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Femtosecond energy-transfer processes in the B800-850 light-harvesting complex of *Rhodobacter sphaeroides* 2.4.1

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The B800-to-B850 energy transfer time in the purified B800-850 light-harvesting complex of *Rhodobacter sphaeroides* 2.4.1 is determined to be 0.7 ps at room temperature. The electronic state dynamics of the principal carotenoid of this species, spheroidene, are examined, both in vivo and in vitro, by direct femtosecond time-resolved experiments and by fluorescence emission yield studies. Evidence is presented which suggests that carotenoid-to-bacteriochlorophyll energy transfer may occur directly from the initially excited carotenoid S_2 state, as well as from the carotenoid S_1 state. Further support for this conjecture is obtained from calculations of energy transfer rates from the carotenoid S_2 state. Previous measurements of in vivo carotenoid and B800 dynamics are discussed in light of the new results, and currently unresolved issues are described.

Introduction

Energy transfer dynamics within the B800-850 light-harvesting complex of purple photosynthetic bacteria have been extensively studied [1–11]. Energy transfer within this complex is remarkable for its high efficiency – in many species, including *Rhodobacter sphaeroides*, energy is transferred from all the bacteriochlorophyll (Bchl) and carotenoid molecules to a final Bchl acceptor with approx. 100% efficiency [2]. The dynamics of energy transfer within this complex are rapid, with carotenoid-to-BChl energy transfer times as short as 200 fs [3].

The absorption spectra of the purified B800-850 complex from *Rb. sphaeroides* 2.4.1, solubilized in the detergents LDAO (lauryldimethylamine *N*-oxide) or in a mixture of LDAO and LDS (lithium dodecyl sulfate), are shown in Fig. 1. The Bchl is partitioned between two populations: one, having its Q_y absorption at 850 nm, is denoted B850, the other, having its Q_y absorption at 800 nm, is denoted B800. In the LDAO-LDS solubilised complex (referred to henceforth as B800-850/LDS), the 800 nm absorption of B800 is nearly absent, and a decrease in absorptivity is also seen in the 580 nm spectral region of the overlapping Q_x bands of B800 and B850 [4,5]. The overall stoichiometry of Bchl in the B800-850 complex solubilised in LDAO (referred to henceforth as B800-850/LDAO) is two B850 molecules per B800 molecule. In addition to Bchl, the complexes contain carotenoids. For the B800-850 complex of *Rb. sphaeroides*, the predominant carotenoid is

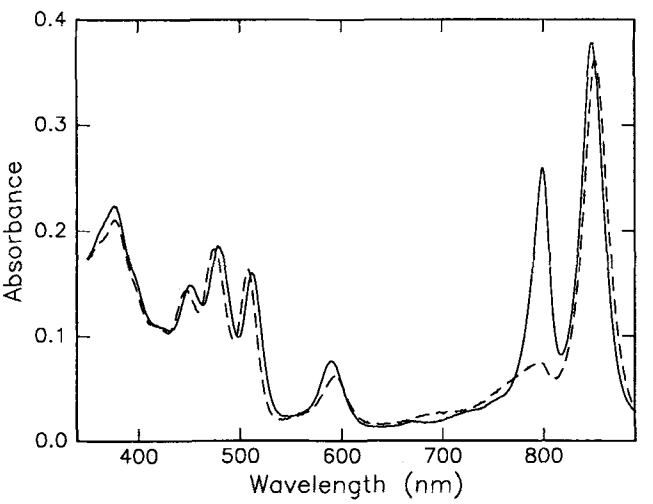

Fig. 1. The absorption spectra of the B800-850 light-harvesting complex from *Rb. sphaeroides* 2.4.1 solubilized in LDAO (———) and in LDAO with LDS added (— — —).

Abbreviations: LDS, lithium dodecyl sulfate; LDAO, lauryldimethylamine *N*-oxide; Bchl, bacteriochlorophyll.

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spheroidene, and its visible absorption in vivo is in the 420 nm to 530 nm spectral region. The ratio of carotenoids to total Bchl is 1 : 2 [6,7].

An understanding of carotenoid electronic state structure is important in discussing carotenoid-to-Bchl energy transfer. The strong visible absorption of carotenoids is thought to arise from the $S_0 \rightarrow S_2$ electronic state transition, while the $S_0 \rightarrow S_1$ electronic state transition is dipole forbidden, or nearly so [12–14]. In this sense, carotenoids are analogous to the well-studied polyenes, which, in the C_{2h} point group, have an S_1 state of A_g symmetry and an S_2 state of B_u symmetry [15]. The ground state of polyenes is of A_g symmetry and the S_0 (1Ag) $\rightarrow S_1$ (2Ag) transition is dipole forbidden, but the S_0 (1Ag) $\rightarrow S_2$ ($1B_u$) transition is allowed, and is typically quite strong [15]. Because exciting carotenoids with visible light results in the initial formation of S_2, some care is required in discussing carotenoid-to-Bchl energy transfer. It has been generally accepted that the S_2-to-S_1 internal conversion is much faster than the carotenoid-to-Bchl energy transfer, so essentially all of the energy transfer would have to occur from the carotenoid S_1 state [14,16,17]. However, a recent direct measurement found the S_2-to-S_1 internal conversion time in β-carotene to be about 0.2 ps [18], and this time is similar to the measured carotenoid-to-Bchl or carotenoid-to-chlorophyll energy transfer times in some bacterial and algal light-harvesting complexes [3,19].

The issue of whether carotenoid-to-chlorophyll energy transfer occurs out of the carotenoid S_2 or S_1 state (or both) is important for mechanistic reasons. Because the carotenoid $S_1 \rightarrow S_0$ transition is weak, the mechanism of carotenoid-to-Bchl energy transfer has been thought to be exchange coupling [20,21]. That is, since the interaction of donor and acceptor transition dipoles is the leading term in Coulomb coupling [22, 23], and since the carotenoid $S_1 \rightarrow S_0$ transition dipole is small (formally zero in the C_{2h} point group), Coulomb coupling is usually discounted for the efficient carotenoid-to-Bchl(Chl) energy transfer. However, were energy to transfer from the carotenoid S_2 state, the large $S_2 \rightarrow S_0$ transition dipole would permit strong Coulomb coupling to the receiving Bchl transition dipole. The energy transfer rate constant contains not only an electronic factor but also one involving nuclear coordinates, which, through overall energy conservation, appears as the integral of the product of donor and acceptor Franck-Condon envelopes. While this factor strongly discriminates against transfer from the carotenoid S_2 state to the Bchl Q_y (S_1) state, it shall be demonstrated that very fast energy transfer times remain possible for transfer from the carotenoid S_2 state to the Bchl Q_x (S_2) state.

Techniques ranging from low temperature emission spectroscopy to femtosecond time-resolved differential absorption spectroscopy have provided many details about the pathways and kinetics of energy flow within the B800-850 complex [1–11]. However, significant questions still remain. Mechanistic details of carotenoid-to-Bchl energy transfer are, as noted, still unresolved. In addition, the energy transfer time for B800-to-B850 transfer has been reported to be about 2.5 ps, both at room temperature [3] and at 4 to 30 K [8]. Additional reports include approx. 3.3 ps at 4 K [9], 1–2 ps at 77 K [10] and less than 1 ps at room temperature [10,11]. Clearly, further investigation is required. Also unresolved is the issue of homogeneity of the carotenoid population in the complex. At the extremes, either all of the carotenoid molecules are able to transfer energy to both B800 and B850 or, alternatively, the carotenoids are distinctly divided between those that transfer only to B800 and those that transfer only to B850.

After a description of the experimental techniques, the following new results are presented: (i) a direct measure of the room temperature B800-to-B850 energy transfer time; (ii) an examination of the dynamics of electronic state internal conversion for the carotenoid spheroidene in vitro; and (iii) a determination of the emission yield for carotenoid fluorescence from both the B800-850 complex and from the in vitro pigment extract of the complex. These new findings, together with those previously published, are then discussed in light of possible models for singlet energy transfer within the purified B800-850 complex of *Rb. sphaeroides*.

Experimental

The femtosecond laser used in the time-resolved experiments has been described previously [3,18,19]. For the experimental results reported here, pump wavelengths of 800 nm, 510 nm or 480 nm and probe wavelengths of 800 nm, 850 nm and 480 to 630 nm were used. Pump-probe cross correlations were between 200 and 300 fs, depending upon the wavelength pair. The data are reported with induced absorptions shown as positive signals. The data were fit to a function given by the convolution of the pump-probe cross correlation with the signal expected from an assumed kinetic model; best fit parameters were determined by a non-linear least-squares fitting program.

The fluorescence quantum yield measurements were performed as described previously [18]. Briefly, the excitation source was a 10 Hz, Nd : YAG-pumped dye laser, and the excitation wavelength was varied between 440 nm and 490 nm. The emission was collected in a 90° geometry, coupled into a fiber bundle, delivered to a spectrograph and dispersed onto an intensified diode array detector. The polarization of the collected light was verified to be completely scrambled by the fiber bundle, so no artifacts were possible from emission anisotropy. The strength of the emitted light depended linearly upon the number of photons absorbed, whether

that number was varied by adjusting the incident intensity or by adjusting the solute concentration. The emission spectra are reported in units of quanta, not energy. Corrections for wavelength dependent instrument response were generated by use of a standard color source, and the emission yields were calibrated from two independent dilution series of Rhodamine 590 in ethanol [24].

The B800-850 complex was purified from chromatophores of *Rb. sphaeroides* 2.4.1 and solubilized in either LDAO (B800-850/LDAO) or in LDAO with LDS added (B800-850/LDS), all according to published procedures [5]. The purification of spheroidene involved a pentane partitioning of the acetone/methanol extracts of whole cells of *Rb. sphaeroides* 2.4.1. The pentane phase was then dried over Na_2CO_3 and rotovapped to dryness. The resulting carotenoid extract was dissolved in petroleum ether (b.p. 40–60 °C) and applied to an alumina column. Purified spheroidene was obtained off the column with an eluting solvent of about 5% ethyl acetate in petroleum ether, and was dissolved in cyclohexane for the in vitro time-resolved experiments. For the emission yield experiments on pigments from the B800-850 complex, the pigments were extracted in acetone, the acetone was evaporated under dry N_2, and the pigments were dissolved in ethanol.

Results

The transient differential absorption of the B800-850/LDAO complex seen with a probe wavelength of 800 nm following a 800 nm pump pulse is shown in Fig. 2. The raw data show an initial induced bleach which decays into an induced absorption. This general behavior matches that observed with 10 ps pulses [10], but the shorter laser pulses used in the current experiment resolve the initial dynamics much more clearly. The induced absorption persists for long times and its decay matches the long-time decay of the transient bleach observed with an 850 nm probe pulse. Because of this match and since B850, as the terminal acceptor in the B800-850 complex, should be the only long-lived singlet excited state species, the long-time response with 800 nm probe light is assigned to induced absorption by the excited state of B850. The dynamics of the B850 excited state population are complicated by excitation annihilation [3] and are difficult to model analytically. Thus the contribution of the B850 excited state to the transient probe signal at 800 nm has been removed by subtracting the transient differential absorption response obtained with 850 nm probe light (and equal 800 nm pump intensities) after the signal amplitudes were matched at long times ($t > 8$ ps, data not shown). The resulting corrected data are also shown in Fig. 2. They are fit with a kinetic model having an instantaneous rise followed by a single exponential decay. The resulting decay time is 0.7 ± 0.2 ps. The relatively large uncertainty in the decay time does not reflect uncertainty in the fit to a given experiment, but rather follows from both an approx. $\pm 10\%$ uncertainty in the matching of the amplitudes of the long time signals with 800 nm and 850 nm probe light, and an approx. $\pm 15\%$ variation found for the decay time among several independent experiments. For the corrected 800 nm probe data the amplitude depended linearly upon excitation intensity and the dynamics were independent of excitation intensity, indicating that annihilation effects within the B800 excited state population were not important in the present experiments.

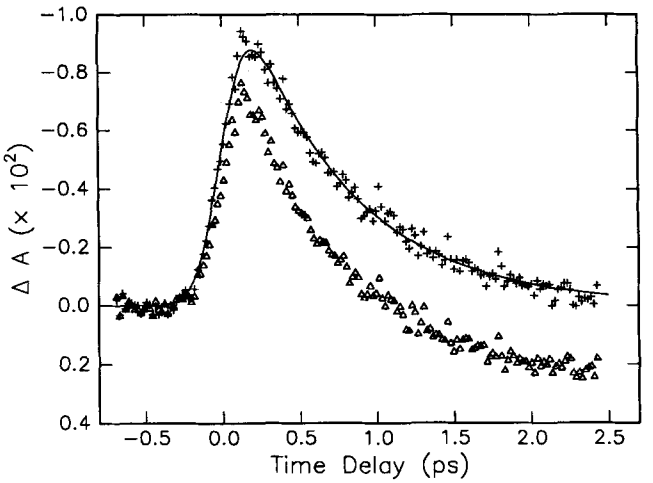

Fig. 2. The time-resolved differential absorption of B800-850/LDAO observed with a probe wavelength of 800 nm following excitation with an 800 nm pump pulse. Both raw data (△) and data corrected for the contribution of B850 excited state (+) are shown. The fit to the latter yields a decay time of 0.7 ps for the B800 excited state. The data were obtained at room temperature.

Absorption transients seen in the room temperature time-resolved study of spheroidene, dissolved in cyclohexane, are presented in Figs. 3–5. The transient differential absorption spectrum of spheroidene 5 ps after excitation with a 480 nm pulse is shown in Fig. 3. This spectrum is assigned as that of the S_1 state (the polyene A_g state) of spheroidene. The ground state recovery of spheroidene in cyclohexane at room temperature was found to be 9.1 ps from data such as those shown in Fig. 4. Kinetic scans with sharp temporal resolution were performed at several different probe wavelengths. The results at three probe wavelengths are shown in Fig. 5. An initial induced transmission is observed at all three wavelengths. This initial bleach quickly decays into a longer time response whose amplitude matches that expected from the transient spectrum presented in Fig. 3. The data in Fig. 5 are fit with a three-state model, namely

$$S_2 \xrightarrow{k_1} S_1 \xrightarrow{k_1} S_0. \quad (1)$$

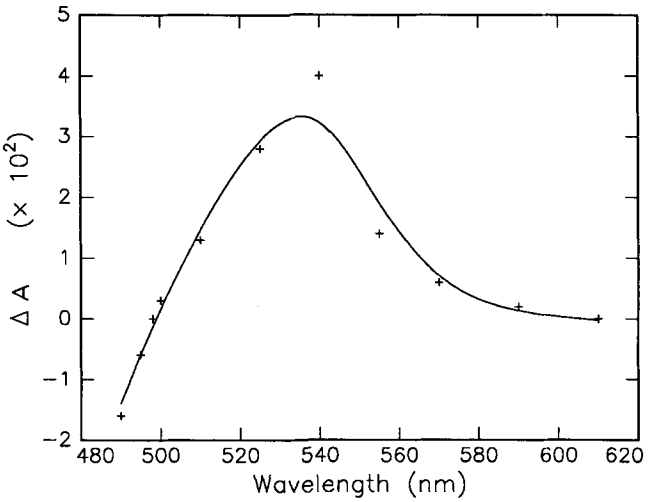

Fig. 3. The transient differential absorption spectrum of spheroidene dissolved in cyclohexane at 5 ps after excitation with a 480 nm pulse. The line is only to guide the eye. The data were obtained at room temperature.

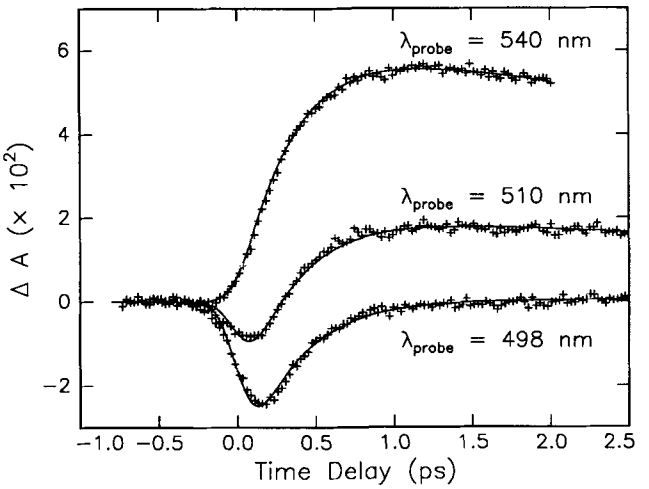

Fig. 5. The time-resolved differential absorption of spheroidene dissolved in cyclohexane at three different probe wavelengths. The pump wavelength was 480 nm. All data are fit with the model of Eqn. 1, with $k_2^{-1} = 9.1$ ps and $k_1^{-1} = 0.34$ ps. The data were obtained at room temperature.

With k_2^{-1} fixed at 9.1 ps, fits to data such as those shown in Fig. 5 determine k_1^{-1} to be 0.34 ps, with an uncertainty of about $\pm 10\%$.

A study of carotenoid emission from the B800-850 complex and from the pigment extract of the complex is complicated by the presence of interfering emissions. In both samples, an unidentified emission, strong relative to that from the carotenoid, is seen peaking in the 700 nm region. This emission peak is apparently not from a carotenoid, since its excitation spectrum does not match the carotenoid absorption spectrum, nor does it match the excitation spectrum of the weaker emission observed in the 500–600 nm region, which we attribute to the carotenoid. The interference is more significant for the B800-850 complex than for the pigment extract, because the carotenoid emission and absorption spectra in vivo are red-shifted by about 20 nm relative to the spectra in vitro. The carotenoid region of the emission and absorption spectra for the B800-850 complex and B800-850 pigment extract are shown in Figs. 6 and 7, respectively. A determination of emission yields by integration of the Gaussian in Fig. 6 or of the emission spectrum in Fig. 7 (with smooth extrapolations to zero) results in carotenoid fluorescence yields of $(1.9 \pm 0.4) \cdot 10^{-4}$ (in vivo, Fig. 6) and $(3.3 \pm 0.6) \cdot 10^{-4}$ (in vitro, Fig. 7). The uncertainties reflect scatter among several independent

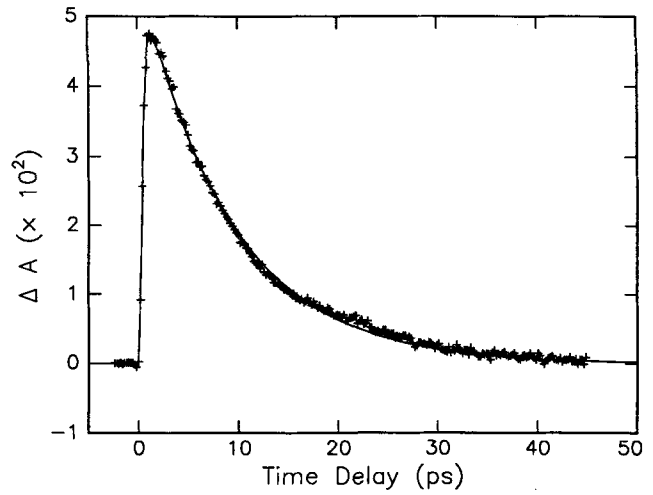

Fig. 4. The recovery of induced absorption at 540 nm following excitation with a 480 nm pulse of spheroidene dissolved in cyclohexane at room temperature. The exponential fit yields a ground state recovery time of 9.1 ps.

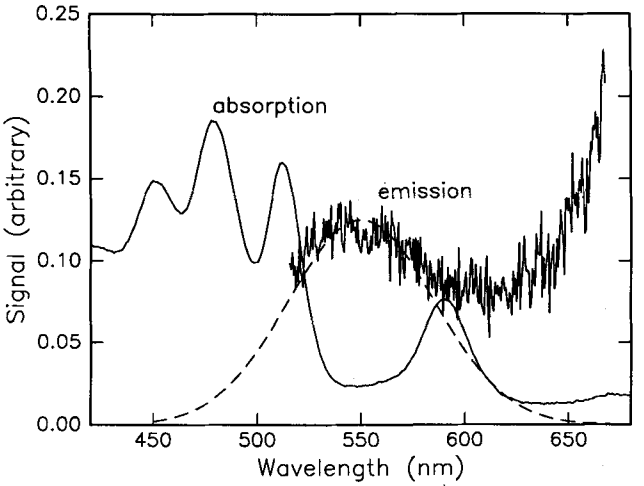

Fig. 6. The absorption and emission spectra of room temperature B800-850/LDAO through the carotenoid spectral region. Integration of the Gaussian fit to the carotenoid emission, when averaged for many experiments, results in an emission yield of $(1.9 \pm 0.4) \cdot 10^{-4}$.

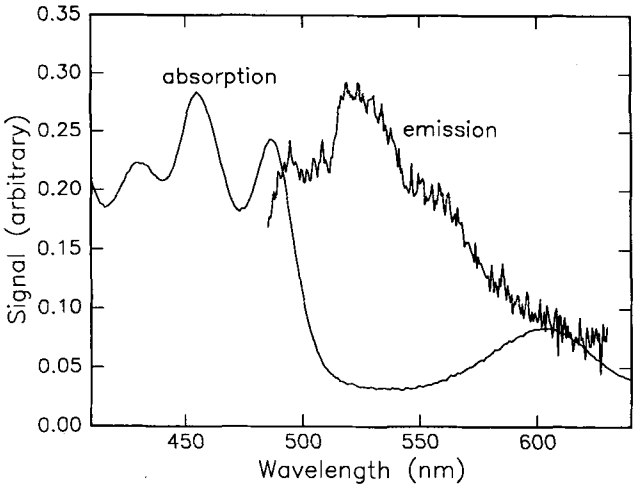

Fig. 7. The absorption and emission spectra of room temperature pigment extract from the B800-850 complex through the carotenoid spectral region. The absorption band near 600 nm is the Q_x absorption of Bchl. The average emission yield from several experiments is $(3.3 \pm 0.6) \cdot 10^{-4}$.

experiments and are the estimated 95% confidence levels of the mean value of the emission yield.

Discussion

In this section, first the B800-to-B850 energy transfer is discussed, then in vitro spheroidene dynamics are examined and, finally, general remarks are presented concerning in vivo energy transfer processes. The dynamics of B800-to-B850 energy transfer have been examined using many different techniques. Low-temperature hole-burning studies [8] and low-temperature emission yield experiments [9] have found the B800 lifetime to be about 2.5 ps and 3.3 ps, respectively, at 4 K. Because the yield for B800-to-B850 energy transfer is unity [2], the decay time of the B800 presumably matches the B800-to-B850 energy transfer time. A direct measure of the rise of the induced transmission at 850 nm due to appearance of excited B850 following excitation of B800 found the room temperature B800-to-B850 transfer time to be about 0.1 ps [11], a result that is probably artificially fast because of annihilation quenching of the B850 excited state population. A value of 0.5 to 1 ps, also at room temperature, was estimated from a measurement of B800 ground state recovery using 10 ps pulses [10]. Of special interest for the current work is a previous experiment from our laboratory [3], where the transient signal was probed at 800 nm following excitation in the carotenoid spectral region with 510 nm pump light. The transient at 800 nm was fit with a rise time of 0.34 ps and a decay time of 2.5 ps; the latter was taken to be the room temperature B800-to-B850 energy transfer time. This result, seen in Fig. 1 of Ref. 3 (also see Fig. 8), has been reproduced with the B800-850/LDAO samples used in the present work.

In the current 800 nm pump/800 nm probe work, excited B800 returns to its ground state in about 0.7 ps at room temperature. This ground state recovery is interpreted as reflecting the B800-to-B850 energy transfer. A direct measure of the energy transfer from the rise of the induced transmission at 850 nm was attempted, but significant early time quenching of the B850 excited state population by annihilation makes the fitting of the 850 nm rise uncertain. At the lowest of excitation intensities a rise time of 0.4 ps was obtained, but even then some annihilation quenching of B850 excited state remained. Thus, this result is regarded as consistent with an energy-transfer-determined 0.7 ps ground state recovery time measured at 800 nm. If the lifetime of excited B800 is 2.5 ps at 4 K, then a subpicosecond B800 lifetime at room temperature is quite reasonable. As previously noted [9,10], if for no other reason, the change in spectral overlap of B800 emission and B850 absorption on going from 4 K to room temperature should shorten the B800-to-B850 energy transfer time.

The present direct measure of the excited state lifetime of B800 must be reconciled with the different dynamics observed for the 800 nm transient when the carotenoid spectral region is pumped with 510 nm light (see above). First, it turns out that the fitting of the 800 nm probe data from the latter experiment contains a formal ambiguity in the absence of independent information about signal amplitudes. Namely, the fit function for the intermediate b in an $a \xrightarrow{k_1} b \xrightarrow{k_2} c$ kinetic

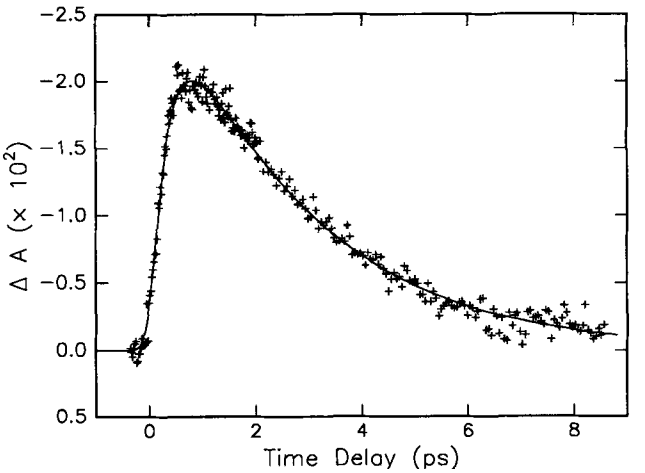

Fig. 8. The transient differential absorption of B800-850/LDAO observed at room temperature with a probe wavelength of 800 nm following excitation with a 510 nm pump pulse. These data have been corrected for the contribution of B850 excited state. See Ref. 3 for details. The fit is according to the kinetic model of Fig. 9. With the times in parentheses in Fig. 9 held fixed (see Fig. 9), the spheroidene S_2-to-B800 and S_1-to-B800 transfer times were found to be 1.7 ps and 3.8 ps, respectively.

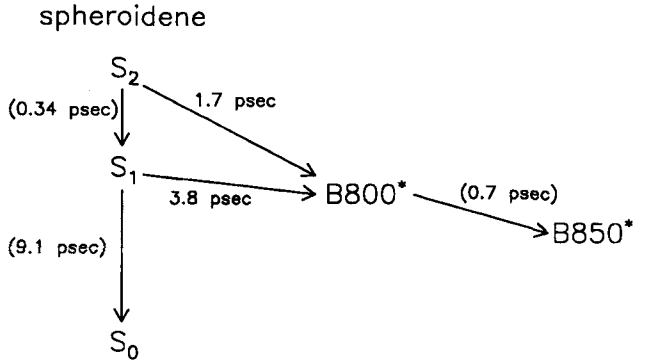

Fig. 9. A schematic representation of the kinetic model used to fit the data shown in Fig. 8. The times in parenthesis were held fixed at values determined for in vitro spheroidene and the in vivo B800-to-B850 transfer time. This model applies only to those carotenoids that are coupled to B800; the majority of carotenoids, which transfer their energy directly to B850 are not included.

model is invariant, except in amplitude, to the interchange of the two rate constants. Thus, while the assignment given in Ref. 3 was a carotenoid-to-B800 energy transfer time of 0.34 ps and a B800-to-B850 transfer time of 2.5 ps, the same data must be (and are) equally well fit with the converse assignment – a carotenoid-to-B800 transfer time of 2.5 ps and a B800-to-B850 transfer time of 0.34 ps. Even so, 0.34 ps differs from the directly measured 0.7 ps, and in fact, the carotenoid pump/800 nm probe data could not be fit successfully upon fixing the B800-to-B850 transfer time to 0.7 ps and allowing only a single carotenoid-to-B800 transfer time to be adjusted. However, measurements of in vitro spheroidene electronic state dynamics (presented below) leave open the possibility that energy transfer may occur from both the carotenoid S_1 and S_2 states. Then, if two carotenoid-to-B800 energy transfer channels are introduced, the 800 nm transient following carotenoid excitation can be fit successfully, as seen in Fig. 8. The kinetic model is shown in Fig. 9. The B800-to-B850 transfer time was fixed to 0.7 ps, the two carotenoid channels were taken to be transfer from the S_2 and the S_1 carotenoid electronic states, and the rate constants for carotenoid internal conversion dynamics were fixed to the in vitro values presented below. The model of Fig. 9 predicts, for those carotenoids coupled to a B800 intermediate, an overall efficiency of 75% for energy transfer from the initially excited carotenoid S_2 state to B850. However, about 3/4 of the carotenoids transfer their energy directly to B850 [9], presumably with near unit efficiency, so the overall carotenoid-to-B850 transfer efficiency is predicted to be 0.94, in good agreement with the 0.95 ± 0.05 experimental result [2,5]. Such a model for the B800 data must be regarded with caution. For example, recent measurements of the effect of electric fields on absorption spectra demonstrate that a much larger difference between ground and excited state dipole moments exists for spheroidene in the B800-850 complex than for spheroidene in a polymer matrix [25]. Such perturbation in the electronic state structure might well change the internal electronic state dynamics of in vivo spheroidene relative to those found in vitro. In addition, if S_2 transfer is Coulomb-mediated and S_1 transfer is exchange-mediated, then it is difficult to understand why transfer times for carotenoid S_2-to-B800 and S_1-to-B800 transfer should be so similar. As an alternative to the two-channel carotenoid-to-B800 transfer model, one could argue that the B800-to-B850 energy transfer time determined by directly exciting B800 need not agree with that determined by pumping the carotenoids and probing at 800 nm. Suppose that a distribution of B800-to-B850 energy transfer times were to exist. Then, if only a fraction of the B800 molecules were coupled to carotenoids, carotenoid pumping would excite only that fraction of the B800 population, but direct pumping of B800 would excite the entire population of B800. The effective B800-to-B850 energy transfer time could differ for these two cases. Yet another model involves single-channel transfer from each carotenoid molecule, with a distribution of carotenoid-to-B800 energy transfer times attributed, for example, to a distribution of carotenoid-B800 separation distances. In addition, the possibility that transient absorption signals may result from vibrational cooling rather than electronic state dynamics cannot be ruled out. From the available data, it is not possible to distinguish among these scenarios. However, the in vitro spheroidene dynamics demonstrate the potential for energy transfer from both the carotenoid S_1 and S_2 states, as required for the two-channel model for carotenoid-to-Bchl energy transfer.

The electronic state internal conversion dynamics of in vitro spheroidene following excitation into S_2 (the polyene B_u state) have been determined from the data in Figs. 3–5. A kinetic model consistent with the data is

$$S_2 \xrightarrow{0.34\,\text{ps}} S_1 \xrightarrow{9.1\,\text{ps}} S_0 \qquad (2)$$

The in vitro fluorescence emission yield results are in general agreement with the model of Eqn. 2; for spheroidene the S_2 radiative lifetime can be calculated, using the procedure of Ref. 26, to be about 1 ns, so a quantum yield for emission of $3.3 \cdot 10^{-4}$ corresponds to a lifetime of 0.33 ps for the S_2 state. The in vivo carotenoid fluorescence emission yield suggests an in vivo lifetime for S_2 of about 0.2 ps. The majority of carotenoids in B800-850/LDAO transfer energy directly to B850 in ≈ 0.3 ps without creating an intermediate B800 excited state [9]. At the very least, the emission data show that the in vivo S_2 lifetime is nearly the same as the appearance time of excited B850 following carotenoid excitation [3]. One could even imagine that the in vivo S_2 lifetime is shortened because of S_2-to-Bchl energy transfer, a decay channel not availa-

ble for in vitro spheroidene. However, a simple, environmentally induced change in S_2 lifetime cannot be excluded. In any case, it is clear that one must seriously consider energy transfer from the carotenoid S_2 state, and not regard the transfer as occurring exclusively from the S_1 state (polyene A_g state).

Energy transfer to Bchl from the carotenoid S_2 state, strongly dipole allowed from the ground state, can occur via transition dipole-transition dipole coupling, but transfer from the carotenoid S_1 state must presumably rely on exchange coupling or Coulomb interactions of higher order than dipolar. It is known that efficient Bchl-to-carotenoid triplet energy transfer occurs in the B800-850 complex of *Rb. sphaeroides* [14]. Triplet energy transfer must occur by exchange coupling, since Coulomb coupling terms are spin forbidden. However, given the long life of a triplet state, efficient triplet energy transfer can occur on a much slower time-scale than required for efficient singlet energy transfer. Thus, the mere existence of efficient triplet energy transfer need not at all imply that the exchange mechanism leads to a transfer rate constant sufficiently large to account for efficient singlet energy transfer. On the other hand, a variety of evidence suggests that Bchl and carotenoid molecules are quite close to one another in the B800-850 complex [14]; then not only might exchange-mediated transfer from the carotenoid S_1 state to Bchl occur, but the Coulomb coupling of the carotenoid S_2 state to Bchl will be very large. When the molecules are separated by only a few angstroms, it is not a good approximation to keep only the transition dipole–transition dipole interaction term [27]. Nevertheless, it remains the leading term in the multipole expansion of the Coulomb interaction, and its magnitude is a good indication of the strength of interaction of the chromophores. The energy transfer rate constant for carotenoid S_2-to-Bchl energy transfer, retaining only transition dipole-transition dipole coupling, can be calculated by the procedure of Förster [22,23]. The Franck-Condon function for the carotenoid S_2 emission is determined by the spectral reflection, weighted by the frequency dependent spontaneous emission probability, of the in vivo carotenoid absorption about an assumed electronic origin of 19400 cm^{-1}. Alternatively, the Gaussian fit to the carotenoid in vivo emission shown in Fig. 6 may be used. These two methods agree within 10%. For a carotenoid-Bchl separation of 10 Å, and an orientation factor matching that obtained from an isotropic distribution of donor-acceptor pairs, the calculated energy transfer time with only transition dipole–transition dipole interaction is 0.1 ps. Of interest is that the receiving Franck-Condon function is more likely that of the Bchl Q_x absorption band, not that of the Q_y band. In this case, the Q_x state presumably undergoes rapid internal conversion into the Q_y state, but this has not been experimentally resolved. For energy transfer into the Bchl Q_x state, the choice of an isotropic orientation factor probably underestimates the chromophore interaction energy because polarization anisotropy measurements suggest that the carotenoid and Bchl Q_x transition dipoles are nearly parallel [2].

In considering the possibility of energy transfer from the carotenoid S_2 state, the issue of overall energy transfer efficiency must be addressed. For B800-850/LDAO from *Rb. sphaeroides* 2.4.1, the overall carotenoid-to-B850 energy-transfer efficiency is 0.95 ± 0.05 [5]. If the transfer occurs from a single carotenoid state, then the transfer time must be rapid in comparison with the natural decay time of the state. With a spheroidene S_2 decay time of about 0.3 ps, energy transfer from this state would call for a transfer time of about 30 fs to account for the observed transfer efficiency. No evidence of such rapid transfer is seen in the time-resolved experiments, nor is such rapid quenching of the in vivo carotenoid S_2 state consistent with the in vivo carotenoid fluorescence emission yield result. Thus, the overall high transfer efficiency requires that energy transfer occur from the carotenoid S_1 state as well. That is, the initially formed carotenoid S_2 state decays by two routes. One is transfer to Bchl, the other is internal conversion into the carotenoid S_1 state. The carotenoid S_1 state must then also transfer energy to Bchl, in competition with the S_1-to-S_0 internal conversion. Since the latter is very much slower than S_2-to-S_1 internal conversion, a relatively slow energy transfer from S_1 can still be efficient.

An additional concern in describing the energy transfer pathways and dynamics in isolated B800-850/LDAO complexes is the role of B800 as an intermediate in overall carotenoid-to-B850 energy transfer. Low temperature fluorescence emission studies [9] indicate that about 1/4 of the total carotenoid excitations pass through an excited B800 intermediate before reaching B850. In LDS-treated complexes, the 800 nm absorption is mostly removed and the overall carotenoid-to-B850 energy transfer efficiency drops from 0.95 ± 0.05 to 0.72 ± 0.03 [2,5]. The suggestion has been made [2] that this decrease in overall transfer efficiency results from a complete decoupling of those carotenoids originally coupled only to B800, while the remainder of the carotenoids, coupled only to B850, continue to transfer energy with nearly 100% efficiency. The rapid carotenoid-to-B850 energy transfer time seen in B800-850/LDS was interpreted to provide further support for a subpopulation exhibiting efficient transfer [3]. But, this interpretation was based on the assumption, then believed, that all the energy transfer occurred from the carotenoid S_1 state with its relatively long lifetime. In that case a subpicosecond carotenoid-to-B850 energy transfer time in B800-850/LDS [3] implies a near unit transfer efficiency, and then a diminished yield implies that some carotenoids are altogether unable to transfer

to B850. However, for energy transfer from the short-lived S_2 state, transfer times of a few hundred femtoseconds do not correspond to highly efficient transfer from that state. Because of the wavefunction overlap requirement, exchange coupling is particularly sensitive to intermolecular separation, and one can imagine a structural situation where partial disruption of the membrane/protein structure during treatment with LDS would quench the exchange coupling energy transfer mechanism, while the Coulomb mechanism would survive. Then, energy transfer would not occur from the carotenoid S_1, and with transfer only from S_2, the carotenoid-to-B850 energy transfer time would have to be about 150 fs to account for about 70% efficiency. This value is not inconsistent with the appearance time of B850 excited state in B800-850/LDS. Thus, a subpopulation of completely uncoupled carotenoids, in fact, need not be required once energy transfer from the carotenoid S_2 state is considered. Note that in this case, the branching ratio for carotenoid-to-B800 and carotenoid-to-B850 transfer in B800-850/LDAO might reflect kinetic competition rather than structural heterogeneity, and in general, some combination of the two effects cannot be ruled out.

Much of the uncertainty regarding possible pathways of in vivo energy transfer could be resolved by studying the dynamics of carotenoid ground state recovery in vivo. In both LDAO and LDS solubilized B800-850 complexes, the carotenoid ground state recovery is multi-component [3]. In Ref. 3 the longer-lived components of the decay were speculatively assigned to the cooling of a vibrationally hot carotenoid ground electronic state that was formed after transfer of electronic energy to bacteriochlorophyll. While this explanation remains possible, in view of the in vitro and in vivo quantum yield and time-resolved studies recently performed on carotenoids [18,28], it now seems more likely that the multiple components present in the in vivo carotenoid ground state recovery data reflect the dynamics of the carotenoid S_1 and S_2 states. Though it is possible to rationalize the ground state recovery data to this model, in practice the complicated nature of the system and the attainable signal-to-noise ratio make difficult an unambiguous interpretation.

Conclusion

Two key new results for energy transfer in the B800-850 complex of *Rb. sphaeroides* 2.4.1 are reported in the current work. The first is that carotenoid-to-chlorophyll energy transfer can occur from both S_2 and S_1 of the spheroidene. This conjecture is supported by in vivo and in vitro time-resolved experiments, by quantum yield measurements and by an estimation of an energy transfer rate constant for spheroidene S_2-to-bacteriochlorophyll energy transfer. The second is the determination of 0.7 ps for the B800-to-B850 energy transfer time at room temperature.

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