Efficient Energy Transfer from the Carotenoid S_2 State in a Photosynthetic Light-Harvesting Complex

Alisdair N. Macpherson,* Juan B. Arellano,† Niall J. Fraser,† Richard J. Cogdell,† and Tomas Gillbro*
*Department of Biophysical Chemistry, Umeå University, SE-90187 Umeå, Sweden; and †Division of Biochemistry and Molecular Biology, IBLS, University of Glasgow, Glasgow, G12 8QQ, United Kingdom

ABSTRACT Previously, the spatial arrangement of the carotenoid and bacteriochlorophyll molecules in the peripheral light-harvesting (LH2) complex from *Rhodopseudomonas acidophila* strain 10050 has been determined at high resolution. Here, we have time resolved the energy transfer steps that occur between the carotenoid's initial excited state and the lowest energy group of bacteriochlorophyll molecules in LH2. These kinetic data, together with the existing structural information, lay the foundation for understanding the detailed mechanisms of energy transfer involved in this fundamental, early reaction in photosynthesis. Remarkably, energy transfer from the rhodopin glucoside S_2 state, which has an intrinsic lifetime of ~120 fs, is by far the dominant pathway, with only a minor contribution from the longer-lived S_1 state.

INTRODUCTION

In photosynthesis, carotenoids are important accessory light-harvesting pigments. Indeed, in certain organisms (e.g., dinoflagellates), most of the solar energy that is used to drive photosynthesis is initially absorbed by carotenoids (Frank and Cogdell, 1996). This light-harvesting process involves singlet-singlet energy transfer from donor carotenoid to acceptor (bacterio-)chlorophyll ((B)Chl) molecules, and can be up to 100% efficient (Frank and Cogdell, 1996). The exact mechanisms involved are, however, poorly understood. To solve this problem, a combination of detailed structural information and accurate, time-resolved measurements are required. The spatial arrangement of the Bchl and carotenoid molecules in the peripheral light-harvesting (LH2) complex from *Rhodopseudomonas acidophila* strain 10050 has been visualized using high-resolution, x-ray crystallography (McDermott et al., 1995). In this study, we have used ultrafast time-resolved spectroscopy to determine the kinetics of singlet-singlet energy transfer from the carotenoids to the lowest energy Bchl molecules in this antenna complex.

Carotenoids have a characteristic strong absorption in the visible region, which is the result of electronic transitions from their ground state, S_0 ($1^1A_g^-$), to their second excited singlet state, S_2 ($1^1B_u^+$) (Hudson et al., 1982). One-photon transitions between the ground and the first excited singlet state, S_1 ($2^1A_g^-$) are symmetry forbidden. The lifetime of the S_2 state is extremely short (<300 fs) (Shreve et al., 1991b; Ricci et al., 1996; Macpherson and Gillbro, 1998a; Akimoto et al., 1999) and is the result of rapid internal conversion from the S_2 to the S_1 state. This ultrashort lifetime has been traditionally thought to be incompatible with the measured, high efficiencies (Frank and Cogdell, 1996) of carotenoid-to-(B)Chl energy transfer. For those carotenoids found in photosynthetic bacteria, relaxation from the S_1 to the ground state typically occurs on a 10-ps time scale (Koyama et al., 1996; Andersson et al., 1996; Frank et al., 1997a,b; Zhang et al., 2000). As the lifetime of the S_1 state is one to two orders of magnitude longer than S_2, it has been tacitly assumed that the primary carotenoid donor state in singlet-singlet energy transfer is S_1 (Gillbro et al., 1993). Kinetic experiments in the last decade, however, have suggested that the S_2 state is also involved (Shreve et al., 1991a; Ricci et al., 1996; Andersson et al., 1996; Krueger et al., 1998b; Desamero et al. 1998).

Previously, the crystal structure of the LH2 complex from *R. acidophila* 10050 has been determined to a resolution of 2.5 Å (McDermott et al., 1995). It has a ring structure and is a nonamer. Each monomeric unit consists of one carotenoid (rhodopin glucoside) (Arellano et al., 1998; Fraser, 1998) and three Bchl molecules (Fig. 1), noncovalently bound to two small apoproteins, α and β. In LH2, the Bchl molecules are arranged as two spectroscopically distinct, physically separate groups and are commonly denoted Bchl-B800 and Bchl-B850. The rhodopin glucoside molecules have an extended S-shape, all-*trans* (Robert and Lutz, 1985) conformation and span the entire depth of the complex. Each carotenoid interacts with Bchl molecules in two, neighboring protomer units. On the cytoplasmic side of LH2, the glycosyl ring of rhodopin glucoside makes specific interactions with several polar residues. The conjugated system of each carotenoid then passes close (~3.4 Å) to the edge of the Bchl-B800 macrocycle in the same protomer unit before coming in close proximity (~3.6 Å) to the α-bound Bchl-B850 molecule in a neighboring $\alpha\beta$ pair.

Here, we have measured the S_2 and S_1 lifetimes of rhodopin glucoside in native and B800 depleted (B850-only) complexes from *R. acidophila* 10050, and in benzyl

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J. B. Arellano's present address: Instituto de Recursos Naturales y Agrobiología de Salamanca, CSIC, 37008 Salamanca, Spain.

Address reprint requests to Dr. Alisdair Macpherson, Department of Biophysical Chemistry, Umeå University, SE-90187 Umeå, Sweden. Tel.: 46-90-786-5368; Fax: 46-90-786-7779; E-mail: Alisdair.Macpherson@chem.umu.se.

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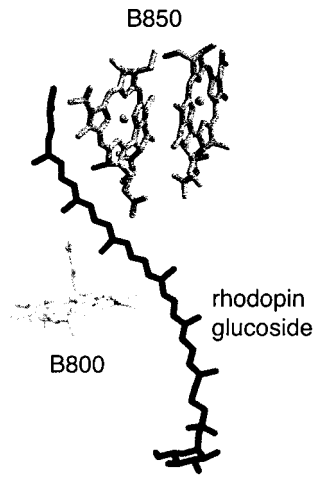

FIGURE 1 The arrangement of the pigments within a monomer unit of LH2 from *R. acidophila* 10050, as viewed parallel to the membrane surface.

alcohol (a simple model of the protein environment). We have also measured the efficiency and kinetics of carotenoid to B850 energy transfer in both the LH2 and B850-only complexes. These results have allowed us to fully assign the extent of energy transfer from the S_2 and S_1 states of rhodopin glucoside to both the Bchl-B800 and Bchl-B850 molecules.

MATERIALS AND METHODS

Sample preparation

Liquid cultures of *R. acidophila* 10050 were grown as described by Pfennig (1969). Cells were harvested 2 days after inoculation by centrifugation. LH2 and B850-only complexes were then prepared according to Fraser et al. (1999).

Rhodopin glucoside was extracted from purified samples of LH2 (Gardiner et al., 1994) by adding methanol to a concentration of 85% before passage through a 0.2-μm PFTE syringe filter (Millex-FGS; Millipore, Bedford, MA). Residual Bchl was removed from the particulate matter trapped in the filter by repeatedly washing it with 85% methanol. Rhodopin glucoside was later released from the filter using acetone and dried under nitrogen before storage at -20°C.

Steady-state spectroscopy

Absorption spectra were measured using a Perkin-Elmer Lambda 40 spectrophotometer (Perkin-Elmer, Norwalk, CT). All measurements were made in a 1-cm quartz cuvette. The samples had an A_{859} of 0.5.

Fluorescence excitation spectra were measured on SPEX Fluorolog 112 and Fluorolog 2 spectrofluorimeters using samples with an A_{490} of ~0.2. The excitation spectra of selected laser dyes were recorded under identical conditions and used to generate a correction file by assuming that their absorptance ($1 - T$) and excitation spectra are identical. The average yields of carotenoid-to-B850 energy transfer in the native and B850-only samples were determined from the average of at least four independent measurements of the efficiency spectra (i.e., the wavelength dependence of the corrected fluorescence excitation intensity, or absorptance ratio) in the range 440–525 nm (530 nm for LH2) at 1-nm intervals. The errors reported are the standard deviation of the efficiency in this range.

Ultrafast spectroscopy

The fluorescence upconversion spectrometer used for kinetic measurements has been described previously (Macpherson and Gillbro, 1998a). The samples were excited at a repetition rate of 82 MHz in the region 480–500 nm by frequency-doubling ~70-fs near-IR pulses from a mode-locked Ti:sapphire laser (Tsunami; Spectra-Physics, Mountain View, CA), pumped by an argon ion laser. To minimize pulse broadening, the thickness of all dispersive elements in the spectrometer were reduced to a minimum and the laser beam was focused into a 0.3-mm BBO type I doubling crystal and recollimated using spherical concave mirrors in an off-axis arrangement. Using this arrangement, a response function shorter than 110 fs could be obtained. The samples were held in a rotating cell with a path length of 0.5 mm. Measurements typically took 1 h, during which time minimal sample degradation occurred. The energy of the excitation pulses was <0.3 nJ. Kinetics were recorded using parallel (rhodopin glucoside S_2 emission) or magic-angle polarization (Bchl emission). Fluorescence transients were fitted to a sum of at least two exponential components with a floating background and were convoluted with a Gaussian response function using the SPECTRA program. The full width at half maximum (fwhm) of the response function was varied in steps of 1 fs until the smallest χ^2 and residuals were obtained. The experimental combination of a high signal-to-noise ratio, short (Gaussian) response function, and ultrafast decay kinetics enabled the carotenoid S_2 lifetimes to be determined with a high precision. The errors in the decay kinetics are reported as standard deviations of three to seven measurements in the range 564–615 nm. The errors in the rise kinetics are estimated uncertainties.

Transient absorption measurements were made using the pump-probe technique. The laser set-up consists of a Ti:Sapphire regenerative amplifier (Spitfire; Positive Light, Los Gatos, CA) pumped by a Nd:YLF laser (Merlin; Positive Light) and seeded by a mode-locked Ti:sapphire laser (Tsunami; Spectra Physics) pumped by a continuous wave frequency-doubled diode-pumped Nd:YAG laser (Millennia V; Spectra Physics). This system gave amplified laser pulses of ~80 fs at 800 nm, with a pulse energy of 0.2 mJ at a repetition rate of 5 kHz. Most of the laser energy (95%) was used to pump an optical parametric amplifier (OPA-800; Spectra Physics). The fourth harmonic of the idler was used for excitation, with a pulse energy of <40 nJ and spot size of ~0.4 mm at the sample, which was held in a static 1-mm-path-length cuvette. The OPA output was double passed through an SF14 prism pair, chopped at 170 Hz, and sent through a computer-controlled optical delay line before sample excitation. The rest of the laser output (5%) was attenuated and focused into a 2-mm-thick sapphire plate to generate a white light continuum, which was further split into probe and reference beams. Spherical mirrors and an achromatic lens were used to focus the white light onto the sample and onto the slit of a computer-controlled monochromator (TRIAX 190; Jobin Yvon Horiba, Edison, NJ), respectively. The intensities of the pump, probe, and reference beams were recorded using separate integrating silicon photodiodes (FD-4; EKSPLA, Vilnius, Lithuańia). Kinetics were recorded with variable delay line step sizes, and the polarization of the pump beam was set at the magic angle with a Berek compensator (New Focus, Santa Clara, CA). The probe beams were also passed through a cube polarizer after sample excitation. The transient absorption kinetics were fitted to a sum of one or two exponential rise components and one or two decay components (depending on wavelength and sample) with a floating background and were convoluted with a Gaussian response function using the SPECTRA program. The response function was wavelength dependent and in the range of 140–200 fs. Absorption spectra were recorded before and after the transient absorption measurements to check that no degradation had occurred. With the exception of rhodopin glucoside in benzyl alcohol, the samples were stable in the laser beam. A stable signal was obtained in

benzyl alcohol by stirring the solution with a wire attached to an electric toothbrush.

RESULTS AND DISCUSSION

The absorption spectrum of LH2 is characterized by two peaks at 800 and 859 nm, associated with the Q_y transitions of the Bchl-B800 and Bchl-B850 molecules, respectively (Fig. 2). A protocol has recently been developed that allows the selective release and removal of the Bchl-B800 molecules from LH2 to give B850-only complexes (Fraser et al., 1999). The Bchl-B800 Q_y band is completely absent in the B850-only complex (Fig. 2). In its place, a broad absorption shoulder, arising from excitonic components and/or vibrational energy levels associated with the Bchl-B850 molecules (Sauer et al., 1996; Alden et al., 1997; Bandilla et al., 1998; Koolhaas et al., 1998; Leupold et al., 1999; Sundström et al., 1999), can be clearly seen. The position and intensity of the Bchl Soret and Q_x transitions are largely unchanged. In LH2, rhodopin glucoside has three prominent absorption bands in the region 400–550 nm. These are blue-shifted by 3–5 nm in the B850-only complex (Fig. 2). This shift is attributed to a loss of interactions between the rhodopin glucoside and Bchl-B800 molecules and/or a change in the polarizability of the carotenoid's local environment (Andersson et al., 1991) and is not the result of differences in the conformation of the carotenoid (Fraser et al., 1999; Gall et al., 1999). By studying the LH2 and B850-only complexes together, it was possible to unravel the energy transfer pathways between the carotenoids and the Bchl-B800 and Bchl-B850 molecules for the first time. The average yields of carotenoid-to-B850 energy transfer in the native and B850-only complexes were determined from their efficiency spectra and found to be 0.56 ± 0.01 and 0.39 ± 0.01, respectively (Fig. 3).

The extent of energy transfer from the S_1 and S_2 states of the carotenoid in LH2 can be determined by comparing their lifetimes in vivo with that in solution of similar microenvironment (i.e., in the absence of energy transfer). Here, benzyl alcohol was used as a simple model of the protein because the absorption maxima and fine structure of rhodopin glucoside in this solvent are essentially the same as that in LH2 (inset of Fig. 2). The S_2 lifetimes of rhodopin glucoside in benzyl alcohol and in the native and B850-only complexes were determined by fluorescence upconversion (Fig. 4 *A*). The average S_2 lifetimes in native (57 ± 2 fs) and B850-only (72 ± 1 fs) complexes are significantly shorter than that in benzyl alcohol (124 ± 8 fs). Typically, carotenoid S_2 lifetimes decrease with increasing refractive index (Ricci et al., 1996; Macpherson and Gillbro, 1998a). However, the lifetime of the rhodopin glucoside S_2 state shows little solvent dependence and is shortest in solvents with a low refractive index (~105 fs in ethanol). This means that the shorter S_2 lifetimes in both the LH2 and B850-only

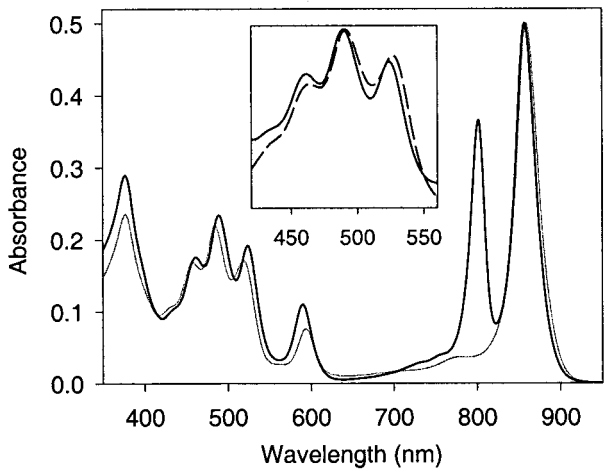

FIGURE 2 Absorption spectra of native (black) and B850-only (gray) complexes from *R. acidophila* 10050. The inset shows the normalized absorption spectra of rhodopin glucoside in benzyl alcohol (– – –) and in LH2 (——).

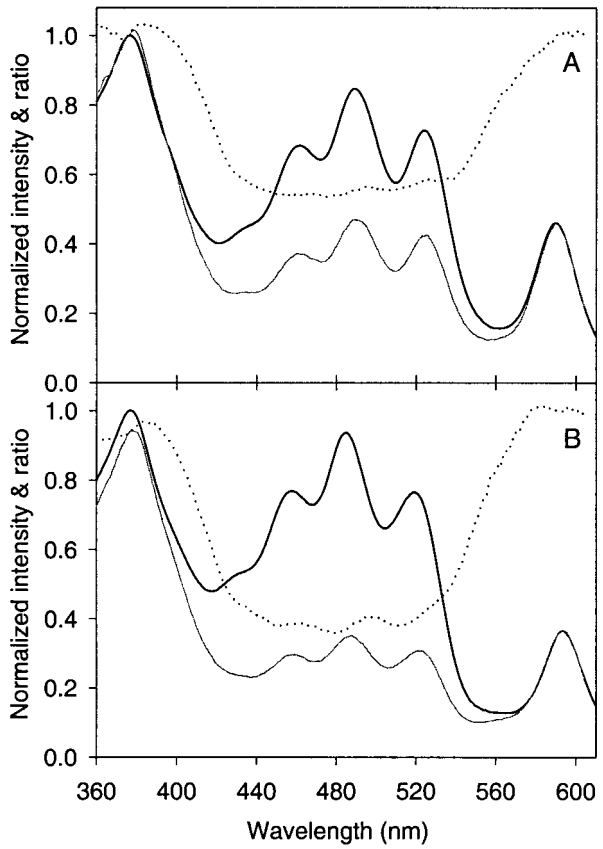

FIGURE 3 The average yield of carotenoid to B850 energy transfer in the native (ϕ_{LH2}; *A*) and B850-only (ϕ_{B850}; *B*) complexes, as determined from their efficiency spectra (*dotted line*). The absorptance (*black line*) and corrected fluorescence excitation (*gray line*) spectra were normalized at the Bchl Q_x transition. ϕ_{LH2} is 0.56 ± 0.01 and ϕ_{B850} is 0.39 ± 0.01.

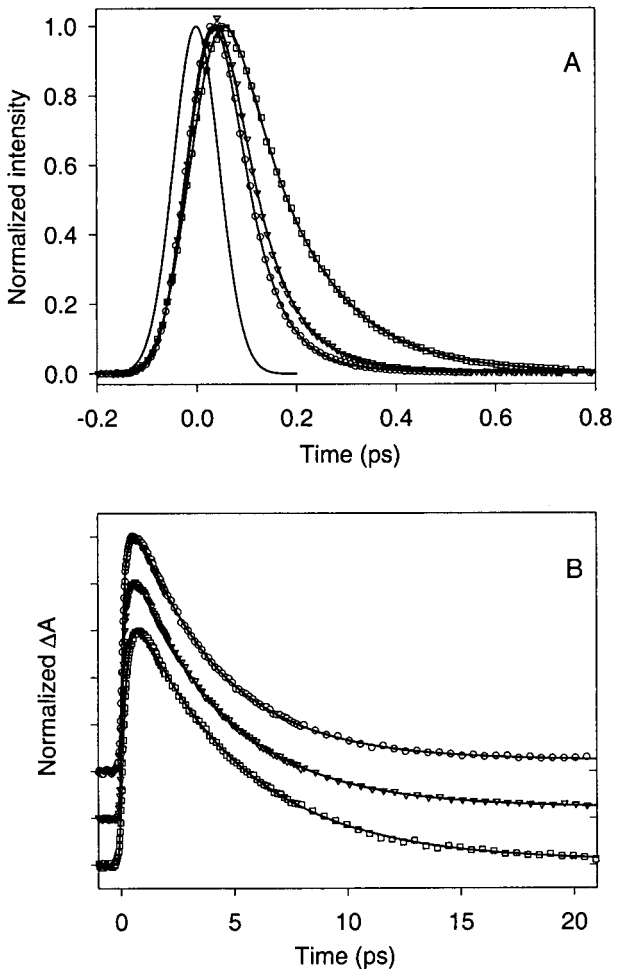

FIGURE 4 The S_2 (*A*) and S_1 (*B*) decay kinetics of rhodopin glucoside in benzyl alcohol (□), and in the native (○) and B850-only (▽) complexes. (*A*) The carotenoid S_2 lifetimes in the native and B850-only complexes, and in benzyl alcohol, determined at an emission wavelength of 589 nm, are 56 fs (97.7% amplitude), 72 fs (99.3%), and 133 fs (98.9%), respectively ($\lambda_{excitation}$ = 491 nm). A Gaussian response function of 108 fs is also shown. (*B*) The S_1 lifetime of rhodopin glucoside is 4.8 ps (98.8%) in benzyl alcohol, 4.1 ps (96.0%) in the B850-only complex, and 3.7 ps (95.7%) in LH2 ($\lambda_{excitation}$ = 488 nm; λ_{probe} = 580 nm). The decay of the S_2 state was determined by fluorescence upconversion, and the S_1 state was monitored using transient absorption spectroscopy.

complexes compared with benzyl alcohol cannot be entirely attributed to differences in the polarity and polarizability of the carotenoid's surroundings. Rather, it suggests that energy transfer occurs from the rhodopin glucoside S_2 state to both the Bchl-B800 and Bchl-B850 molecules.

The role of the carotenoid S_1 state in energy transfer was also investigated by measuring its excited-state absorption decay kinetics in benzyl alcohol and in the native and B850-only complexes (Fig. 4 *B*). The S_1 lifetime is 4.8 ± 0.2 ps in benzyl alcohol, and it is 4.1 ± 0.1 ps and 3.7 ± 0.1 ps in the B850-only and native complexes, respectively. To determine whether these small changes in lifetime arise from differences in local environment, additional S_1 lifetime measurements were made in a range of solvents. The S_1 lifetime of rhodopin glucoside has a solvent dependence similar to that of the S_2 state and increases with the refractive index. The S_1 lifetime is 4.1 ± 0.1 ps in diethylether, 4.2 ± 0.1 ps in ethanol, 4.3 ± 0.1 ps in acetone, and 4.9 ± 0.2 ps in carbon disulfide (results not shown). These results are consistent with previous measurements made by Frank et al. (1997a) in petroleum ether (H. A. Frank, University of Connecticut, personal communication, 2000). Contrary to what was found for the S_2 state, the carotenoid S_1 lifetime in the B850-only complex falls within the range, but at the low end, of the solution measurements. Thus, from the decay kinetics alone, it is not clear whether any energy is transferred from S_1 to the Bchl-B850 molecules. The S_1 lifetime of rhodopin glucoside in LH2 is, however, shorter than in any solvent or the B850-only complex, suggesting that the Bchl-B800 molecules can accept energy from the S_1 state. If this energy pathway does exist in LH2, it should be possible to switch it off by elevating the energy of the acceptor transition. This was tested by measuring the S_1 lifetime in B850-only complexes that had their B800 sites reconstituted with either Bchl (Q_y transition ~800 nm) or Chl (663 nm). The S_1 lifetimes in the control Bchl-B800 and Chl-B800 reconstituted complexes are 3.7 ± 0.1 ps and 4.1 ± 0.1 ps, respectively (A. N. Macpherson, N. J. Fraser, H. Scheer, T. Gillbro, and R. J. Cogdell, manuscript in preparation). This confirms that there is indeed limited energy transfer from the rhodopin glucoside S_1 state to the Bchl-B800 molecules.

The rhodopin glucoside excited-state decay kinetics suggest that there is significant energy transfer from the S_2 state and a minor contribution from S_1. To confirm this, the time-dependent arrival of excitation energy at the Bchl molecules was studied by fluorescence upconversion. Excitation of the carotenoid leads to an ultrafast rise in fluorescence from the Bchl-B850 molecules. In LH2, at the far blue edge of the B850 emission (840 nm), the rise kinetics are bi-exponential (τ_1 = 58 ± 10 fs; τ_2 = 0.9 ± 0.2 ps). At the same emission wavelength, the B850-only complex displays just a single rise phase (τ = 79 ± 6 fs; Fig. 5 *A*). Moving further to the red, the fast rise component slows down from 133 fs at 868 nm to ~180 fs at 900 nm (Macpherson et al., 1998). A similar change in the rise kinetics with emission wavelength has also been observed for the B800-B820 complex from *R. acidophila* 7050 (Krueger et al. 1998b). This effect is attributed to relaxation within the B850 band and appears at the blue edge of the B850 emission as a short decay component (see the B850-only kinetics, Fig. 5 *A*). More importantly, the shortest B850 rise components are only slightly longer than the S_2 lifetimes of rhodopin glucoside in both the native and B850-only complexes, confirming direct energy transfer from the carotenoid S_2 state to the Bchl-B850 molecules. In the LH2

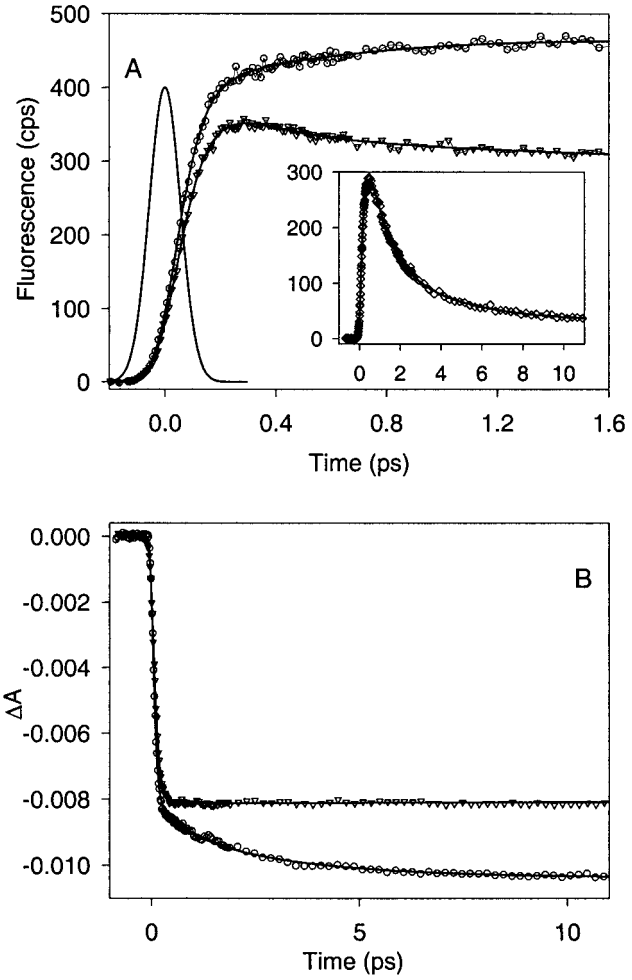

FIGURE 5 (*A*) The time-dependent rise in fluorescence emission centered at 840 nm from the Bchl-B850 molecules in native (○) and B850-only (▽) complexes, after carotenoid excitation at 490 nm. In LH2, the rise kinetics are bi-exponential (τ_1 = 58 fs; τ_2 = 0.9 ps), whereas the B850-only complex has just a single rise phase (τ = 79 fs). The inset shows the ultrafast rise (τ = 210 fs) and slower decay phases (τ_1 = 0.9 ps; τ_2 = 3.6 ps; $\tau_3 > 100$ ps) of fluorescence from the Bchl-B800 molecules in LH2 at 795 nm ($\lambda_{excitation}$ = 501 nm). (*B*) The kinetics of carotenoid to B850 energy transfer in the native (○) and B850-only (▽) complexes, studied over a longer time range, by transient absorption spectroscopy. The LH2 rise contains a slow component of 3.6 ps at 860 nm, attributed to energy transfer from the carotenoid S_1 state to the Bchl-B800 molecules ($\lambda_{excitation}$ = 486 nm).

kinetics, a slow rise phase (τ = 0.9 ps), identical to the known B800-to-B850 transfer time (Kennis et al., 1997; Ma et al., 1997; Herek et al., 2000), is also observed. This implies that there is indirect energy transfer from the rhodopin glucoside S_2 state to the Bchl-B850 molecules via B800. The existence of this pathway in LH2 was investigated by monitoring the time-dependent changes in B800 emission after carotenoid excitation (inset of Fig. 5 *A*). The ultrafast arrival of excitation energy at the Bchl-B800 molecules confirms that they receive energy from the S_2 state of rhodopin glucoside.

The S_1 lifetime measurements suggest that limited energy transfer occurs from the carotenoid S_1 state to the Bchl molecules. From the upconversion measurements, the existence of slower rise components with small amplitude could not be ruled out because of singlet-triplet annihilation (Bradforth et al., 1995). To overcome this problem, the kinetics of carotenoid to B850 energy transfer were also studied by transient absorption spectroscopy. At 865 nm, the time-dependent rise in bleaching/stimulated emission of the Bchl-B850 Q_y band in the B850-only complex is monoexponential (Fig. 5 *B*). This clearly shows that there is no energy transfer from the S_1 state of rhodopin glucoside to the Bchl-B850 molecules. In LH2, however, the kinetics are multi-exponential. In addition to the rise components assigned to direct and indirect (via B800) carotenoid S_2-to-B850 energy transfer, a third slower phase is also observed. At 860 nm, this component has a lifetime of 3.6 ps, consistent with energy transfer from the carotenoid S_1 state. The lifetime of this rise phase is, however, wavelength dependent and is ~6 ps at 870 nm. The B850-only complex does not show an equivalent slow rise phase at any probe wavelength tested. Although we do not understand the origin of this effect, this rise component cannot be attributed solely to energy transfer from S_1.

The efficiency of carotenoid-to-B850 energy transfer in LH2 is determined by the rates of energy transfer from the S_2 and S_1 states of the carotenoid (to both the Bchl-B800 and Bchl-B850 molecules) relative to those for internal conversion (S_2-S_1, S_1-S_0). The possible fates of excitation energy after leaving the S_2 state of rhodopin glucoside in LH2 are summarized in Fig. 6. The yield of energy transfer from S_2, ϕ_{ET2}, can be expressed as

$$\phi_{ET2} = (k_2 + k_3)/(k_1 + k_2 + k_3), \quad (1)$$

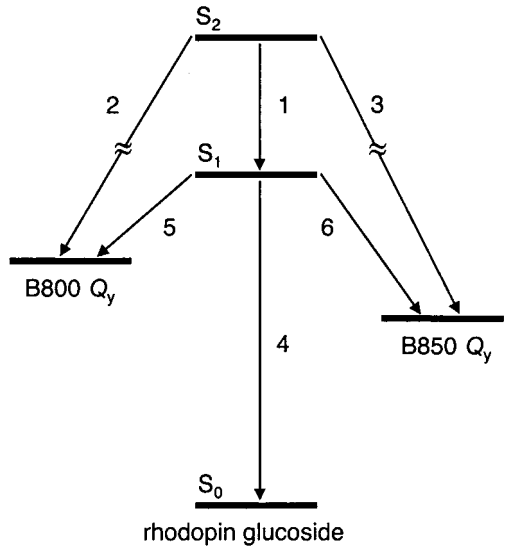

FIGURE 6 Schematic diagram showing the possible fates of excitation energy after leaving the S_2 state of rhodopin glucoside in LH2.

whereas the yield of energy transfer from S_1, ϕ_{ET1}, is given by

$$\phi_{ET1} = ((k_5 + k_6)/(k_4 + k_5 + k_6)) \times (1 - \phi_{ET2}). \quad (2)$$

Using the data from our steady-state efficiency and excited-state lifetime measurements, we can now calculate the rate constants k_1 to k_6. In the B850-only complex, we have shown that energy is only transferred from the carotenoid S_2 state, and k_6 is, therefore, zero. The efficiency of carotenoid-to-B850 energy transfer in the B850-only complex, ϕ_{B850}, can then be written as

$$\phi_{B850} = k_3/(k_1 + k_3). \quad (3)$$

In the B850-only complex, $\phi_{B850} = 0.39$ and $k_1 + k_3 = (72\ \text{fs})^{-1}$. From Eq. 3, the rates k_1 and k_3 can therefore be calculated, and they are given in Table 1. In LH2, $k_1 + k_2 + k_3 = (57\ \text{fs})^{-1}$. Thus, k_2 and ϕ_{ET2} (Eq. 1) can also be determined, assuming that k_1 and k_3 are the same as for the B850-only complex (Table 1). In the absence of energy transfer from the S_1 state of rhodopin glucoside, $k_4 = (4.1\ \text{ps})^{-1}$. In LH2, $k_4 + k_5 = (3.7\ \text{ps})^{-1}$. This allows k_5 and ϕ_{ET1} (Eq. 2) to be calculated (Table 1).

Combining $\phi_{ET2} = 0.51 \pm 0.05$ and $\phi_{ET1} = 0.05 \pm 0.02$ gives a total carotenoid-to-B850 energy transfer yield of 0.56 ± 0.05 in LH2. Within the limits of experimental error, this is the same as that determined from the steady-state efficiency spectrum (Fig. 3 *A*). Moreover, the calculated rate k_1 $(118 \pm 4\ \text{fs})^{-1}$ and the experimentally determined S_2 lifetime of rhodopin glucoside (124 ± 8 fs) in benzyl alcohol are essentially the same. This means that any contribution from protein-induced distortions in the conformation of rhodopin glucoside to the quenching of the S_2 lifetime in vivo must be relatively small and, at least for the S_2 state, benzyl alcohol is a satisfactory model system. The difference between the S_1 lifetimes of rhodopin glucoside in the B850-only complex and in benzyl alcohol, however, indicates that the environment provided by benzyl alcohol is not a true reflection of that found in LH2. This discrepancy can be explained if the lifetime of the longer-lived S_1 state is more sensitive to conformational distortions (Hudson et al., 1982) introduced by binding to the protein than the S_2 state.

TABLE 1 Rate constants and energy transfer yields for the deactivation processes that occur in LH2 after excitation of rhodopin glucoside to the second excited singlet state (S_2)

Energy deactivation pathway	Rate, $k \times 10^{12}$ (s^{-1})	Energy transfer yield, ϕ
$S_2 \rightarrow S_1$ (1)	8.5 ± 0.3	
$S_2 \rightarrow$ B800 Q_y (2)	3.6 ± 0.8	0.20 ± 0.04
$S_2 \rightarrow$ B850 Q_y (3)	5.4 ± 0.2	0.31 ± 0.02
$S_1 \rightarrow S_0$ (4)	0.24 ± 0.01	
$S_1 \rightarrow$ B800 Q_y (5)	0.034 ± 0.010	0.05 ± 0.02
$S_1 \rightarrow$ B850 Q_y (6)	0	0

In this work, we have fully described the major energy transfer events that occur between the initial carotenoid donor (S_2) state and the lowest-energy Bchl acceptor transition (Bchl-B850 Q_y) in the LH2 complex from *R. acidophila* 10050. Remarkably, given its ultrashort lifetime of ~120 fs, the rhodopin glucoside S_2 state functions as an efficient donor in singlet-singlet energy transfer. This observation is in agreement with other fluorescence upconversion measurements that suggested appreciable energy transfer occurs from the S_2 state (Ricci et al., 1996; Krueger et al., 1998b; Walla et al., 2000) and is not restricted to this antenna alone. Measurements on light-harvesting complexes from other species of green and purple bacteria, LHCII from higher plants, and synthetic dyad molecules of carotenoids covalently linked to tetrapyrroles have directly shown that the yield of energy transfer from S_2 typically ranges between 0.3 and 0.7 (A. N. Macpherson, unpublished data; Macpherson and Gillbro, 1998b). An exception to this general trend is the carotenoid peridinin, where quenching of the S_2 state is negligible (A. N. Macpherson, unpublished data) and the primary route of energy transfer is from S_1 (Bautista et al., 1999). In those natural and artificial photosynthetic systems where energy transfer yields approach unity, it is clear that energy from the S_1 state must also be effectively harvested.

Our work also shows that the Bchl-B800 and Bchl-B850 molecules in this LH2 complex are almost equally involved in accepting excitation energy from the carotenoids. Contrary to theoretical predictions based on the structure of LH2 (Krueger et al., 1998a,b), the Bchl-B800 molecules receive energy from both the carotenoid S_2 ($\phi_{ET} = 0.20$) and S_1 ($\phi_{ET} = 0.05$) states. The most efficient carotenoid to Bchl energy transfer process in this antenna is that from the rhodopin glucoside S_2 state to the Bchl-B850 molecules ($\phi_{ET} = 0.31$). This finding confirms previous structure-based calculations that predicted highly efficient energy transfer from the carotenoid S_2 state to the Bchl-B850 Q_x transition via a Coulombic coupling mechanism (Nagae et al., 1993; Freer at al., 1996; Krueger et al., 1998b; Damjanovic et al., 1999). The inability of the Bchl-B850 molecules to capture energy from the S_1 state is, however, unexpected. Before this observation can be fully understood, additional experiments need to be undertaken. We do, however, point out that the spectral overlap between the carotenoid S_1 emission and the Bchl-B800 and Bchl-B850 Q_y absorption bands is thought to favor transfer to Bchl-B850 (Zhang et al., 2000). Furthermore, the edge-to-edge distances between the rhodopin glucoside molecules and the Bchl-B800 and Bchl-B850 tetrapyrrole rings are essentially the same (Freer et al., 1996). It is unlikely, therefore, that either of these factors could explain the preferential transfer of energy from the carotenoid S_1 state to the Bchl-B800. Factors that could account for the observed behavior are a less favorable alignment of donor and acceptor transition dipoles and a larger center-to-center distance (Scholes et al.,

1997) between each rhodopin glucoside and its nearest Bchl-B850 molecule (14.2 Å) compared with that for Bchl-B800 (10.2 Å) (Freer et al., 1996).

Using the kinetic picture presented here, together with the available structural information on LH2, it is now possible to quantitatively evaluate prospective mechanisms of energy transfer from both the carotenoid S_2 and S_1 states to the Bchl-B800 and Bchl-B850 molecules.

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REFERENCES

Akimoto, S., I. Yamazaki, S. Takaichi, and M. Mimuro. 1999. Excitation relaxation of carotenoids within the S_2 state probed by the femtosecond fluorescence up-conversion method. *Chem. Phys. Lett.* 313:63–68.

Alden, R. G., E. Johnson, V. Nagarajan, W. W. Parson, C. J. Law, and R. J. Cogdell. 1997. Calculations of spectroscopic properties of the LH2 bacteriochlorophyll-protein antenna complex from *Rhodopseudomonas acidophila*. *J. Phys. Chem. B.* 101:4667–4680.

Andersson, P. O., R. J. Cogdell, and T. Gillbro. 1996. Femtosecond dynamics of carotenoid-to-bacteriochlorophyll *a* energy transfer in the light-harvesting antenna complexes from the purple bacterium *Chromatium purpuratum*. *Chem. Phys.* 210:196–217.

Andersson, P. O., T. Gillbro, L. Ferguson, and R. J. Cogdell. 1991. Absorption spectral shifts of carotenoids related to medium polarizability. *Photochem. Photobiol.* 54:353–360.

Arellano, J. B., B. B. Raju, K. R. Naqvi, and T. Gillbro. 1998. Estimation of pigment stoichiometries in photosynthetic systems of purple bacteria: special reference to the (absence of) second carotenoid in LH2. *Photochem. Photobiol.* 68:84–87.

Bandilla, M., B. Ücker, M. Ram, I. Simonin, G. Gelhaye, G. McDermott, R. J. Cogdell, and H. Scheer. 1998. Reconstitution of the B800 bacteriochlorophylls in the peripheral light-harvesting complex B800–850 of *Rhodobacter sphaeroides* 2.4.1 with Bchl*a* and modified (bacterio-)chlorophylls. *Biochim. Biophys. Acta.* 1364:390–402.

Bautista, J. A., R. G. Hiller, F. P. Sharples, D. Gosztola, M. Wasielewski, and H. A. Frank. 1999. Singlet and triplet energy transfer in the peridinin-chlorophyll *a* protein from *Amphidinium carterae*. *J. Phys. Chem. A.* 103:2267–2273.

Bradforth, S. E., R. Jimenez, F. van Mourik, R. van Grondelle, and G. R. Fleming. 1995. Excitation transfer in the core light-harvesting complex (LH1) of *Rhodobacter sphaeroides*: an ultrafast fluorescence depolarization and annihilation study. *J. Phys. Chem. B.* 99:16179–16191.

Damjanovic, A., T. Ritz, and K. Schulten. 1999. Energy transfer between carotenoids and bacteriochlorophylls in light-harvesting complex II of purple bacteria. *Phys. Rev. E.* 59:3293–3311.

Desamero, R. Z. B., V. Chynwat, I. van der Hoef, F. J. Jansen, J. Lugtenburg, D. Gosztola, M. R. Wasielewski, A. Cua, D. F. Bocian, and H. A. Frank. 1998. Mechanism of energy transfer from carotenoids to bacteriochlorophyll: light-harvesting by carotenoids having different extents of π-electron conjugation incorporated into the B850 antenna complex from the carotenoidless bacterium *Rhodobacter sphaeroides* R-26.1. *J. Phys. Chem. B.* 102:8151–8162.

Frank, H. A., V. Chynwat, R. Z. B. Desamero, R. Farhoosh, J. Erickson, and J. Bautista. 1997a. On the photophysics and photochemical properties of carotenoids and their role as light-harvesting pigments in photosynthesis. *Pure Appl. Chem.* 69:2117–2124.

Frank, H. A., and R. J. Cogdell. 1996. Carotenoids in photosynthesis. *Photochem. Photobiol.* 63:257–264.

Frank, H. A., R. Z. B. Desamero, V. Chynwat, R. Gebhard, I. van der Hoef, F. J. Jansen, J. Lugtenburg, D. Gosztola, and M. R. Wasielewski. 1997b. Spectroscopic properties of spheroidene analogs having different extents of π-electron conjugation. *J. Phys. Chem. A.* 101:149–157.

Fraser, N. J. 1998. Modified pigments and mechanisms of energy transfer in LH2 complexes from purple bacteria. Ph.D. thesis. University of Glasgow, Glasgow, UK. 212pp.

Fraser, N. J., P. J. Dominy, B. Ücker, I. Simonin, H. Scheer, and R. J. Cogdell. 1999. Selective release, removal and reconstitution of Bchl*a* molecules into the B800 sites of LH2 complexes from *Rhodopseudomonas acidophila* 10050. *Biochemistry.* 38:9684–9692.

Freer, A. A., S. M. Prince, K. Sauer, M. Z. Papiz, A. M. Hawthornthwaite-Lawless, G. McDermott, R. J. Cogdell, and N. W. Isaacs. 1996. Pigment-protein interactions and energy transfer in the antenna complex of the photosynthetic bacterium *Rhodopseudomonas acidophila*. *Structure.* 4:449–462.

Gall, A., N. J. Fraser, M.-C. Bellissent-Funel, H. Scheer, B. Robert, and R. J. Cogdell. 1999. Bacteriochlorin-protein interactions in native B800–B850, B800 deficient, and B800-Bchl*a* reconstituted complexes from *Rhodopseudomonas acidophila* strain 10050. *FEBS Lett.* 449: 269–272.

Gardiner, A. T., R. J. Cogdell, and S. Takaichi. 1994. The effect of growth conditions on the light-harvesting apparatus in *Rhodopseudomonas acidophila*. *Photosynth. Res.* 38:159–167.

Gillbro, T., P. O. Andersson, R. S. H. Liu, A. E. Asato, S. Takaishi, and R. J. Cogdell. 1993. Location of the carotenoid $2A_g^-$ state and its role in photosynthesis. *Photochem. Photobiol.* 57:44–48.

Herek, J. L., N. J. Fraser, T. Pullerits, P. Martinsson, T. Polìvka, H. Scheer, R. J. Cogdell, and V. Sundström. 2000. B800→B850 energy transfer mechanism in bacterial LH2 complexes investigated by B800 pigment-exchange. *Biophys. J.* 78:2590–2596.

Hudson, B. S., B. E. Kohler, and K. Schulten. 1982. Linear polyene electronic structure and potential surfaces. *In* Excited States, Vol. 6. E. C. Lim, editor. Academic Press, New York. 22–95.

Kennis, J. T. M., A. M. Streltsov, S. T. E. Vulto, T. J. Aartsma, T. Nozowa, and J. Amesz. 1997. Femtosecond dynamics in isolated LH2 complexes of various species of purple bacteria. *J. Phys. Chem. B.* 101:7827–7834.

Koolhaas, M. H. C., R. N. Frese, G. J. S. Fowler, T. S. Bibby, S. Georgakopoulou, G. van der Zwan, C. N. Hunter, and R. van Grondelle. 1998. Identification of the upper exciton component of the B850 bacteriochlorophylls of the LH2 antenna complex using a B800-free mutant of *Rhodobacter sphaeroides*. *Biochemistry.* 37:4693–4698.

Koyama, Y., M. Kuki, P. O. Andersson, and T. Gillbro. 1996. Singlet excited states and the light-harvesting function of carotenoids in bacterial photosynthesis. *Photochem. Photobiol.* 63:243–256.

Krueger, B. P., G. D. Scholes, and G. R. Fleming. 1998a. Calculations of couplings and energy transfer pathways between the pigments of LH2 by the ab initio transition density cube method. *J. Phys. Chem. B.* 102: 5378–5386.

Krueger, B. P., G. D. Scholes, R. Jimenez, and G. R. Fleming. 1998b. Electronic excitation energy transfer from carotenoid to bacteriochlorophyll in the purple bacterium *Rhodopseudomonas acidophila*. *J. Phys. Chem. B.* 102:2284–2292.

Leupold, D., H. Stiel, J. Ehlert, K. Nowak, K. Teuchner, B. Voigt, M. Bandilla, B. Ücker, and H. Scheer. 1999. Photophysical characterization of the B800-depleted light-harvesting complex B850 of *Rhodobacter sphaeroides*: implications to the ultrafast energy transfer 800→850 nm. *Chem. Phys. Lett.* 301:537–545.

Ma, Y.-Z., R. J. Cogdell, and T. Gillbro. 1997. Energy transfer and exciton annihilation in the B800–850 antenna complex of the photosynthetic purple bacterium *Rhodopseudomonas acidophila* (strain 10050): a femtosecond transient absorption study. *J. Phys. Chem. B.* 101:1087–1095.

Macpherson, A. N., J. B. Arellano, N. J. Fraser, R. J. Cogdell, and T. Gillbro. 1998. Ultrafast energy transfer from rhodopin glucoside in the light-harvesting complexes of *R. acidophila*. *In* Photosynthesis: Mechanisms and Effect, Vol. 1. G. Garab, editor. Kluwer, Dordrecht, The Netherlands. 9–14.

Macpherson, A. N., and T. Gillbro. 1998a. Solvent dependence of the ultrafast S_2-S_1 internal conversion rate of β-carotene. *J. Phys. Chem. B.* 102:5049–5058.

Macpherson, A. N., and T. Gillbro. 1998b. Ultrafast singlet energy transfer in a caroteno-pyropheophorbide dyad: a biomimetic model for photosynthetic light-harvesting? *In* Ultrafast Phenomena XI. T. Elsaesser, J. G. Fujimoto, D. A. Wiersma, and W. Zinth, editors. Springer-Verlag, Berlin. 672–674.

McDermott, G., S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, and N. W. Isaacs. 1995. Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature.* 374:517–521.

Nagae, H., T. Kakitani, T. Katoh, and M. Mimuro. 1993. Calculation of the excitation transfer-matrix elements between the S_2 and S_1 state of the carotenoid and the S_2 and S_1 state of bacteriochlorophyll. *J. Chem. Phys.* 98:8012–8023.

Pfennig, N. 1969. *Rhodopseudomonas acidophila* sp.n., a new species of the budding purple non-sulfur bacteria. *J. Bacteriol.* 99:597–602.

Ricci, M., S. E. Bradforth, R. Jiminez, and G. R. Fleming. 1996. Internal conversion and energy transfer dynamics of spheroidene in solution and in the LH1 and LH2 light-harvesting complexes. *Chem. Phys. Lett.* 259:381–390.

Robert, B., and M. Lutz. 1985. Structures of antenna complexes of several *Rhodospirillales* from their resonance Raman spectra. *Biochim. Biophys. Acta.* 807:10–23.

Sauer, K., R. J. Cogdell, S. M. Prince, A. A. Freer, N. W. Isaacs, and H. Scheer. 1996. Structure-based calculations of the optical spectra of the LH2 bacteriochlorophyll-protein complex from *Rhodopseudomonas acidophila*. *Photochem. Photobiol.* 64:564–576.

Scholes, G. D., R. D. Harcourt, and G. R. Fleming. 1997. Electronic interactions in photosynthetic light-harvesting complexes: the role of carotenoids. *J. Phys. Chem. B.* 101:7302–7312.

Shreve, A. P., J. K. Trautman, H. A. Frank, T. G. Owens, and A. C. Albrecht. 1991a. Femtosecond energy transfer processes in the B800–850 light-harvesting complex of *Rhodobacter sphaeroides* 2.4.1. *Biochim. Biophys. Acta.* 1058:280–288.

Shreve, A. P., J. K. Trautman, T. G. Owens, and A. C. Albrecht. 1991b. Determination of the S_2 lifetime of β-carotene. *Chem. Phys. Lett.* 178: 89–96.

Sundström, V., T. Pullerits, and R. van Grondelle. 1999. Photosynthetic light-harvesting: reconciling dynamics and structure of purple bacterial LH2 reveals function of photosynthetic unit. *J. Phys. Chem. B.* 103: 2327–2346.

Walla, P. J., J. Yom, B. P. Krueger, and G. R. Fleming. 2000. Two-photon excitation spectrum of light harvesting complex II and fluorescence upconversion after one- and two-photon excitation of the carotenoids. *J. Phys. Chem. B.* 104:4799–4806.

Zhang, J.-P., R. Fujii, P. Qian, T. Inaba, T. Mizoguchi, Y. Koyama, K. Onaka, Y. Watanabe, and H. Nagae. 2000. Mechanism of the carotenoid-to-bacteriochlorophyll energy transfer via the S_1 state in the LH2 complexes from purple bacteria. *J. Phys. Chem. B.* 104:3683–3691.