $S_2$  states of the carotenoid may transfer singlet energy directly to BChl.<sup>13,18,23</sup>

There are two important excited states of BChl, which can act as potential acceptors of the excitation energy.<sup>1,2</sup> 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.<sup>4,16</sup>

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.

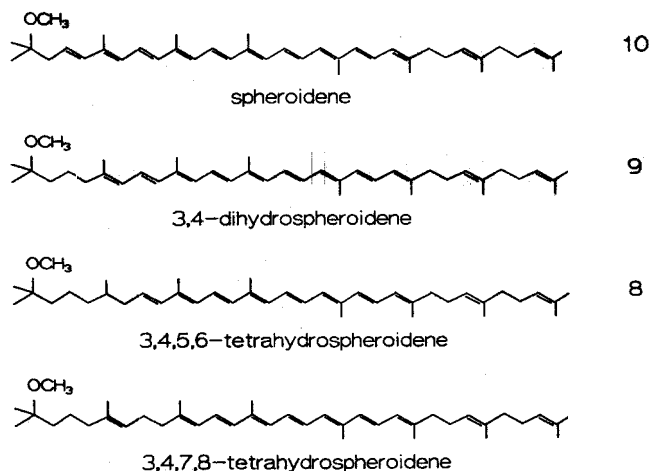


Figure 1. The four carotenoids used in this study. The numbers on the right indicate the number of conjugated carbon-carbon double bonds.

incorporated into the B850 light-harvesting complex from the carotenoidless mutant, photosynthetic bacterium, *Rb. sphaeroides* R-26.1. These molecules have variable extents of  $\pi$ -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.<sup>12</sup> 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.<sup>12</sup> 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.<sup>24</sup> 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.<sup>12</sup> 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.<sup>25,26</sup>

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 $\beta$ -cholan-24-oic acid-3 $\alpha$ , 12 $\alpha$ -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 mechanism<sup>27</sup> 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 species<sup>28</sup> and therefore must operate over shorter distances than the coulombic interaction. The ex-

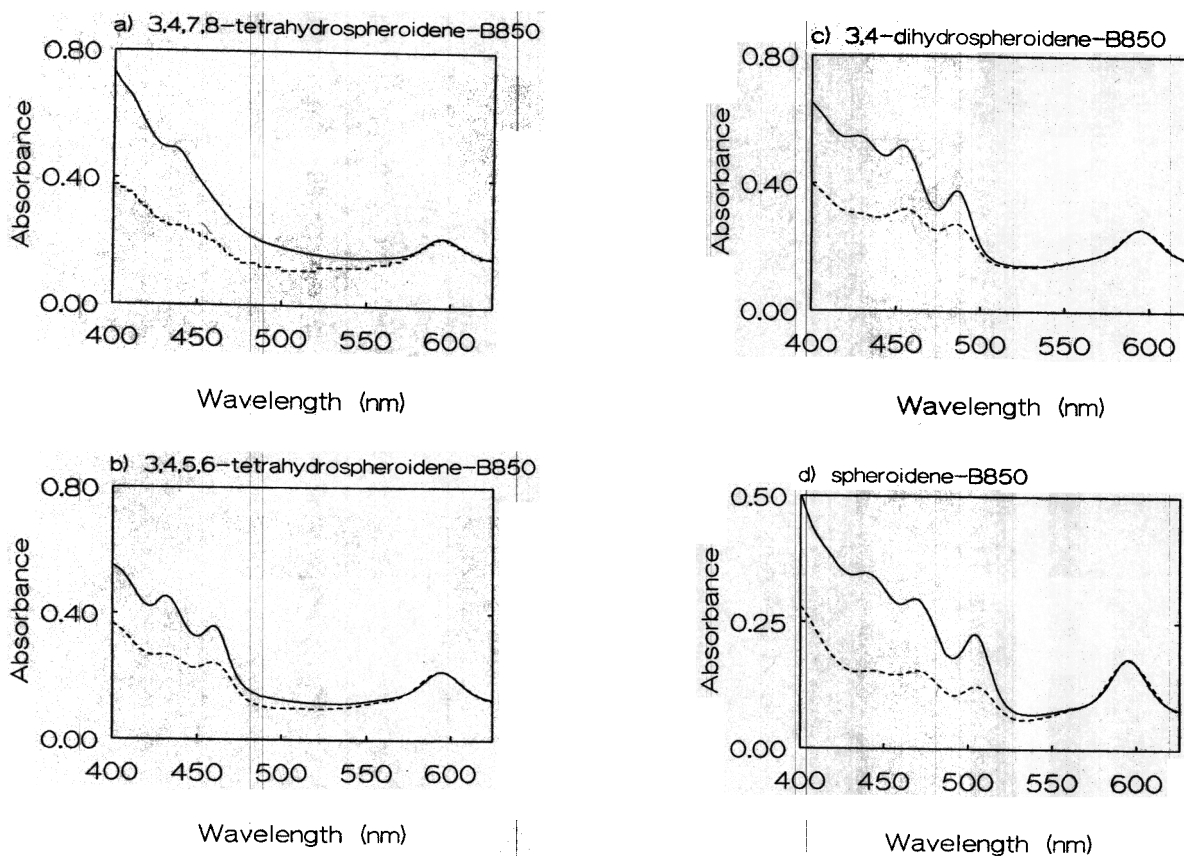


Figure 2. Absorption (solid line) and fluorescence excitation (dashed line) spectra for: (a) 3,4,7,8-tetrahydro-spheroidene; (b) 3,4,5,6-tetrahydro-spheroidene; (c) 3,4-dihydro-spheroidene; and (d) spheroidene incorporated into the B850 complex from *Rhodobacter sphaeroides* R-26. The left y-axis corresponds 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 transfer<sup>29</sup> 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  $J_{\text{exchange}}$  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,  $\epsilon_{ET}$ , 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	$\epsilon_{ET}$ (%)	$2^1A_g$ state spectral origin ( $\text{cm}^{-1}$ )	Relative value of $J_{\text{exchange}}$
3,4,7,8-Tetrahydro-spheroidene	7	$54 \pm 2$	18 400	0.15
3,4,5,6-Tetrahydro-spheroidene	8	$66 \pm 4$	16 700	0.58
3,4-Dihydro-spheroidene	9	$71 \pm 6$	15 300	0.91
Spheroidene	10	$56 \pm 3$	14 000	1.0

The calculated value of  $J_{\text{exchange}}$  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  $\nu$  is the spectral frequency. The efficiency of energy transfer,  $\epsilon_{ET}$ , 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.<sup>2</sup> The  $2^1A_g$  lifetimes of several carotenoids have been measured to be in the range 5.2 ps to 25.4 ps.<sup>23,30,32</sup> The  $2^1A_g$  lifetime of spheroidene is 9.1 ps.<sup>23</sup>

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 polyenes<sup>13</sup> as a guide, the  $2^1A_g$  spectral origins were assigned to the highest energy inflection in the emission lineshapes *in vitro*.<sup>12</sup> In order to see the connection between the value of the overlap integral,  $J_{\text{exchange}}$ , and the measured efficiencies,  $\epsilon_{ET}$ , 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  $J_{\text{exchange}}$  value was then numerically evaluated by summing the products of the intensities of the spectral traces weighted by their corresponding  $\nu^{-4}$  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.<sup>12,18</sup> 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.<sup>32</sup> 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  $\text{cm}^{-1}$ .<sup>18,32</sup>

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  $\pi$ -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.<sup>8,10</sup> 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  $J_{\text{exchange}}$  based on the equation given above for  $\epsilon_{ET}$  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

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

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



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.

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