Quenching Capabilities of Long-Chain Carotenoids in Light-Harvesting-2 Complexes from Rhodobacter sphaeroides with an Engineered Carotenoid Synthesis Pathway

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ABSTRACT: Six light-harvesting-2 complexes (LH2) from genetically modified strains of the purple photosynthetic bacterium Rhodobacter (Rb.) sphaeroides were studied using static and ultrafast optical methods and resonance Raman spectroscopy. These strains were engineered to incorporate carotenoids for which the number of conjugated groups \(N = N_{\text{cong}} + N_{\text{cong+}}\) varies from 9 to 15. The R. sphaeroides strains incorporate their native carotenoids spheroidene \(N = 10\) and spheroidenone \(N = 11\), as well as longer-chain analogues including spirilloxanthin \(N = 13\) and diketospirilloxanthin \(N = 15\) normally found in Rhodospirillum rubrum. Measurements of the properties of the carotenoid-first singlet excited state \(S_1\) in antennas from the Rb. sphaeroides set show that carotenoid-bacteriochlorophyll \(a\) (BChl \(a\)) interactions are similar to those in LH2 complexes from various other bacterial species and thus are not significantly impacted by differences in polypeptide composition. Instead, variations in carotenoid-to-BChl \(a\) energy transfer are primarily regulated by the \(N\)-determined energy of the carotenoid \(S_1\) excited state, which for long-chain \(N \geq 13\) carotenoids is not involved in energy transfer. Furthermore, the role of the long-chain carotenoids switches from a light-harvesting supporter (via energy transfer to BChl \(a\)) to a quencher of the BChl \(a\) \(S_1\) excited state \(B850^*\). This quenching is manifested as a substantial (\(\sim 2\)-fold) reduction of the \(B850^*\) lifetime and the \(B850^*\) fluorescence quantum yield for LH2 housing the longest carotenoids.

INTRODUCTION

The light-harvesting machinery of most photosynthetic purple bacteria is comprised of two types of pigment–protein complexes, LH1 and LH2. Peripheral LH2 absorbs light and transfers excitation energy to inner LH1, which is closely associated with the reaction center.\(^{1,2}\) LH2 complexes have circular structures built from identical protein subunits, each of which is a heterodimer of \(\alpha\) and \(\beta\) polypeptides spanning a photosynthetic membrane. Each \(\alpha\beta\) heterodimer houses three bacteriochlorophyll \(a\) (BChl \(a\)) and one carotenoid. The type of carotenoid that is bound to LH2 can vary significantly between bacterial species and habitat (growth conditions). High resolution X-ray structures have been obtained for LH2 from two nonsulfur species, Rhodopseudomonas (Rps.) acidiphila strain 10050 and Phaeospirillum (Phs.) molischianum.\(^{3-6}\) Those structures, the 6 Å projection structure of the Rhodobacter (Rb.) sphaeroides LH2\(^+\) and recent electron-microscopy work on LH2 from sulfur purple bacterium Allochromatium vinosum show that the complexes adopt circular forms with \(8-13\) \(\alpha\beta\) subunits depending on species.\(^{3-6,8}\)

All LH2s possess two spectrally distinct forms of BChl \(a\) designated B850 and B800. B850 is an array of BChl \(a\) molecules built from pairs in close contact within each \(\alpha\beta\) subunit that have macrocycles perpendicular to the membrane plane. Electronic interactions within a pair and between pairs in the LH2 ring shift the \(S_0 \rightarrow S_1\) absorption wavelength to \(\sim 850\) nm. B800 is a monomer-like set of BChl \(a\) molecules that are oriented parallel to the membrane plane and located between polypeptides of adjacent subunits; their \(S_0 \rightarrow S_1\) absorption wavelength is \(\sim 800\) nm.\(^{3-5}\)

The main absorption of carotenoids is generally between 400 and \(550\) nm and is associated with the strongly allowed \(S_0 \rightarrow S_1\) electronic transition. Optical transitions between \(S_0\) (\(1^1A_{\text{g}}^+\)) and \(S_1\) (\(2^1B_{\text{g}}^+\)) lowest singlet excited state are forbidden due to the lack of a change in state symmetry \((g \leftrightarrow u)\) and pseudoparity \((+ \leftrightarrow -)^{9}\). The negligible transition dipole moment of the \(S_1\) \((2^1A_{\text{g}}^-)\) state also implies that variations in carotenoid environment should not significantly affect its excited-state energy or lifetime unless the structure is modified. The energies of both excited states

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depend on the double-bond conjugation length (N): the more extended the π-electron system, the lower the energy. Prior ultrafast absorption studies of various LH2 complexes showed that although transitions involving the S1 (21Ag−) state are not allowed, this state contributes along with S2 (11Bu+) to the overall yield of energy transfer from carotenoid to B850 (ΦCar→BChl), especially in LH2s with carotenoids that have modest C=C lengths (N = 9−10).10−12 Herzberg−Teller (vibronic) coupling involving S2 (11Bu+) and S1 (21Ag−) apparently imparts some dipole-allowed character to processes involving S1.13−16

Carotenoids play a dual role in photosynthetic antenna systems. Carotenoid absorption in LH2 fills the empty spectral window in BChl a absorption between 450 and 550 nm and transfer the absorbed energy to B800 and B850, thereby enhancing overall light harvesting. Carotenoids also serve a photoprotective role by efficiently quenching the triplet excited state of the BChls (B800 and B850) to inhibit formation of reactive oxygen species.17,18 Regarding the first role, past studies have shown that ΦCar→BChl significantly varies in LH2s among various purple bacterial species. The yields descend from ~95% for LH2s that contain neurosporene (N = 9) or spheroidene (N = 10)10,19−21 to 55−65% for rhodopin, rhodopin glucoside or lycopene (N = 11)10,19,20 to 30−40% for a mixture of carotenoids (N = 11−13),22,23 or for spirilloxanthin (N = 13), ketospirilloxanthin (N = 14, NCar=C = 13, NC=C = 1) or diketospirilloxanthin (N = 15, NCar=C = 13, NC=C = 2).21,24 The relative contributions of NC=C and NC=C to energy transfer, various S0, S1, and S2 properties and effective conjugation length for long chain carotenoids such as diketospirilloxanthin has been discussed recently.24

Figure 1. Typical spheroidene/spheridenonene carotenoid biosynthesis pathway and the spirilloxanthin pathway incorporated in the mutant strains. The dashed squared boxes highlight the major carotenoids incorporated into the individual LH2 complexes (blue, the LH2s studied in detail in this research; cyan, the LH2s studied in detail previously24). The chemical structures of carotenoids assembled into the LH2 complexes; red color highlights carotenoid’s double bond conjugation, N.

The substantial variations in ΦCar−→BChl suggest (1) not all LH2 proteins have optimal interactions with the carotenoid, (2) not all carotenoids are equally suited to serve dual roles in LH2, or (3) a combination of the above. In the first case, factors by which differing amino acid sequences in the various bacterial species could modulate carotenoid-BChl a electronic communication include structural alteration of interpigment distance and orientation, or purely electronic environmental effects on carotenoid excited-state energy/lifetime, with the latter having electrostatic or specific (e.g., hydrogen-bonding) origin. In the second case, the key is the inherent excited-state properties of the carotenoid dictated by the number and arrangement of C=C and C═O bonds.

Challenges in pinpointing the contributions of the above-noted factors have been considerably ameliorated by recent advances that afford the ability to study a wide range of carotenoids in a common protein environment in a single bacterial species. In particular, genetic engineering of the carotenoid synthesis pathway of Rb. sphaeroides has provided LH2s that present a fixed polypeptide architecture and electrostatic milieu housing carotenoids that have N between 9 and 15.21,24 In these engineered strains, the native three-step phytoene desaturase (from the spheroidene synthesis pathway)25 along with additional mutations to other enzymes to truncate carotenoid synthesis at a certain conjugation length (Figure 1). Two studies21,24 that employ these strains have shown that the overall trend in ΦCar−→BChl...
with carotenoid conjugation length $N$ obtained previously using different bacterial species (with different amino acid sequences) is reproduced when employing the common Rb. sphaeroides protein scaffolding. The studies have also afforded detailed insights into trends in both ground- and excited-state carotenoid properties with increasing $N_{\text{Car}}$ versus $N_{\text{Chl}}$. One conclusion is that variations in $\Phi_{\text{Fus,max}}$ with carotenoid conjugation length in the various native (or mutant) bacterial species do not arise from substantial peptide-induced alterations in carotenoid properties or carotenoid-BChl $a$ interactions. Thus, future work can focus more tightly on the alterations in carotenoid properties or carotenoid-BChl $a$ interactions.

The studies reported herein complement and extend the prior work on the engineered Rb. sphaeroides strains. The static optical studies used to obtain $\Phi_{\text{Fus,max}}$ for LH2s containing modest-length carotenoids ($N = 9–11$) are joined by time-resolved studies of carotenoid excited-state dynamics employed for strains with the longer analogues ($N = 13–15$). Additionally, the main focus of the current work is examination, for the entire collection of strains spanning $N = 9–15$, of the excited-state properties of the LH2-bound carotenoids and the BChl $a$ molecules with which they interact. The latter properties include the fluorescence quantum yield and singlet excited-state lifetime of the BChl $a$ array B850. The results are compared with those from prior studies of LH2s that house the same or similar carotenoids within the different protein environments provided by the various bacterial species. Collectively the results extend fundamental insights into carotenoid function in photosynthetic antenna systems.

**METHODS AND MATERIALS**

**Rb. sphaeroides Strains and Growth Conditions.** The $\Delta$crtC, $\Delta$crtA, $\Delta$crtI::crtIPa$crtD$, and $\Delta$crtI::crtIPa$crtI::crtIPa$crtD$ strains of Rb. sphaeroides were constructed and, except in one case noted below, grown anaerobically (photosynthetically) as described by Chi et al. After reaching stationary stage the cultures were harvested and pelleted by centrifugation. The primary carotenoids produced by these strains are as follows:\(^{21,24}\) $\Delta$crtC (neurosperone; $N = 9$), $\Delta$crtA (spheroidene; $N = 10$), $\Delta$crtI::crtIPa$crtC$ (lycopene; $N = 11$), and $\Delta$crtI::crtIPa$crtD$ (rhodopin, $N = 11$), $\Delta$crtI::crtIPa$crtI::crtIPa$crtD$ when grown anaerobically (spirilloxanthin; $N = 13$, $N_{\text{Car}} = 13$, $N_{\text{Chl}} = 0$). When $\Delta$crtI::crtIPa$crtI::crtIPa$crtD$ is grown semiaerobically it gives a mixture of keto-carotenoids including 2-ketoanhydrorhopbodovin (5443 $N = 13$, $N_{\text{Car}} = 12$, $N_{\text{Chl}} = 1$), 2-ketoanhydrorhopbodovin (5443 $N = 14$, $N_{\text{Car}} = 13$, $N_{\text{Chl}} = 0$), and 2,2′-diketoanhydrorhopbodovin (5443 $N = 15$, $N_{\text{Car}} = 13$, $N_{\text{Chl}} = 0$).

**Isolation and Purification of LH2.** Pellets of bacterial cells were resuspended in 20 mM tris(hydroxymethyl)-amino-

methane (Tris) buffer at $pH = 8.0$. The membranes were isolated by ultracentrifugation and then pelleted by centrifugation. Subsequently, the pellet was resuspended in 20 mM Tris buffer ($pH = 8.0$) to $OD_{500} \approx 20$ (1 cm path) and mixed with lauryl dimethyamine-oxide (LDAO) to a final concentration of $\sim 0.5\%$ for 20 min at room temperature (RT). The insoluble material was pelleted by centrifugation. Further purification of the complexes was carried out using an anion exchange chromatographic column (Q Sepharose High Performance, GE Health-care) equilibrated with 20 mM Tris buffer ($pH = 8.0$) with 0.06% LDAO by applying 50 mM gradient steps of NaCl from 150 and 500 mM. The protein-containing fraction was eluted with 300–400 mM NaCl. For comparative purposes LH2 complexes were also purified according to protocol used by Chi et al.,\(^{21}\) in which the buffer is 4-(2-hydroxyethyl)-1-piperazine-

neethanesulfonic acid (HEPES) and the detergent is n-dodecyl $\beta$-maltoside (DDM).

**Steady-state and time-resolved fluorescence measurements** were performed on LH2s from both preparations and tabulated. However, LH2 complexes in the HEPES-DDM show modest variability in B800/B850 ratio (as shown in Chi et al.), perhaps due to slow oxidation of the B800 BChl $a$ that gives rise to modest differences in some photophysical properties. Thus, only results from the Tris-LDAO preparations are used in the figures and detailed discussion of excited-state properties. The only exception is the resonance Raman studies of the LH2-bound carotenoids because they are largely blind to chemical modifications of the B800 BChls.

**Steady-State and Femtosecond Transient Absorption Spectroscopy.** Time-resolved pump–probe absorption experiments were carried out using a Helios femtosecond transient absorption (TA) spectrometer (Ultrafast Systems LCC, Sarasota, FL) coupled to a Spectra-Physics femtosecond laser system described previously.\(^{25}\) Excitation wavelengths were preferentially set to excite the first vibronic band of the carotenoid absorption in each case: $\Delta$crtC (491 nm), $\Delta$crtA (511 nm), $\Delta$crtI::crtIPa$crtC$ (524 nm). The energy of the excitation beam was kept between 200 and 400 nJ for studies at RT, corresponding to an intensity of $\sim 0.5–1 \times 10^{14}$ photons/cm$^2$. For 77 K studies, in order to minimize permanent photobleaching of the sample, the energy of the pump was lowered to 100 nJ ($\sim 3 \times 10^{13}$ photons/cm$^2$).

All steady-state absorption measurements were performed using a Shimadzu UV-1800 spectrophotometer. The absorbance of the samples at the maximum of carotenoid absorption band was set between 0.1 and 0.5 in a 5 mm cuvette (depending on the availability of sample). For low-temperature measurements, the buffered-LH2 solution was mixed with glycerol in 1:1 (v/v) ratio, transferred to a 1 cm square plastic cuvette and frozen in a VNF-100 liquid nitrogen cryostat (Janis, Woburn, MA).

**Steady-State Fluorescence Spectroscopy.** Fluorescence and fluorescence–excitation spectra were recorded at RT using a Horiba-Spek Nanolog fluorometer. The spectra were recorded with detection at 90° to excitation and corrected for the instrument spectral response. The excitation and detection bandwidths were 2–4 nm. To avoid front-face and inner-filter effects, the samples were adjusted to an absorbance $\leq 0.1$ at the excitation and emission wavelengths. Fluorescence quantum yields ($\Phi_f$) were determined by comparing the integrated absorbance-corrected emission spectrum to that for free base tetraphenyl porphyrin ($\Phi_f = 0.09$ in degassed toluene and 0.07 in nondegassed toluene)\(^{26}\) excited at the same wavelength; data were acquired using excitation at the BChl $a$ Soret (375 nm) and Q$_\lambda$ (590 nm) bands and averaged. The efficiency of carotenoid-to-bacteriochlorophyll energy transfer ($\Phi_{\text{Fus,max}}$) was obtained from the ratio of the amplitudes of the carotenoid features in the B850 fluorescence–excitation spectrum to those at the same wavelengths in the absorbance spectrum after normalizing at the Q$_\lambda$ maximum ($\sim 850$ nm).

**Resonance Raman (RR) Spectroscopy.** The RR spectra were obtained at RT of the isolated carotenoids in tetrahydrofuran (THF) and of the LH2 complexes resuspended either in Tris-LDAO or HEPES-DDM solution as described below.
previously. Briefly, the RR spectra were acquired with a Spex 1877 triple spectograph (Horiba, Japan) equipped with holographically etched 1800 groove/mm gratings in the third stage and UV-enhanced charge-coupled device (EEV1152-UV; Princeton Instruments). The excitation wavelength of 532 nm was provided by Verdi-V6, diode-pumped solid-state laser (Coherent) with power set to 5–6 mW and a beam diameter of ∼0.5 mm. The scattered light was collected at 90° using a 50 mm f/1.4 Cannon camera lens. The spectra were acquired with 1–2 h of signal averaging (20 × 180 s to 40 × 180 s scans). The spectral resolution was ∼2 cm⁻¹ at a Raman shift of 200 cm⁻¹. The spectra data were calibrated using the known frequencies of indene.

Time-Resolved Fluorescence Spectroscopy. The time-correlated single photon counting (TCSPC) setup consisted of a stand-alone Simple-Tau 130 system (Becker&Hickl, Germany), equipped with the following: a PMC-100–20 detector (GaAs version) that has an instrument response function (IRF) with a full width at half-maximum (fwhm) of <200 ps, a PHD-400 high speed Si pin photodiode (as triggering module), a motorized Oriel Cornerstone 130 1/8 m monochromator (Newport) with manually controlled, micrometer-adjustable entrance and exit slits and ruled 1200 l/mm grating blazed at 750 nm (having spectral resolution of 21 nm with fully open entrance slit), and a manual filter wheel. Excitation pulses at 590 nm were produced by an Inspire100 ultrafast optical parametric oscillator (Spectra-Physics) pumped with Mai-Tai, an ultrafast Ti:sapphire laser (Spectra-Physics), generating ∼90 fs laser pulses at 820 nm with a frequency of 80 MHz. The frequency of the excitation beam was lowered to 8 MHz (125 ns between excitations) by a 3980 Pulse Selector equipped with a 3986 controller (Spectra-Physics). Isotropic excitation of the sample was achieved by depolarizing the excitation beam using an achromatic depolarizer (DPU-25, Thorlab). The intensity of excitation beam (focused to an ∼1 mm circular spot at the sample) was set to ∼10⁹ photons/cm² per pulse to ensure annihilation-free conditions. The signal was recorded at 90° to the excitation. The LH2 complexes were suspended in the appropriate buffer-detergent solution to an absorbance of ≤0.1 in the B850 band.

Data Processing and Fitting. Group velocity dispersion in the TA data sets was corrected using Surface Explorer Pro software provided by Ultrafast Systems by building a dispersion correction curve from a set of initial times of transient signals obtained from single wavelength fits of representative kinetics. Kinetic modeling (global analysis) of TA data was performed using CarpetView (Light Conversion Ltd., Vilnius, Lithuania). The instrument temporal response function was assumed to have a Gaussian-like shape with the fwhm of 150–250 fs and was used as a fixed parameter in the fitting procedure. The focus of the analysis was to obtain trends in the photophysical characteristics of the carotenoids and BChls for the various LH2s. Thus, a method commonly used for global analysis of TA data for LH2 complexes was employed in which the time evolution of the system is described by a sequential series of steps following photoexcitation. The spectral components thereby obtained are typically called evolution associated difference spectra (EADS). The EADS typically may not correspond to the spectra of the true transient states but provide useful information on the time evolution of the system.

## RESULTS

### Static Absorption Studies.

The RT and 77 K absorption spectra of the LH2 complexes from four (of six) *Rh. sphaeroides* strains are shown in Figure 2A, C, E, and G. At RT, the B800 peak position shows a small variation between 848 and 850 nm (Table 1). Upon lowering the temperature to 77 K, a bathochromic shift of ∼4 nm of the B850 band is seen in all cases (Table 1). The B800/B850 peak-intensity ratio varies between 0.7 and 0.8 at RT, a typical range for properly assembled LH2 complexes. Although the carotenoid absorption reveals a clear vibronic structure at RT, lowering the temperature to 77 K substantially enhances resolution of the vibronic bands. Temperature also affects the B800/B850 amplitude ratio, increasing to ∼1 for some complexes at 77 K.

A point relevant to analysis of carotenoid function in LH2 is whether binding to the protein affects the B800/B850 ratio. Generally, the B800/B850 ratio for RH sets from ∼0.7 to ∼0.8 at RT, as shown in Figure 2A, C, E, and G. At RT and at 77 K, the B800 peak position is at 800 nm in all of the complexes, while the B850 peak position shows a small variation between 848 and 850 nm (Table 1). Upon lowering the temperature to 77 K, a bathochromic shift of ∼4 nm of the B850 band is seen in all cases (Table 1). The B800/B850 peak-intensity ratio varies between 0.7 and 0.8 at RT, a typical range for properly assembled LH2 complexes. Although the carotenoid absorption reveals a clear vibronic structure at RT, lowering the temperature to 77 K substantially enhances resolution of the vibronic bands. Temperature also affects the B800/B850 amplitude ratio, increasing to ∼1 for some complexes at 77 K.
Table 1. Spectroscopic Properties of BChls (B850) and Primary Carotenoids in LH2 Complexes Studied Here and Previously

| Rh. sphaeroides strain (other species) | primary carotenoids (%)<sup>a</sup> | N<sup>b</sup> | N<sub>C=C</sub> | N<sub>C=O</sub> | Car S<sub>0</sub>(0→0)<sup>c</sup> (nm) | Car S<sub>2</sub>(0→0)<sup>c</sup> (nm) | B850 (nm) | B850 (nm) | τ<sub>2</sub> (ns)<sup>d</sup> | Φ<sub>F,exc</sub> (%) | Φ<sub>Car→B850</sub> (%) | ref |
|--------------------------------------|-----------------------------------|-----|----------|----------|----------------|----------------|----------|----------|----------------|----------------|----------------|-----|
| ΔcrtC                                | neurosporene (100)                | 9   | 9        | 0        | 492            | 493            | 847      | 853      | 1.33 (0.86) | 10.7 (9.7)  | 87              | this work |
| (G1C)                                | neurosporene (100)                | 9   | 9        | 0        | 492            | (494)          | 847      | (854)    | 1.38 (1.13) | 11.0 (8.5)  | 88              | this work |
| ΔcrtA                                | spheroidene (96)                 | 10  | 10       | 0        | 511            | 514            | 848      | 851      | 1.38          | 11.0          | 88              | this work |
| wild type                            | spheroidene (100)                | 10  | 10       | 0        | 511            | (515)          | 848      | (852)    | 1.38          | 11.0          | 88              | this work |
| ΔcrtI::crtI<sup>e</sup> ΔcrtC        | lycopene (91)                    | 11  | 11       | 0        | 524            | 527            | 848      | 854      | 1.1 (0.76)  | 8.5 (7.5)    | 66              | this work |
| (DPP240[pERW12])                    | lycopene                        | 11  | 11       | 0        | 526            |               |          |          | ~55          | 8.1 (6.8)    | 65              | this work |
| ΔcrtI::crtI<sup>e</sup> ΔcrtD        | rhodopin, lycopene (34), MeO/OH-lycopenes (36) | 11  | 11       | 0        | 524            | 529            | 849      | 856      | 1.1 (0.8)   | 8.1 (6.8)    | 65              | this work |
| (Rps. acidophila 10050)              | rhodopin glucoside (100)         | 11  | 11       | 0        | 524            | (529)          | 856      | (868)    | 1.0          | 8.1 (6.8)    | 65              | this work |
| ΔcrtI<sup>f</sup> (PS)<sup>j</sup>  | spirilloxanthin (71)             | 13  | 13       | 0        | 555            | 556            | 849      | 852      | 0.74 (0.71) | 4.8 (5.7)    | 41              | this work |
| ΔcrtI<sup>f</sup> (SA)<sup>k</sup>  | diketospirilloxanthin (62), Ketospirilloxanthin (16) | 15  | 13       | 2        | 849            | 853            | 849      | 853      | n.r.<sup>j</sup> | 42            | 24              | |

<sup>a</sup>From Chi et al.21<sup>b</sup>Total number of conjugated double bonds (N = N<sub>C=C</sub> + N<sub>C=O</sub>).<sup>c</sup>0→0) S<sub>0</sub> → S<sub>2</sub> absorption band.<sup>d</sup>B850 excited-state lifetime from fluorescence decay.<sup>e</sup>B850 fluorescence quantum yield.<sup>f</sup>Carotenoid-to-BChl <sub>a</sub> energy transfer efficiency from excitation vs absorptance (1−T) spectra.<sup>g</sup>Room temperature.<sup>h</sup>LDAO vs DDM preparations.<sup>i</sup>At 6 K.<sup>j</sup>Photosynthetically (anaerobically) grown.<sup>k</sup>Semiaserobically grown.<sup>l</sup>Not resolved.
solvent-dissolved carotenoid should give insights into potential structural differences. This point was explored as described in detail below.

The pigment complement of each LH2 complex studied here was analyzed previously. The carotenoid content in most cases was found to be relatively simple and consist primarily of a single carotenoid (Table 1). The ΔcrtI::crtP crtD LH2 is more complex and contains several carotenoids with similar absorption spectra; these carotenoids have the same number of conjugated C=C bonds (Ncut = 11; Table 1). Consequently, if the protein does not distort the carotenoid backbone, the carotenoid absorption profile in LH2 should be mimicked by the (the sum of) absorption spectra of individual carotenoids in solution. The spectra should only differ by a general wavelength shift due to the higher polarizability of protein binding pocket. Such a situation is demonstrated in Figure 3 for representative LH2 samples. The expected absorption spectrum of the LH2-bound carotenoid was obtained by subtraction of the projected spectra in the LH2s. The expected absorption spectrum of the LH2-bound BChl has a maximum in the range 856−862 nm.21

**Figure 3.** Reconstruction of the carotenoid absorption band in the absorption spectra of the LH2 complexes. The black line represents the LH2 absorption; the blue line is the carotenoid absorption spectrum taken in acetonitrile/THF solvent mixture; the green dashed line is an anticipated absorption spectrum of a carotenoidless LH2.24 The red profiles are predicted absorption spectra of the carotenoids bound into the LH2s.

The above results show that the energy of the ν₁ mode is indeed a linear function of 1/N in both solvent and protein environments; moreover, the lines for the carotenoids in the protein and organic solvent are parallel. This finding implies that the energetic shift of the ν₁ mode is the same for all the carotenoids and thus, most likely results from the effect of increasing medium polarizability. Therefore, upon binding to the protein, the effective conjugation of all the carotenoids remains unchanged from organic media, otherwise the two linear functions would not be parallel.37

**Static Fluorescence and Fluorescence−Excitation Studies.** The fluorescence spectra of each _Rh. sphaeroides_ LH2 (in Tris-LDAO) was measured using excitation in the BChl Soret band (385 nm) and Qₐ band (590 nm). The fluorescence spectra are shown in Figure 2. Emission is observed predominantly from the BChl a array B850 with a maximum in the range 856−862 nm. The spectra are basically the same as those found previously for these LH2s in HEPES-DDM.21 The fluorescence−excitation spectra (Figure 2) were obtained upon monitoring emission on the long-wavelength side of the B850 fluorescence band. These spectra are accompanied by 1−T spectra and normalized at the ~850 nm maximum, a wavelength for which the B850 BChls are excited directly and exclusively. Comparison of the amplitudes of the normalized fluorescence−excitation spectra and 1−T spectra in the region of the carotenoid absorption gives a measure of Φ_{Car−BChl} for each LH2. It is evident from Figure 2 that Φ_{Car−BChl} decreases as the double-bond conjugation length (N) of the carotenoid increases. This finding is in agreement with previous results for the same or similar carotenoids in LH2
from various bacterial strains (Table 1).10,19−21,24,39,40 The effect has been attributed to two factors: a reduced carotenoid- BChl a spectral (energy) overlap and enhanced internal conversion of the excited carotenoid to the ground state. With increase of carotenoid conjugation, the relevant excited states become closer in energy to the ground state and internal conversion becomes progressively more rapid (in agreement with energy gap law for nonradiative decay) and at some point may become competitive with energy transfer. The quantum yield of fluorescence (Φf) from excited B850 (B850*) was also measured for each Rb. sphaeroides LH2 at RT. Samples were excited in the Soret (375 nm) and Qx (590 nm) bands and the values averaged (Table 1). The Φf values of 10.7−11.0% for strains ∆crtC and ∆crtA that incorporate neurosporene (N = 9) and spheroidene (N = 10), respectively, are comparable to the Φf = 9.9% reported for Rb. sphaeroides WT,31 which appears to be the sole value for an LH2 in the literature. The Φf values drop to 8.1−8.5% for the strains (∆crtI::crtI PA∆crtC and ∆crtI::crtI PA∆crtD) that produce primarily lycopene and/or rhodopin (N = 11). The values drop further still to 5.5% for strain ∆crtI::crtI PA grown photosynthetically that produces primarily spirilloxanthin (N = 13) and to 4.8% for the same strain grown semiaerobically with incorporation of significant diketospirilloxanthin (N = 15). Thus, increasing conjugation length of the carotenoid not only reduces the efficiency of energy transfer from carotenoid to B850 (ΦCar→BChl), but also a property (Φf) of the B850* excited state that is produced. The next section describes further exploration of both facets of LH2 functionality via time-resolved studies.

**TA Studies.** Femtosecond-time-resolved absorption studies were performed to obtain more detailed information on the
carotenoid $S_1 (2^1A_g^−)$ excited state, particularly its lifetime in the various LH2s (versus in organic media). Such information provides additional information on carotenoid-to-BChl $a$ energy transfer.

The left column of Figure 5 shows the TA spectra of the LH2s taken at different delay times in the VIS spectral range at RT. The samples were excited into the carotenoid (0–0) vibronic band (Table 1). The early time 100 fs TA spectra show characteristic features associated with the $S_1 (1^1B_u^+)$ state of the carotenoid—bleaching of the ground-state absorption band (GSB), appearing simultaneously with a weak $S_2 (1^1B_u^+)$ → $S_0 (1^1A_g^−)$ stimulated emission.

A couple hundred of femtoseconds after the initial appearance of the GSB, an excited-state absorption (ESA) of the carotenoid $S_1 (2^1A_g^−)$ state emerges. It is visible as a pronounced feature with maximum at 540 nm for $\Delta$ crtC, 557 nm for $\Delta$ crtA, 580 nm for $\Delta$ crtI::crtIPa $\Delta$ crtC and at 581 nm for $\Delta$ crtI::crtIPacrtD. The $S_1 (2^1A_g^−)$ → $S_0$ ESA band disappears within a few picoseconds, seemingly more quickly in the LH2s that contain carotenoids with shorter $C=\pi C$ conjugation, such as neurosporene and spheroidene. Subsequent to the decay of the $S_1 (2^1A_g^−)$ → $S_0$ ESA band, the remaining spectral features associated with the carotenoids show some characteristics resembling those of the so-called carotenoid $S^*$ state, previously suggested to be a precursor of the carotenoid triplet state. Lowering the temperature to 77 K impacts the TA spectra of the LH2-bound carotenoids in several ways. In all cases except $\Delta$ crtC, the position of the $S_1 (2^1A_g^−)$ → $S_0$ ESA band shifts bathochromically by 3–6 nm and its amplitude relative to the GSB feature decreases.

The temporal characteristics of carotenoid (and BChl $a$) excited states were investigated by applying global analysis of the TA data sets. As described in the Methods and Materials section, the data were fit assuming a sequential kinetic model for the excited-state evolution to give EADS. Although EADS are typically not those of the true states (species) involved the spectral evolution and EADS associated with the $S_1 (1^1A_g^−)$ excited state can be easily recognized as is dominated by the $S_1 (1^1A_g^−)$ → $S_0$ ESA band. This type of global analysis was applied to the data sets depicted in Figure 5. The EADS from analysis of RT TA data are given in Figure 6. The left column shows EADS for each LH2, the right column shows corresponding EADS concentrations, matched by the same time delays.

Figure 5. TA spectra of the LH2 complexes in the VIS spectral range upon excitation at their carotenoid band. The spectra were recorded at various delay times after excitation and taken at RT (left column) and at 77 K (right column).

Figure 6. Global analysis results of the RT TA data sets given in Figure 5. The fitting applied a sequential model of the excitation decay path and gives EADS with the corresponding decay lifetimes (left column). The contribution of a particular EADS in the raw TA spectrum at a certain delay time is given by the EADS concentration (right column).
color. The fitting required either 5 or 6 EADS to obtain satisfactory fits.

The first EADS with the lifetime marked as <IRF (shorter than the fwhm of the IRF) corresponds to the initial GSB and the $S_1(1^1B_{g}^\text{ff}\,\text{ff}) \rightarrow S_1(1^1A_{g}^\text{ff})$ stimulated emission, appearing up to 650 nm. The EADS time constant corresponds to the lifetime of the $S_1(1^1B_{g}^\text{ff})$ state, but the value is obscured by both the instrument time resolution and interference from strong solvent/buffer contribution (stimulated Raman bands, etc.). The EADS with 280–340 fs time constant, which is not observed in the LH2s with spheroidene and neurosporene, shows spectral and temporal characteristics of the ESA from a vibrationally nonequilibrated $S_1(2^2A_{u}^\text{ff})$ state, commonly called a hot $S_1$. The EADS with a lifetime ranging from about 1.2 to 3.4 ps is associated with decay of the vibrationally equilibrated $S_1(2^2A_{u}^\text{ff})$ state. The spectral profile of this EADS is dominated by the carotenoid $S_1(2^2A_{u}^\text{ff}) \rightarrow S_\text{sol} \text{ ESA band}$. The EADS with a lifetime of 12–25 ps has been previously assigned to the enigmatic carotenoid $S^*\text{ state}$. The next EADS with lifetime of 870–1200 ps is associated with excited state of the B850 BChls (B850*). This contribution results mostly from bleaching of the weak BChl $a_{Q\text{b}}$ band at ~590 nm; because the amplitude is small, the time constant should be considered as only a rough measure of the B850* lifetime. The latter has been investigated in detail by monitoring the B850* fluorescence decay via TCSPC (see Table 1 and results below). The EADS with infinite lifetime corresponds to a very small signal in the region of carotenoid absorption and is most likely associated with carotenoid triplet state.

Global analysis results of the TA data sets obtained at 77 K are shown in Figure 7. The analysis does not reveal kinetic components associated with the hot $S_1$ state for the LH2 complexes from ΔcrtI::crtIPa strains. These issues are discussed in more detail below. The lifetime of the $S_1(2^2A_{u}^\text{ff})$ state relative to that of B850* (and B800*) regulates the rate and yield of energy transfer from the former to produce the latter. The lifetime of the $S_1(2^2A_{u}^\text{ff})$ state, which is sensitive to the rate and yield of energy flow to the BChls, is essentially the same in LH2s from various bacteria housing the same carotenoid, including the engineered Rb. sphaeroides strains studied here. These conclusions are supported by the $\Phi_{\text{Car} \rightarrow \text{BChl}}$ values across the LH2s, which measure the extent of energy transfer to B850 from both the carotenoid $S_1(2^2A_{u}^\text{ff})$ and $S_2(1^1B_{g}^\text{ff})$ states. Therefore, differences in polypeptide properties are not a significant contributor to regulation of energy flow from carotenoid to the BChls in LH2 complexes.

Detailed knowledge about the $S_1(2^2A_{u}^\text{ff})$ excited state lifetime of each carotenoid in solvent and in the LH2 complex (Table 2) allows the efficiency of energy transfer to B850 from $S_1(2^2A_{u}^\text{ff})$ to be estimated from eq 1.

$$\text{Eff}_{\text{Car} \rightarrow \text{B850}} = \left(1 - \frac{\tau_{\text{Sol}}}{\tau_{S_1}}\right) \times 100$$

Figure 7. Global analysis results of 77 K TA data sets given in Figure 5. The fitting applied a sequential model of excitation decay path and gives EADS with corresponding decay lifetimes (left column). The contribution of a particular EADS in the raw TA spectrum at a certain delay time is given by the EADS concentration (right column).

Various bacterial species that bind longer-conjugation carotenoids, is underpinned by the electronic properties of the carotenoid. Specifically, the energy of the carotenoid $S_1(2^2A_{u}^\text{ff})$ state relative to that of B850* (and B800*) regulates the rate and yield of energy transfer from the former to produce the latter. The efficiency of energy transfer to B850 from both the carotenoid $S_1(2^2A_{u}^\text{ff})$ and $S_2(1^1B_{g}^\text{ff})$ states. Therefore, differences in polypeptide properties are not a significant contributor to regulation of energy flow from carotenoid to the BChls in LH2 complexes.
determination of S2 (11Bu state to energy transfer is deduced using the assumption

Here \( \tau_{\text{B850}} \) and \( \tau_{\text{S1}} \) are the lifetimes of the S1 (21Ag state in the LH2 and in organic solvent, respectively. Because temporal resolution of the TA measurements precludes precise determination of S1 (1B\text{n}) state lifetime, the contribution of this state to energy transfer is deduced using the assumption

that the overall yield of energy transfer from carotenoid to B850 (\( \Phi_{\text{Car} \rightarrow \text{B850}} \)) is given by the experimental value obtained from comparison of absorbance (1−T) and fluorescence excitation spectra and that this is equal to the yield obtained from analysis of carotenoid excited-state lifetime data. The overall \( \Phi_{\text{Car} \rightarrow \text{B850}} \) is given by eq 2.

\[
\Phi_{\text{Car} \rightarrow \text{B850}} = \Phi_{\text{Car} \rightarrow \text{S2} \rightarrow \text{B850}} + (100 - \Phi_{\text{Car} \rightarrow \text{S2} \rightarrow \text{B850}} - \Phi_{\text{Car} \rightarrow \text{S}^* \rightarrow \text{B850}}) \times \frac{\text{Eff}_{\text{CarS1} \rightarrow \text{B850}}}{100}
\]

In eq 2, all values are given as percent, and the term in parentheses reflects the fraction of the S2 (1B\text{n}) state that decays to the S1 (21Ag) state. Within that term, \( \Phi_{\text{Car} \rightarrow \text{S}^* \rightarrow \text{S}^*} \) is yield of formation of so-called S* state from the S2 (1B\text{n}) state. It is generally accepted that the S* state formation appears to be similar across all studied LH2 complexes studied here. The value of S* state formation is assumed to be 10% in all cases on the basis of the ratio of bleaching of the ground-state absorption associated with the S* TA signal to the overall bleaching observed immediately after the excitation flash.

The results of this analysis, provided in Table 3, show that actual contribution of the S1 (21Ag) state in the overall

| carotenoid  | \( N^a \) | \( N_{C=C} \) | \( N_{C=O} \) | environment | \( S_1 \) (cm⁻¹) [nm] | \( \tau_{S1} \) (ps) | \( \tau_{S2} \) (ps) | \( \Delta \tau_{S2} \) (ps) | \( \Delta \tau_{C} \) (ps) | \( \Delta \tau_{D} \) (ps) | reference |
|-----------|------|------|------|-------------|-----------------|----------------|----------------|----------------|----------------|----------------|--------|
| neurosporene | 9 | 9 | 0 | v.s. | 14 170−14 400 [695−706] | 21−24 | 34.8 | 45 | 1.2 | 1.2 | n.e. | 44, 45 |
| spheroidene | 10 | 10 | 0 | v.s. | 13 400 [746] | 7.7−9.3 | 11.5 | 45 | 1.4 | 1.6 | n.e. | 44, 44, 49 |
| lycopene | 11 | 11 | 0 | v.s. | 12 500 [800] | 4−4.7 | 3.4 | 5.9 | 3.2 | | | 48, 52 |
| rhodopin | 11 | 11 | 0 | benzene | 12 450 [803] | 2.9 | 3.2 | 5.9 | 3.4 | | | |
| spirilloxanthin | 13 | 13 | 0 | v.s. | 11 350−11 500 [870−881] | 1.3−1.7 | 2.0 | | 3.3 | | | 45, 53, 54 |
| diketospirilloxanthin | 15 | 13 | 2 | v.s. | 11 000−11 100 [900−909] | 0.8 | 1.2 | | 1.1 | 1.8 | 2.6 | |

\( ^a \text{Total number of conjugated double bonds (} N = N_{C=C} + N_{C=O}. ^b \text{Energy value defined via measuring } S_1 \rightarrow S_2 \text{ energy gap. ^c Carotenoid } S_1 (21Ag) \text{ state lifetime, ^d B850-to-carotenoid time constant (inverse rate constant) in LH2 assuming that unquenched B850 lifetime is 1.35 ns. ^e Room temperature. ^f Various solvents. ^g Rb. sphaeroides strain. ^h Not evident. } \)

Figure 8. Temporal characteristics of the B850 BChl fluorescence in the LH2 complexes from six Rh. sphaeroides strains: Fluorescence decay curves recorded under annihilation free condition after excitation at 590 nm at RT; IRF, instrument response function.
Table 3. Carotenoid $S_1 (2^1A_g)$ State Lifetimes and Contributions of Carotenoid Excited States to Energy Transfer to BChl $a$

| carotenoid      | $N^b$ | $N_{C=C}$ | $N_{C=O}$ | $r_{53}$ (ps) | $E_{fla} S_1 \rightarrow B850$ (%)$^d$ | $\Phi_{fla} S_1 \rightarrow B850$ (%)$^e$ | $\Phi_{fla} S_2 \rightarrow B850$ (%)$^f$ | $\Phi_{fla} \rightarrow B850$ (%)$^g$ |
|-----------------|-------|-----------|-----------|-------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| neurosporene    | 9     | 9         | 0         | 22.5        | 1.2                                     | 95                                     | 27                                     | 60                                     | 87                                     |
| spheroidene     | 10    | 10        | 0         | 8           | 1.4                                     | 84                                     | 18                                     | 70                                     | 88                                     |
| lycopene        | 11    | 11        | 0         | 4.4         | 3.4                                     | 23                                     | 7                                      | 59                                     | 66                                     |
| rhodopin        | 11    | 11        | 0         | 3.3         | 2.9                                     | 12                                     | 3                                     | 62                                     | 65                                     |
| spirilloxanthin | 13    | 13        | 0         | 1.5         | 1.4                                     | 10                                     | 0                                     | 46                                     | 46                                     |
| diketospirilloxanthin | 15 | 13       | 2         | 0.8         | 1.1                                     | 0                                     | 0                                     | 41                                     | 41                                     |

“Total number of conjugated double bonds ($N = N_{C=C} + N_{C=O}$). $^b$ $S_1 (2^1A_g)$ state lifetime in solvent calculated as midrange of literature values (RT) given in Table 2. $^c$ $S_1 (2^1A_g)$ state lifetime in LH2 at RT obtained in this work. $^d$ Carotenoid-to-BChl $a$ energy transfer efficiency for $S_1 (2^1A_g)$ obtained from difference between lifetime in solvent and in LH2; this value is the percentage of $S_1 (2^1A_g)$ state produced from $S_0 (1^1B_{2g})$ that gives energy transfer to B850 calculated via eq 1. $^e$ The quantum yield of energy transfer from $S_1 (2^1A_g)$ to B850; this is the actual contribution of carotenoid $S_1 (2^1A_g)$ state to the overall energy transfer (per photon absorbed) to produce the $S_0 (1^1B_{2g})$ state obtained using eq 2. $^f$ Quantum yield of energy transfer from the carotenoid $S_1 (1^1B_{2g})$ state to B850; this value is the actual contribution of the carotenoid $S_1 (1^1B_{2g})$ state to overall energy transfer. $^g$ Overall carotenoid-to-BChl $a$ energy transfer yield obtained from comparison of absorbance $(1−T)$ and fluorescence excitation spectra.

$\Phi_{fla} \rightarrow B850$ is actually not the dominant factor even for carotenoids such as neurosporene for which the $\Phi_{fla} S_1 \rightarrow B850$ pathway is very efficient in that it comprises a substantial fraction of the $S_1 (2^1A_g)$ state decay.

The steady-state fluorescence studies reveal trends in the yield of B850 fluorescence yield. This point is shown in Figure 9A, which reveals a nominally linear relationship (red dashed line) of $\Phi_{fla}$ and $\Phi_{fla} \rightarrow B850$ (Table 1). At first glance, one might not expect a connection between these two quantities because the $\Phi_{fla}$ values were obtained using direct excitation of B850 BChl array (and the B800 BChls) in the $Q_b$ band and thus should not be affected by the yield of energy transfer from carotenoid to BChl $a$. Rather one might have expected $\Phi_{fla}$ to remain at the $\sim 11%$ level found for the short-chain carotenoids neurosporene and spheroidene (Figure 9A black dashed line).

The results in Figure 9A suggest that the B850 $\Phi_{fla}$ is sensitive to one or more carotenoid characteristics that also affect energy transfer to B850. One such property is energy of the carotenoid $S_1 (2^1A_g)$ excited state (Table 2). Figure 9B shows the relationship between this energy and the B850 $\Phi_{fla}$. The $\Phi_{fla}$ value drops as carotenoid $S_1 (2^1A_g)$ state falls from $>13,000$ (spheroidene, neurosporene) to $\sim 12,500$ cm$^{-1}$ (rhodopin, lycopene) and further still for the two longer-chain carotenoids as the energy descends to the $\sim 11,100$ cm$^{-1}$ level for diketospirilloxanthin.

The results in Figure 9A and B suggest that (1) photons absorbed by B850 or B800 in the upper excited states (e.g., $Q_b$ band) do not result in quantitative formation of the lowest ($Q_a$) excited state of the BChl $a$ array (B850*); because of rapid competing energy transfer to the carotenoid and/or (2) B850* itself is quenched by energy transfer to the carotenoid. The latter quenching contribution also would be expected to give rise to a reduction in the B850* excited-state lifetime. In this connection, Table 2 and Figure 9C show that the B850* lifetime is $\sim 1.3$ ns for the LH2s with short-chain carotenoids neurosporene ($N = 9$) and spheroidene ($N = 10$) and decreases substantially to $\sim 0.75$ ns for the LH2 containing the much longer diketospirilloxanthin ($N = 15$). The data depicted in Figure 9C suggest that quenching of B850* by diketospirilloxanthin is reasonable because the $S_1 (2^1A_g)$ excited-state energy of this carotenoid ($11,100$ cm$^{-1}$) has dropped below that of B850* ($11,760$ cm$^{-1}$). The same is true for spirilloxanthin, for which the $S_1 (2^1A_g)$ energy is $11,300$ cm$^{-1}$ and the B850* lifetime is $\sim 0.9$ ns. What is surprising is that there is a modest B850* reduction to $\sim 1.1$ ns for lycopene and rhodopin, which have an $S_1 (2^1A_g)$ energy of $\sim 12,500$ cm$^{-1}$. The latter energy is near that of B800 and the suspected position of the “upper” exciton level of B850* that carries little oscillator strength, the bulk of which is in the “lower” component that gives rise to the B850 band. This upper state has been proposed to serve as an energetic “bridge” for energy flow from the B800 to B850 sets of BChl $a$ molecules.

Interestingly, the trends in excited-state lifetime (Figure 9C) and $\Phi_{fla}$ (Figure 9A) roughly parallel each other in that in both cases values for lycopene and rhodopin are intermediate between spheroidene (plus neurosporene) and diketospirilloxanthin, with spirilloxanthin slightly above the diketo-analogue. Such trends cannot be explained by thermal repopulation of the upper excited states (e.g., the upper exciton level of B850*), which should be negligible even at room temperature. Energy flow from the upper exciton states of B850 (or B800) to carotenoid could contribute to the $\Phi_{fla}$ reduction trend if sufficiently fast (despite apparent poor dipole–dipole coupling) to compete with relaxation to B850*, but to first order would not contribute to the B850* lifetime reduction because the two exciton levels are normally considered to be discrete states. One possible explanation for these observations is that the B850 and carotenoid excited-state manifolds are tightly coupled and that altering the carotenoid alters the excited-state mixing and thereby affects the B850* photophysical properties more so than might meet the eye from the optical spectra. Another explanation is that carotenoid $S_1 (2^1A_g)$ state of energies lie lower than placed by comparison of wavelength of the $S_1 (2^1A_g) \rightarrow S_0 (1^1B_{2g})$ TA feature and the wavelength (energy) of the $S_0 (1^1A_{2g}) \rightarrow S_0 (1^1B_{2g})$ ground-state absorption band. The accuracy of that method has been debated, as $S_1 (2^1A_g)$ energies obtained via this TA technique are often somewhat lower than placed by comparison of wavelength of the $S_1 (2^1A_g) \rightarrow S_0 (1^1B_{2g})$ TA feature and the wavelength (energy) of the $S_0 (1^1A_{2g}) \rightarrow S_0 (1^1B_{2g})$ ground-state absorption band. The accuracy of that method has been debated, as $S_1 (2^1A_g)$ energies obtained via this TA technique are often somewhat lower than placed by comparison of wavelength of the $S_1 (2^1A_g) \rightarrow S_0 (1^1B_{2g})$ TA feature and the wavelength (energy) of the $S_0 (1^1A_{2g}) \rightarrow S_0 (1^1B_{2g})$ ground-state absorption band. Regardless, such considerations do not diminish the fact that the longest carotenoids, because of their low $S_1 (2^1A_g)$ energies, quench B850*.

In our previous study of two representatives of the engineered *Rhodobacter sphaeroides* LH2 family — those incorporating carotenoids with long ($N = 13–15$) conjugation lengths — we speculated that a direct quenching of the B850* by carotenoids such as spirilloxanthin and diketospirilloxanthin may occur and seems energetically favorable. However, the B850* lifetimes in that study were obtained
from TA measurements. In those measurements like those described here on the set of Rh. sphaeroides LH2s (in either Tris-LDAO or HEPES-DDM), the B850* lifetime for the longest carotenoids (N = 13–15) are consistently somewhat under 1 ns and those for the shortest carotenoids (N = 9–10) are consistently somewhat over 1 ns. However, it has been uncertain whether such variations in B850* lifetimes obtained from such TA data indeed reflects carotenoid quenching or arises from differences in time-resolved measurements (e.g., differing contributions of excited-state annihilation) and/or from unforeseen aspects of this unique set of LH2s. The comprehensive studies reported herein have employed the least invasive excited-state monitoring techniques using fluorescence decay with weak excitation flashes. The time-resolved measurements have been complemented by static B850* fluorescence-yield studies (that use weaker excitation) that are sparse in the LH2 literature and that afford the same trends as the B850* lifetime studies (Table 2). These collective measurements demonstrate that shortening of the B850* lifetime with longer-chain carotenoids is real and most likely arises from direct quenching of the excited state of the B850 BChl a array by carotenoid.

Although direct quenching of BChl a singlet excited states by carotenoids in protein environments has not been reported previously to our knowledge, such carotenoid-mediated quenching via excitation energy transfer has been seen for the related pigment chlorophyll a in representative photosynthetic protein complexes from higher plants such as LHCII59 or from cyanobacteria such as IsiA60 and in the Hlip proteins.63,64 Quenching by excited-state mixing has been suggested in Chl a light-harvesting protein as LHClI65 and in synthetic carotenoid-tetrapyrrrole dyads.66,67 It should also be emphasized that direct quenching of B850* by carotenoids in LH2 systems cannot be monitored by recording of the rise and decay of the carotenoid ESA band associated with the S1 (2A1g−) state that serves as the energy quencher, as was possible for the cyanobacterial HliD protein.59,64 This is so because the effective concentration of carotenoids in the S1 (2A1g−) state depends on the relative rates of the formation (population) and decay of this excited state. The formation is driven by the B850* quenching rate constant (kq, Table 2), which is quite small (the process is slow) compared to the extremely large (rapid) S1 (2A1g−) decay. Consequently, the effective concentration of excited carotenoids will be extremely small at a given time in LH2.

**CONCLUSIONS**

There are two principal conclusions from this study. First, the essential factors that determine electronic communication and thus energy flow between carotenoid and BChl a molecules in LH2 complexes are the inherent electronic properties of the carotenoid; polypeptide properties that vary from strain to strain are far less important in regulation of energy transfer between the carotenoids and BChl a molecules. Second, if the carotenoid possesses π-electron conjugation that is long enough to afford an S1 (2A1g−) energy comparable to or lower than the S1 energy of excited BChl-a array B850*, quenching of B850* by the carotenoid occurs. In this regard, the S1 (2A1g−) energy of the longer chain carotenoids bearing keto groups (e.g., ketospiorilloxanthin and diketospiorilloxanthin), is sufficiently low due to the large NCar-C (plus Ncar-car) that effects of the protein involving the keto groups will have little consequence.

**ASSOCIATED CONTENT**

Supporting Information

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Full references for references with >10 authors (PDF)

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Notes

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