

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

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**ABSTRACT** Previously, the spatial arrangement of the carotenoid and bacteriochlorophyll molecules in the peripheral light-harvesting (LH2) complex from *Rhodospseudomonas 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  $\sim 120$  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 *Rhodospseudomonas 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,  $\alpha$  and  $\beta$ . 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 ( $\sim 3.4$  Å) to the edge of the Bchl-B800 macrocycle in the same protomer unit before coming in close proximity ( $\sim 3.6$  Å) to the  $\alpha$ -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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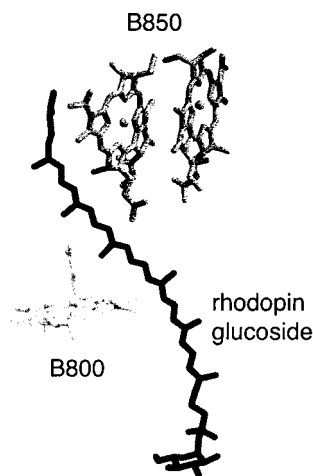


FIGURE 1 The arrangement of the pigments within a monomer unit of LH2 from *R. acidophila* 10050, as viewed parallel to the membrane surface.

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- $\mu$ m PTFE 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  $-20^\circ\text{C}$ .

### 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  $\sim 0.2$ . The excitation spectra of selected laser dyes were recorded under identical conditions and used to generate a correction file by assuming that their absorbance ( $1 - T$ ) 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 absorbance 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  $\sim 70$ -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  $\chi^2$  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  $\sim 80$  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  $\sim 0.4$  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; EKSPILA, Vilnius, Lithuania). 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

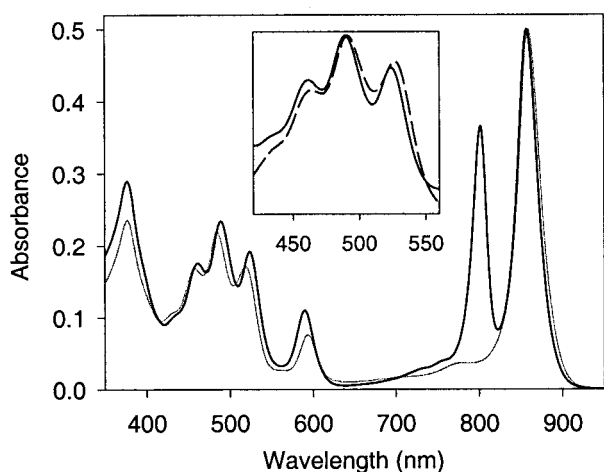


FIGURE 2 Absorption spectra of native (black) and B850-only (gray) complexes from *R. acidophila* 10050. The inset shows the normalized absorption spectra of rhodopin glucoside in benzyl alcohol (---) and in LH2 (—).

their efficiency spectra and found to be  $0.56 \pm 0.01$  and  $0.39 \pm 0.01$ , 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 ( $57 \pm 2$  fs) and B850-only ( $72 \pm 1$  fs) complexes are significantly shorter than that in benzyl alcohol ( $124 \pm 8$  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 ( $\sim 105$  fs in ethanol). This means that the shorter  $S_2$  lifetimes in both the LH2 and B850-only

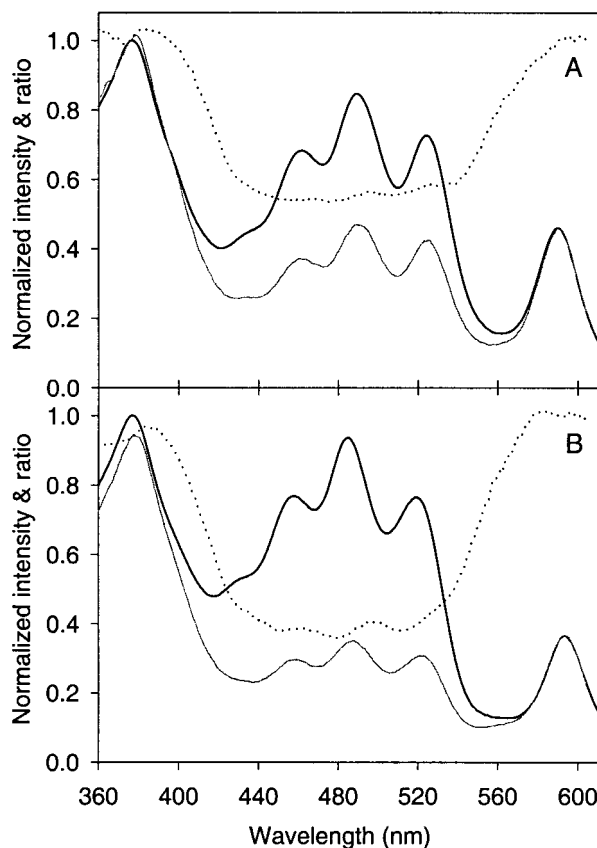


FIGURE 3 The average yield of carotenoid to B850 energy transfer in the native ( $\phi_{LH2}$ ; A) and B850-only ( $\phi_{B850}$ ; B) complexes, as determined from their efficiency spectra (dotted line). The absorbance (black line) and corrected fluorescence excitation (gray line) spectra were normalized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

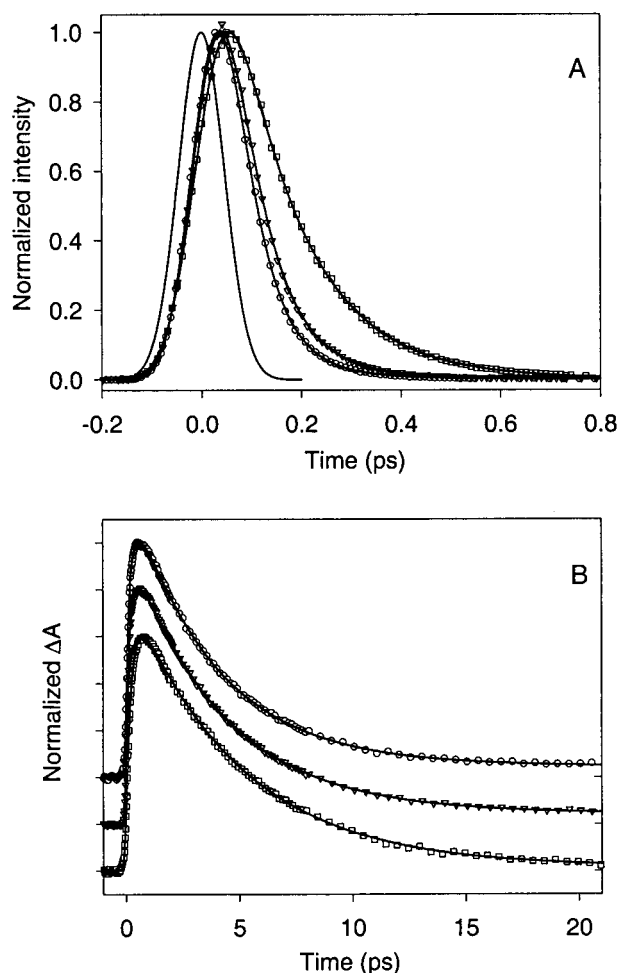


FIGURE 4 The  $S_2$  (A) and  $S_1$  (B) decay kinetics of rhodopin glucoside in benzyl alcohol ( $\square$ ), and in the native ( $\circ$ ) and B850-only ( $\nabla$ ) complexes. (A) The carotenoid  $S_2$  lifetimes in the native and B850-only complexes, and in benzyl alcohol, determined at an emission wavelength of 589 nm, are 56 fs (97.7% amplitude), 72 fs (99.3%), and 133 fs (98.9%), respectively ( $\lambda_{\text{excitation}} = 491$  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 ( $\lambda_{\text{excitation}} = 488$  nm;  $\lambda_{\text{probe}} = 580$  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  $4.8 \pm 0.2$  ps in benzyl alcohol, and it is  $4.1 \pm 0.1$  ps and  $3.7 \pm 0.1$  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  $4.1 \pm 0.1$  ps in diethylether,  $4.2 \pm 0.1$  ps in ethanol,  $4.3 \pm 0.1$  ps in acetone, and  $4.9 \pm 0.2$  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  $\sim 800$  nm) or Chl (663 nm). The  $S_1$  lifetimes in the control Bchl-B800 and Chl-B800 reconstituted complexes are  $3.7 \pm 0.1$  ps and  $4.1 \pm 0.1$  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 ( $\tau_1 = 58 \pm 10$  fs;  $\tau_2 = 0.9 \pm 0.2$  ps). At the same emission wavelength, the B850-only complex displays just a single rise phase ( $\tau = 79 \pm 6$  fs; Fig. 5 A). Moving further to the red, the fast rise component slows down from 133 fs at 868 nm to  $\sim 180$  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



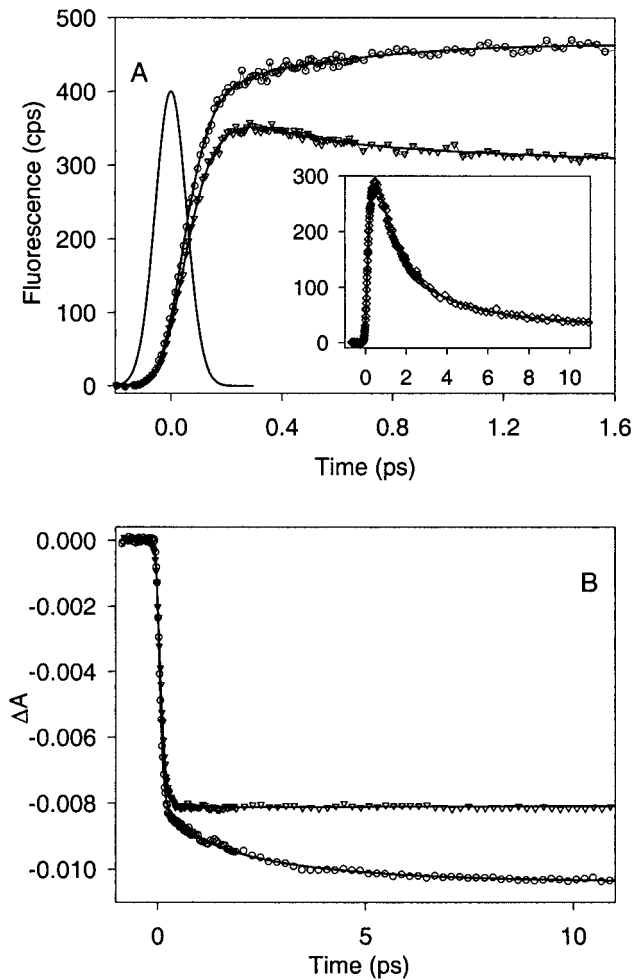


FIGURE 5 (A) The time-dependent rise in fluorescence emission centered at 840 nm from the Bchl-B850 molecules in native (○) and B850-only (▽) complexes, after carotenoid excitation at 490 nm. In LH2, the rise kinetics are bi-exponential ( $\tau_1 = 58$  fs;  $\tau_2 = 0.9$  ps), whereas the B850-only complex has just a single rise phase ( $\tau = 79$  fs). The inset shows the ultrafast rise ( $\tau = 210$  fs) and slower decay phases ( $\tau_1 = 0.9$  ps;  $\tau_2 = 3.6$  ps;  $\tau_3 > 100$  ps) of fluorescence from the Bchl-B800 molecules in LH2 at 795 nm ( $\lambda_{\text{excitation}} = 501$  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 ( $\lambda_{\text{excitation}} = 486$  nm).

kinetics, a slow rise phase ( $\tau = 0.9$  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 mono-exponential (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  $\sim 6$  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  $S_2$ ,  $\phi_{\text{ET2}}$ , can be expressed as

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

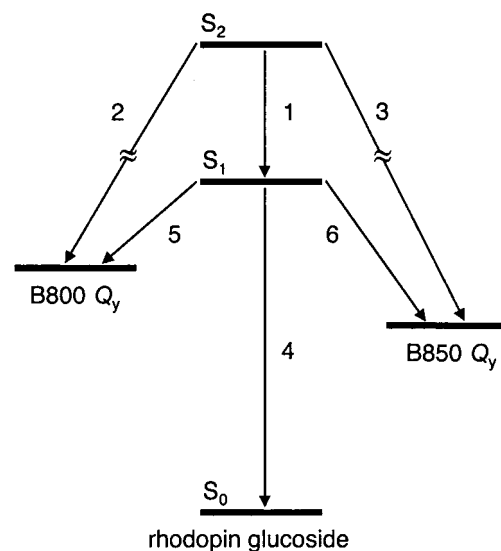


FIGURE 6 Schematic diagram showing the possible fates of excitation energy after leaving the  $S_2$  state of rhodopin glucoside in LH2.

whereas the yield of energy transfer from  $S_1$ ,  $\phi_{ET1}$ , 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,  $\phi_{B850}$ , 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  $\phi_{ET2}$  (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  $\phi_{ET1}$  (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  $0.56 \pm 0.05$  in LH2. Within the limits of experimental error, this is the same as that determined from the steady-state efficiency spectrum (Fig. 3A). Moreover, the calculated rate  $k_1$  ( $118 \pm 4 \text{ fs})^{-1}$  and the experimentally determined  $S_2$  lifetime of rhodopin glucoside ( $124 \pm 8 \text{ fs}$ ) 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, $\phi$
$S_2 \rightarrow S_1$ (1)	$8.5 \pm 0.3$	
$S_2 \rightarrow \text{B800 } Q_y$ (2)	$3.6 \pm 0.8$	$0.20 \pm 0.04$
$S_2 \rightarrow \text{B850 } Q_y$ (3)	$5.4 \pm 0.2$	$0.31 \pm 0.02$
$S_1 \rightarrow S_0$ (4)	$0.24 \pm 0.01$	
$S_1 \rightarrow \text{B800 } Q_y$ (5)	$0.034 \pm 0.010$	$0.05 \pm 0.02$
$S_1 \rightarrow \text{B850 } Q_y$ (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  $\sim 120 \text{ fs}$ , 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 et 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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