Chapter 12

Energy Transfer from Carotenoids to Bacteriochlorophylls

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Summary

The photosynthetic apparatus contains light-harvesting (LH) pigment-protein complexes that capture light energy from the sun and transfer it efficiently to the reaction center. In photosynthetic bacteria, carotenoids supplement the non-optimal LH capacity of bacteriochlorophyll (BChl) in the 400–500 nm region of the visible spectrum. Thus, carotenoid-to-BChl energy transfer provides an essential process for enhancing the ability of these systems to capture light energy and convert it into useful work. Carotenoids have at least two states involved in energy transfer to BChl. These are the S_2 state into which absorption from the ground state, S_0, is strongly allowed, and a low-lying, S_1 state into which absorption is forbidden by symmetry. These two states represent the primary energy donors for carotenoid-to-BChl energy transfer. The S_2 state transfers energy with an efficiency between 30 and 70%, the value of which is only slightly dependent on the structure of the carotenoid. The S_1-mediated energy transfer pathway depends strongly on the π-electron conjugation length of the carotenoid. This route is essentially closed for carotenoids with eleven or more conjugated carbon-carbon double bonds because in these cases the S_1 energy of the carotenoid lies too low to enable transfer to BChl.

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Besides the main S_2 and S_1 pathways, the past few years of investigations have raised the prospect of other carotenoid excited states participating in energy transfer. The possibilities include vibrationally hot S_1 states, a state denoted S* thought to be formed by a branched deactivation pathway from S_2, and a state with symmetry representation $1B_u^-$ predicted on the basis of theoretical computations to lie between S_1 and S_2. This chapter reviews the evidence for these states and discusses their possible involvement as energy donors in the process of light-harvesting in photosynthetic bacteria.

I. Introduction

Carotenoids in antenna complexes of purple bacteria have multiple functions. Besides their key roles in photoprotection and structure stabilization, purple bacteria utilize carotenoids as efficient LH agents covering the 400–550 nm spectral region. This part of the spectrum is of crucial importance, because it matches the maximum of the solar irradiance curve at the Earth's surface, and it is where the main photosynthetic pigment of purple bacteria, BChl *a*, has very low absorption (Fig. 1). Purple bacterial antenna systems are ideal for studying carotenoids as LH pigments in photosynthetic pigment-protein complexes. This is because detailed structural knowledge exists and provides an ideal platform for experimental and theoretical investigations of mechanisms and pathways of energy transfer between carotenoids and BChl.

To date, the structures of a few LH complexes have been reported. In 1995, the 2.5 Å structure of an outer antenna complex called LH2 from *Rhodopseudomonas* (*Rps.*) *acidophila* containing the carotenoid rhodopin glucoside (McDermott et al., 1995) became a landmark study of purple bacterial antenna complexes. A 2.4 Å structure of the LH2 complex from *Rhodospirillum* (*Rsp.*) *molischianum* containing lycopene followed shortly after (Koepke et al., 1996). Subsequently, the structure of the related complex LH3 (McLuskey et al., 2001) was reported, as well as the structure of the LH1 antenna complex surrounding the reaction center (Roszsak et al., 2003). These studies revealed the common theme of a ring-shaped protein motif containing either two (LH1) or three (LH2, LH3) BChl *a* molecules, and one carotenoid molecule per fundamental unit (Gall et al., 2006). The BChl *a* molecules in LH2 are arranged in two layers. One consists of two strongly coupled BChl *a* molecules absorbing at 850 nm (B850). The second is a monomeric BChl *a* absorbing at 800 nm (B800). For other complexes, the strongly-coupled BChl *a* absorb at different wavelengths, giving rise to a B875 band in LH1 or a B820 band in the LH3 complex. The monomeric BChl *a* molecule absorbs at 800 nm in all complexes except LH1 which lacks this BChl *a* completely. The carotenoids are in close contact with both types of BChl *a* molecules allowing them to serve as efficient energy donors in the light-harvesting process.

The detailed knowledge about the arrangement of pigments in purple bacterial antenna complexes provided a structural basis for both experimental and theoretical studies of energy transfer between carotenoids and BChl *a*. The studies of carotenoid to BChl *a* energy transfer are also facilitated by the fact that many antenna complexes from purple bacteria accumulate only one major carotenoid. However, different species of purple bacteria utilize distinct ca-

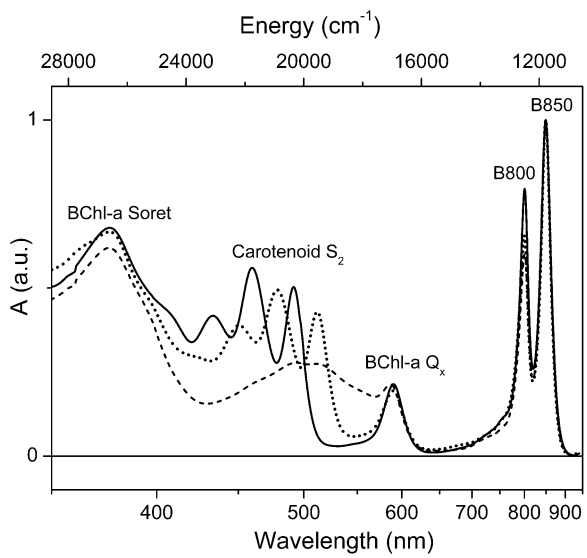

Fig. 1. Absorption spectra of LH2 complexes from *Rba. sphaeroides* containing carotenoids with different conjugation lengths: neurosporene, N = 9 (solid); spheroidene, N = 10 (dotted); spheroidenone N = 10 + C=O (dashed). All spectra are normalized to the maximum of the B850 band.

Abbreviations: BChl *a* – bacteriochlorophyll *a*; LDS – lithium dodecyl sulfate; CD – circular dichroism; LH – light harvesting; RC – reaction center

rotenoid types in their antenna complexes (Fig. 2). It was known long before X-ray structures were resolved that the structure of a particular carotenoid affects its ability to carry out energy transfer to BChl *a*. Using fluorescence excitation spectroscopy, carotenoid-to-BChl energy transfer efficiencies of 80–100% have been reported for *Rhodobacter* (*Rba.*) *sphaeroides* containing spheroidene (Cogdell et al., 1981; van Grondelle et al., 1982; Angerhofer et al., 1995), while values between 35–70% have been obtained for *Rps. acidophila* containing rhodopin glucoside (Angerhofer et al., 1986, 1995; Chadwick et al., 1987). The carotenoid-BChl energy transfer yield drops to ~30% for LH1 of *Rsp. rubrum* containing spirilloxanthin (Rademaker et al., 1980). These values were later confirmed and/or refined by a variety of time-resolved spectroscopic techniques that enabled the pathways of energy transfer between carotenoids and BChl *a* to be followed. Despite the fact that this topic has been studied extensively for more than ten years, and summaries of the achievements obtained during this period can be found in specialized reviews (Frank and Cogdell, 1996; Ritz et al., 2000; Fraser et al., 2001; Polívka and Sundström, 2004), the details concerning the mechanisms, pathways, participating donor and acceptor states and the molecular factors controlling energy transfer are still a matter of considerable debate. This is mainly because the excited state properties of carotenoids in solution, which serve as models for interpreting the data obtained from carotenoid excited-state dynamics in purple bacterial antenna complexes, are still not sufficiently well known.

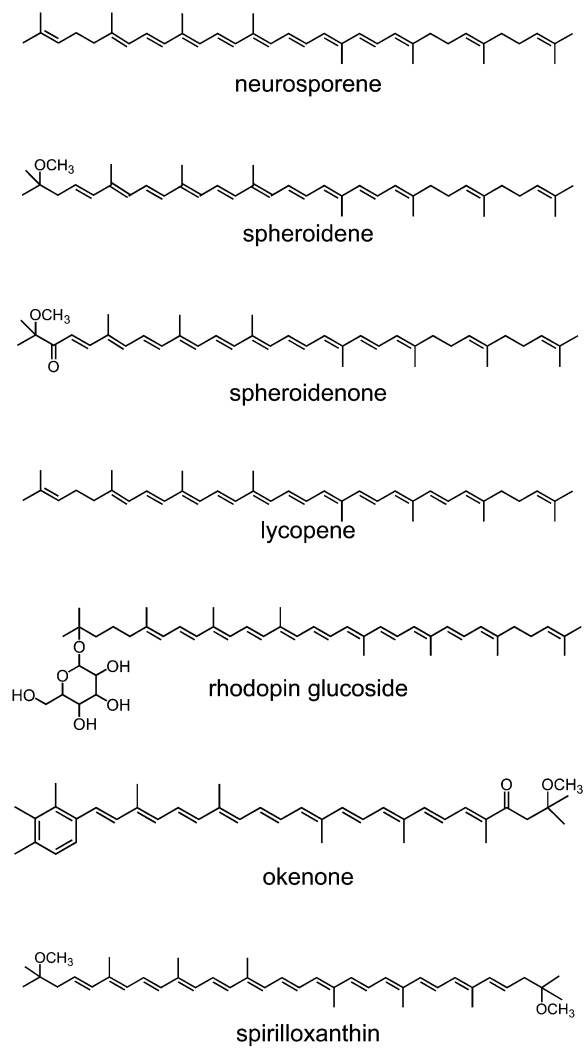

Fig. 2. Structures of carotenoids commonly occurring in LH complexes of purple bacteria.

II. Carotenoid Excited States

The LH function of carotenoids in the LH2 complex relies on their ability to transfer energy from the low-lying excited states to both Q_x and Q_y states of BChl *a*. Consequently, knowledge of the details of the carotenoid excited state manifold, including energies of the excited states and dynamics following the excitation is a necessary prerequisite to understanding carotenoid-BChl energy transfer in antenna complexes of purple bacteria. Much of our current understanding of mechanisms and pathways of carotenoid-BChl energy transfer stems from numerous experimental studies of carotenoids in solution. Until the late nineties much of the photophysics of carotenoids was interpreted in terms of the allowed S_2 state ($1B_u^+$ in C_{2h} symmetry) responsible for the strong absorption of carotenoids in the 400–550 nm region, and the symmetry-forbidden S_1 state ($2A_g^-$), which is located a few thousand reciprocal centimeters below the S_2 state. Thus, in contrast to other naturally-occurring dyes, the lowest excited state is a dark state. A number of spectroscopic studies have demonstrated that after being promoted to the S_2 state a carotenoid molecule will relax in less than 300 fs to the S_1 state. The lifetime of the S_1 state is determined by conjugation length of carotenoid and varies from 300 ps for short conjugated chains to ~1 ps for the longest (Polívka and Sundström, 2004).

Recent reports, however, have suggested that a

few other states denoted as $1B_u^-$ and $3A_g^-$ (Furuichi et al., 2002) and predicted by calculations on polyenes (Tavan and Schulten, 1987), or S* and $S^‡$ (Gradinaru et al., 2001; Papagiannakis et al., 2002; Larsen et al., 2003), and whose origin and properties remain to be firmly established, may be located either between or in proximity to the S_1 and S_2 states. All of these states represent potential donors in the process of carotenoid-BChl energy transfer in LH2 complexes. In addition, novel excited state properties were recently discovered for carotenoids containing a conjugated carbonyl group. Unlike other carotenoids their photophysics is strongly affected by the polarity of the environment. This behavior was attributed to a presence of another excited state having a charge transfer character (Frank et al., 2000; Zigmantas et al., 2004), and added to the complexity of the carotenoid excited state manifold. Because some carbonyl carotenoids, e.g., spheroidenone or okenone (Fig. 2), are constituents of purple bacterial antenna complexes and reaction centers, the charge transfer state may also be involved in energy transfer processes taking place in these organisms. A scheme showing the current view of the carotenoid excited state ordering together with possible energy transfer pathways to BChl is depicted in Fig. 3. A detailed description of carotenoid excited states in solution is beyond the scope of this chapter and can be found in specialized reviews (Frank et al., 2001; Hashimoto et al., 2004; Koyama et al., 2004; Polívka and Sundström, 2004).

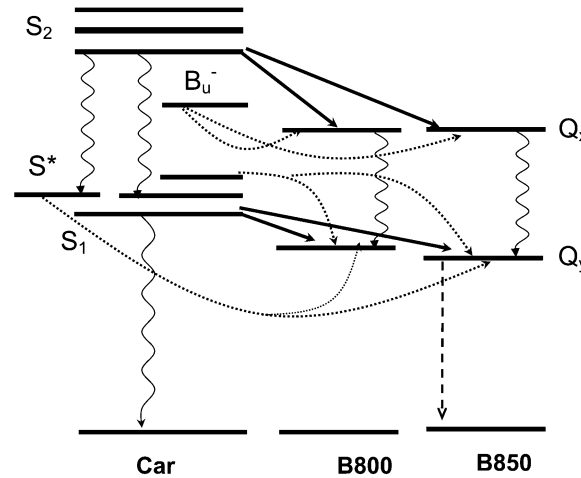

Fig. 3. Scheme of energy levels of carotenoid and BChl in light harvesting complexes of purple bacteria. Wavy arrows denote intramolecular relaxation processes, while the dashed arrow represents the long-lived BChl *a* fluorescence. Solid arrows represent the dominating energy-transfer channels involving the S_2 and S_1 states. The dotted lines represent minor energy-transfer channels that usually contribute only fractionally to the total energy transfer: the pathway via higher vibrational levels of the S_1 state, the pathway via the S* state and the B_u^- pathway were proposed to be active for some LH2 complexes. See text for details.

III. Energy Transfer in Light-Harvesting 2 Complexes

The large complexity of carotenoid excited states described in the previous section was not revealed until the end of the last century. Thus prior suggestions regarding mechanisms and pathways of carotenoid-BChl energy transfer in LH2 complexes involved only the S_1 and S_2 states. Moreover, because of the extremely fast deactivation of the S_2 state, the S_1 state was suggested to be the primary donor responsible for carotenoid-BChl energy transfer. The forbidden nature of transitions to and from the S_1 state imply a negligible transition dipole moment of the S_1 state and led to the suggestion that the Dexter electron exchange mechanism should be active in this process (Naqvi, 1980; van Grondelle, 1985; Cogdell and Frank, 1987). However, time-resolved experiments performed in the early nineties showed that following excitation of carotenoids, the B850 Q_y state was populated in less than 200 fs, indicating that, despite its very short lifetime, the S_2 state participates as a donor in carotenoid-BChl energy transfer (Trautman et al., 1990; Shreve et al., 1991). This suggestion was supported by calculations carried out by Nagae et al. (1993) who showed that for the carotenoid neurosporene both the S_1 and S_2 states can efficiently participate in energy transfer. When LH2 structures at atomic resolution became available, a number of experimental and theoretical investigations of energy transfer mechanisms and pathways between carotenoids and BChls bolstered the initial proposal that both the S_1 and S_2 states are involved in energy transfer. It was also concluded that the precise pathways and directions of energy flow are governed mainly by the conjugation length of the carotenoid. In addition, new experimental approaches and improved methods of data analysis have revealed alternate pathways that may contribute substantially to the overall carotenoid-BChl energy transfer in LH complexes of purple bacteria.

A. Energy Transfer via the S_2 State

Absorption spectra of carotenoids in LH2 complexes resemble those obtained for carotenoids in solution,

except for a red shift of ~1000 cm^{-1} caused by interaction with the protein environment (Fig. 1). The vibrational sub-structure of the carotenoid S_0-S_2 spectra of LH2 complexes is usually very similar to that obtained in solution, although for some carotenoids the protein environment represents a confinement of the carotenoid structure, leading to a better resolution of vibrational bands of the spectra (Polívka et al., 2002; Georgakopolou et al., 2004). Early experimental data on energy transfer via the S_2 state was obtained by fluorescence up-conversion (Ricci et al., 1996). S_2 emission decays of spheroidene in LH2 complexes of *Rba. sphaeroides* yielded a time constant of 80 fs. Comparing this result with the markedly longer decays in solution (150–250 fs), it was concluded that energy transfer via the S_2 state takes place with a time constant of 170 fs, which corresponded to an efficiency of 47%.

Thus, these experiments clearly demonstrated that energy transfer via the S_2 state can successfully compete with fast S_2-S_1 internal conversion. On the basis of spectral overlap, it was also concluded that energy transfer via the S_2 state occurs to the Q_x state of BChl *a* (Ricci et al., 1996). Similar results were reported for the LH3 complex from *Rps. acidophila* containing rhodopin glucoside, which has a longer conjugation length than spheroidene (Fig. 2). Analysis of up-conversion kinetics supported by calculations yielded an S_2-Q_x energy transfer rate of $(120–150\ fs)^{-1}$. Based on this, the S_2-Q_x channel was concluded to be the dominating one, accounting for 60% of the total energy transfer efficiency (Krueger et al., 1998). Later, a similar value was obtained when up-conversion experiments were carried out on the LH2 complex of *Rps. acidophila* (Macpherson et al., 2001). An observed 51% efficiency of S_2-mediated energy transfer is close to that obtained for LH3, suggesting that small structural differences between LH2 and LH3 complexes have no effect on carotenoid-BChl energy transfer. Instead, energy transfer efficiency appears to be primarily determined by the structure of the carotenoid.

The fast and efficient S_2 energy transfer pathway was also observed by transient absorption measurements. Although the data obtained from this method are more complicated to analyze than the data obtained from fluorescence up-conversion, recently introduced advanced methods of global fitting data analysis (van Stokkum et al., 2004) allowed for reliable separation of signals originating from different excited states. Application of these methods to transient absorption data measured in a broad spectral range for the LH2 complex from *Rps. acidophila* determined the efficiency of the S_2-mediated energy transfer to be 45–50%; the range is given by the model used for the global fitting of the transient absorption data (Papagiannakis et al., 2006a). Essentially the same value was obtained by Rondonuwu et al. (2004) who concluded that S_2 state of rhodopin glucoside in LH2 from *Rps. acidophila* transfers energy to BChl *a* with 48% efficiency.

A different method of data treatment using evolutionary target analysis determined the efficiency of the S_2 pathway in the same system to be 42% (Wohlleben et al., 2003). Thus, for the LH2 complex of *Rps. acidophila*, independent experiments utilizing different types of data analysis gave values of S_2-mediated energy transfer efficiency in the range of 42–51%. This mild discrepancy between different experiments points to an intrinsic problem in determining the S_2-mediated energy transfer efficiencies. The calculation of efficiency requires a precise knowledge of the rate constant for S_2-S_1 internal conversion in LH2. However, this depends on the environment, and it is thus impossible to determine an accurate value for the intrinsic (without energy transfer) lifetime of the S_2 state in the protein environment. This problem, discussed in detail by Macpherson et al. (2001), puts a limitation on the precision with which the S_2 energy transfer efficiency may be determined. Another limitation is the time resolution of the experiments. The measured S_2 lifetimes of rhodopin glucoside in LH2 complexes are on the sub-100 fs time scale, which in most cases is faster than the limits of time resolution of the instrument. However, recent experiments employing 10 fs pulses to study carotenoid to BChl *a* energy transfer in the LH2 complex of *Rps. acidophila* yielded a value for the efficiency of the S_2-mediated energy transfer of 49% (Polli et al., 2006), confirming the values obtained from earlier experiments. It is also worth noting that the 70 fs value of the S_2 lifetime of rhodopin glucoside in LH2 was also obtained on the basis of modeling homogeneous linewidths of the absorption and CD bands (Georgakopolou et al., 2004), further supporting the results obtained from time-resolved experiments.

Besides the LH2 complex from *Rps. acidophila,* Papagiannakis et al. (2002) studied S_2-mediated energy transfer from spheroidene in the LH2 from *Rba. sphaeroides* and obtained an energy transfer efficiency of 57%, which is in good agreement with the up-conversion data obtained by Ricci et al. (1996).

Another system studied by transient absorption spectroscopy is the LH2 complex from *Chromatium purpuratum* which binds the carotenoid okenone (Fig. 2). The data obtained on this complex are more complicated to analyze, because the long conjugated chain of okenone (Fig. 2) shifts the 0-0 band of the S_2 state so much to the red that it partially overlaps with the Q_x band of BChl *a* (Andersson et al., 1996). Consequently, partial excitation of the Q_x band makes the determination of energy transfer efficiency difficult. Moreover, okenone possesses a conjugated carbonyl group that makes the excited state properties sensitive to the polarity of environment (Frank et al., 2000b; Zigmantas et al., 2004). Andersson et al. (1996) reported the S_2-mediated energy transfer time from okenone to be 50–100 fs, which taking into account the ~150 fs S_2 lifetime of okenone in solution, gave energy transfer efficiencies over 50%. These experiments were recently repeated by Polli et al. (2006) with the significantly better time resolution of 10 fs. These authors compared the excited state dynamics of okenone in a few solvents and in LH2 and concluded that the protein environment is best mimicked by CS_2. By comparing the S_2 lifetimes of okenone in CS_2 and in LH2 they obtained an energy transfer rate constant of $(56\ fs)^{-1}$ yielding a 65% efficiency of the S_2 channel (Polli et al., 2006).

An even higher efficiency of 70% for the S_2-mediated energy transfer was reported by Zhang et al. (2001) for the LH2 complex from *Rba. sphaeroides* G1C which contains neurosporene (conjugation length N=9). Comparing the results on LH2 complexes containing carotenoids with N=9-13, there is a trend to higher efficiency of S_2 energy transfer for shorter carotenoids. This trend is supported by experiments on LH2 of the carotenoidless mutant *Rba. sphaeroides* R26.1 incorporated with spheroidene analogs of different conjugation lengths (Desamero et al., 1998). Using S_2-S_1 internal conversion rates measured in solution, overall carotenoid-BChl energy transfer efficiencies, and S_1-mediated energy transfer efficiencies, the efficiencies of S_2 energy transfer were calculated. Although the LH2s with spheroidene analogs having N=8,9 were observed to have slightly less efficient S_2 energy transfer than LH2 with spheroidene (N=10), the systematic decrease of S_2 efficiency from 50% (spheroidene) to 12% for the analog having N=13, supported the observed trend of decreasing S_2-mediated energy transfer efficiency with increasing conjugation length (Desamero et al., 1998). On the other hand, essentially no change in the efficiency of the S_2 channel was found by Rondonuwu et al. (2004), who explored a series of LH2 complexes possessing different carotenoids. For the S_2-mediated energy transfer, no dependence on carotenoid structure and conjugation length was found. A narrow 46–48% range of values was determined for neurosporene (N=9) and spheroidene (N=10) in LH2 from *Rba. sphaeroides*, lycopene (N=11) in LH2 from *Rsp. molischianum*, and rhodopin glucoside (N=11) in LH2 from *Rps. acidophila* (Koyama et al., 2004; Rondonuwu et al., 2004). For neurosporene and spheroidene these values are significantly lower than those obtained in other experiments (Zhang et al., 2001; Papagiannakis et al., 2002), which is likely caused by additional decay channels included in the analysis performed by Rondonuwu et al. (2004) who assumed other carotenoid excited states act as energy donors in addition to the S_2 state. These potential additional energy transfer channels are discussed below.

Although the precise values of the S_2 transfer efficiencies are still a matter of debate, mainly because of possible involvement of other states, it is clear that the S_2 energy transfer route operates with efficiencies in the range 30–70% in all purple bacterial antenna complexes studied so far. Thus, the S_2 channel successfully competes with S_2-S_1 internal conversion for a number of LH2 complexes. This competition of S_2 energy transfer with the S_2-S_1 internal conversion has been rationalized theoretically. The contribution of the electron-exchange Dexter energy transfer mechanism to the S_2 energy transfer pathway has been computed to be negligible with the Förster-type mechanism dominating (Nagae et al., 1993; Krueger et al., 1998; Damjanovic et al., 1999). Even without detailed structural information, Nagae et al. (1993) calculated the Coulombic couplings between the S_0-S_2 transition of neurosporene and the Q_x transition of BChl *a* for a few hypothetical configurations. They concluded that S_2 energy transfer can be faster than 100 fs, if the proper orientation of donor and acceptor is realized (Nagae et al., 1993). Determination of the LH2 structure provided detailed information about the mutual orientation of pigments within LH2, allowing more precise calculations of couplings between carotenoids and BChl *a*. Krueger et al., applied an advanced method, the so-called transition density cube method, to calculate full Coulombic couplings between pigments in both LH3 (Krueger et al., 1998a) and LH2 (Krueger et al., 1998b) complexes from *Rps. acidophila*. This method replaces the vector

description of the transition dipole moments by three-dimensional transition density volumes, and gives a more accurate account of the interaction between molecules (Krueger et al., 1998b). The couplings of the S_0-S_2 transition of rhodopin glucoside with all possible transitions of neighboring BChl *a* were calculated for the LH3 complex, yielding values larger than 100 cm^{-1} for β-B820 Q_x, B800 Q_y and also for the B800 Q_y transition of BChl *a* located in the neighboring building unit. However, due to the small value of the spectral overlap integral for the Q_y states, only the S_2-B820 Q_x yielded an appreciable energy transfer rate constant of $(240\ fs)^{-1}$ (Krueger et al., 1998a). Similar results were obtained for the LH2 complex. Although the actual couplings were slightly different from those calculated for the LH3 complex, the S_2-B850 Q_x channel again represented the dominant route with an energy transfer rate constant of $(135\ fs)^{-1}$ (Krueger et al., 1998b). The results of these calculations are in very good agreement with the observed depopulation rates of the S_2 state, but predict no S_2 transfer to the B800 BChl *a* molecule which contradicts experimental observation (Macpherson et al., 2001).

On the other hand, similar calculations using full Coulombic couplings performed on the basis of the LH2 structure of *Rsp. molischianum* containing the carotenoid lycopene, yielded appreciable couplings of the S_0-S_2 transition with both the B850 and B800 BChls, resulting in comparable S_2 energy transfer rate constants of $\sim(200\ fs)^{-1}$ for both B850 and B800 acceptors (Damjanovic et al., 1999). A similar result was obtained by calculations of the lycopene-BChl couplings in LH2 by means of the collective electronic oscillators algorithm (Tretiak et al., 2000). It is interesting to note that these calculations also proposed strong coupling between the B_x (Soret) band of both B800 and B850 BChls and the S_2 state of lycopene, indicating a possibility of efficient energy transfer from BChl *a* Soret to lycopene (Tretiak et al., 2000), but no experimental evidence for such transfer has been given so far. Also, calculations of couplings were carried out for either isolated molecules in LH2 complexes or in a dielectric medium simulating the mean field created by the protein environment. The effect of the dielectric medium on couplings involving the S_2 state were quite significant, underlining the importance of protein effects (Tretiak et al., 2000).

An interesting aspect of the carotenoid-BChl energy transfer in LH2 is the fact that by proper shaping of excitation pulses it is possible to control the ratio between S_2-S_1 internal conversion and energy transfer. However, as was shown for rhodopin glucoside in LH2 from *Rps. acidophila*, only a decrease in energy transfer efficiency can be achieved. Herek et al. (2002) showed that the S_2 population of rhodopin glucoside can be steered to the S_2-S_1 channel, as demonstrated by increase of the signal due to the S_1 state by a factor 1.4. In the coherent control experiments carried out by Herek et al. (2002) the increase in proportion of S_2-S_1 internal conversion was achieved by optimizing the envelope and phase of the excitation pulses (Herek et al., 2002; Wohlleben et al., 2005). Recently, Papagiannakis et al. (2006a) showed that also incoherent effects, such as annihilation and saturation can lead to similar control of the S_2-S_1/S_2-BChl ratio, demonstrating that the actual value of S_2 energy transfer efficiency depends also on the intensity of excitation pulses.

B. Energy Transfer via the S_1 State

Despite the forbidden nature of the S_1 state, energy transfer via the S_1 route is straightforward to study by means of transient absorption spectroscopy because of the strong S_1-S_n band readily observed in the visible spectral region (Polívka and Sundström, 2004). The position and shape of this band is characteristic of each carotenoid (Fig. 4) and the dynamics of this band can be used to determine the S_1 lifetimes of carotenoids in various environments. Thus, comparison of S_1 lifetimes in solution and in antenna complexes allows for determining energy transfer efficiencies via the S_1 state. To study the effect of conjugation length on energy transfer in LH2 complexes, this method was applied to the *Rba. sphaeroides* R26.1 mutant with incorporated spheroidene analogs of different conjugation lengths (Desamero et al., 1998). Although for conjugation lengths $N \geq 11$ the S_1 energy transfer was undetectable, quenching of the S_1 state due to energy transfer was observed for shorter carotenoids. This result was explained in terms of spectral overlap between hypothetical S_1 fluorescence and the B850 absorption band, which becomes small for longer carotenoids. A similar study was carried out by Zhang et al. (2000) using three LH2 complexes with different carotenoids. On the basis of measured S_1 lifetimes of neurosporene (N=9), spheroidene (N=10) and lycopene (N=11) in solution and LH2, a significant decrease of S_1 energy transfer efficiency was observed; it dropped from 94% (neurosporene) to 82 % (spheroidene) and further to

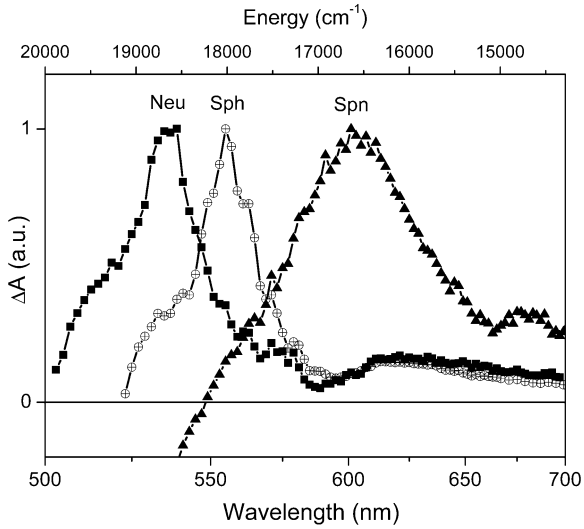

Fig. 4. Transient absorption spectra in the S_1-S_n region measured 1 ps after excitation into the lowest vibrational band of the carotenoid S_2 state for LH2 complexes from *Rba. sphaeroides* with neurosporene (Neu), spheroidene (Sph) and spheroidenone (Spn). All spectra are normalized to the S_1-S_n maximum.

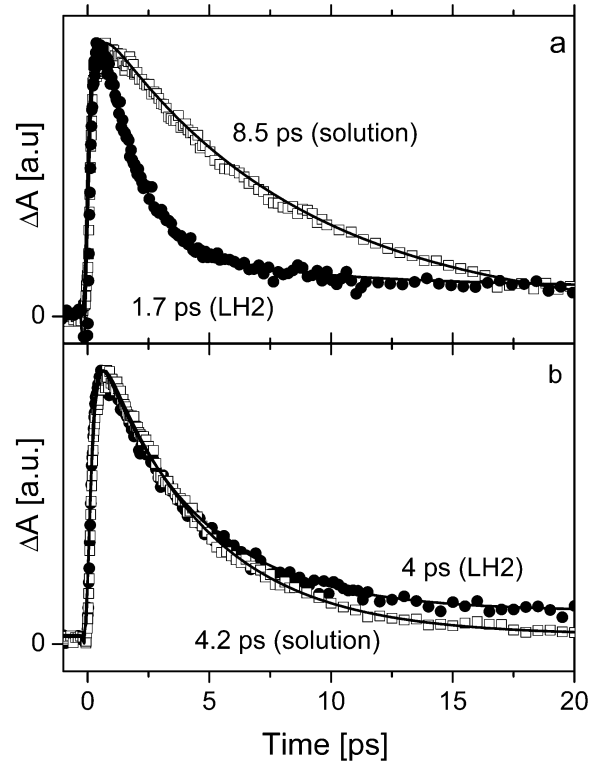

Fig. 5. Kinetics recorded at the maximum of the S_1-S_n transition after excitation of the 0-0 band of the S_0-S_2 transition of the carotenoids. The S_1 decays of the carotenoids in solution (open symbols) and in the LH2 complex (full symbols) are compared for the LH2 complexes from *Rba. sphaeroides* (a) and *Rps. acidophila* (b). The corresponding fits of the kinetics are represented by solid lines. Excitation wavelengths are 515 nm (*Rba. sphaeroides*) and 525 nm (*Rps. acidophila*).

less than 30 % (lycopene), as the conjugation length increased from 9 to 11.

The significant drop in energy transfer via the S_1 state was also confirmed for spheroidene and rhodopin glucoside in LH2 complexes from *Rba. sphaeroides* and *Rps. acidophila* by means of measurements of S_1-S_n kinetics either after two-photon excitation of the S_1 state (Walla et al., 2000) or after one-photon excitation of the S_2 state (Polívka et al., 2002). These authors confirmed the ~1.7 ps S_1 lifetime of spheroidene in LH2 indicating energy transfer. For rhodopin glucoside, however, no change in S_1 lifetime was observed upon going from solvent to LH2, demonstrating that in this complex the S_1 state does not transfer energy (Fig. 5). A few other studies confirmed this trend and established that LH2 complexes utilizing carotenoids with N≥11 are incapable of energy transfer via the S_1-route as its efficiency does not exceed 5% (Macpherson et al., 2001; Wohlleben et al., 2003; Koyama et al., 2004; Rondonuwu et al., 2004; Polli et al., 2006). The importance of the carotenoid structure was shown in a study of a *Rba. sphaeroides* mutant incorporating the longer (N=11) lycopene instead of spheroidene (N=10) naturally present in wild type LH2. When lycopene is present in the complex, the efficiency of S_1-mediated energy transfer drops to a few percent, while more than 80% is achieved when spheroidene is present in the wild type (Billsten et al., 2002a). Further evidence that carotenoids with N=11 are on the edge of capability to transfer energy via the S_1 state was provided by studies of LH2 complexes from *Rba. sphaeroides* R26.1 reconstituted with spirilloxanthin (N=13) (Papagiannakis et al., 2003a) or LH2 complexes from *Chromatium purpuratum* containing okenone (N~12) (Polli et al., 2006). These studies showed that no (or very little) S_1-mediated energy transfer is achieved by these long carotenoids as essentially no S_1-mediated energy transfer was observed for spirilloxanthin, and an upper limit of 6% was found for okenone.

The obvious relation between the conjugation length and efficiency of the S_1-mediated energy transfer can be explained by a decrease of the S_1 energy with increasing conjugation length. To achieve efficient energy transfer the S_1 energies of carotenoids must be higher than those of the acceptor states.

While the determination of the energy is a trivial task for the S_2 state, the forbidden nature of the S_1 state prevents a direct measurement of the S_1 energy. In the late nineties a few papers appeared describing different methods for determining S_1 energies of carotenoids: 1) measurement of the extremely-weak S_1 emission (Fujii et al., 1998); 2) measurements of resonance Raman profiles (Sashima et al., 1998); 3) time-resolved measurements of the S_1-S_2 spectra (Polívka et al., 1999); and 4) two-photon absorption (Krueger et al., 1999). Detection of S_1 fluorescence and measurements of resonance Raman profiles, however, could not provide unambiguous information about S_1 energies in LH2 and related LH systems. However, since it was known that S_1 energies are usually insensitive to solvent properties (except for carbonyl carotenoids that are not typical for purple bacterial LH complexes; Polívka and Sundström, 2004), it was reasonable to assume that the S_1 energies in LH2 complexes should be very close to those determined for carotenoids in solution. Under this assumption, S_1 energies of carotenoids with $N \leq 10$ were calculated to be high enough to allow sufficient overlap between carotenoid S_1 emission and Q_y bands of BChl *a*.

This conclusion was later verified by direct measurements of the S_1 energies of neurosporene, spheroidene and rhodopin glucoside in LH2 complexes by recording the S_1-S_2 spectra. S_1 energies of 14500 cm^{-1}, 13400 cm^{-1}, and 12550 cm^{-1} were determined for neurosporene, spheroidene, and rhodopin glucoside, respectively (Polívka et al., 2002, 2004), showing that the difference of 850 cm^{-1} between S_1 energies of spheroidene and rhodopin glucoside makes a significant change in the energy transfer pathways in LH2 complexes. These experiments also justified the approximation of S_1 energies in LH2 complexes using energies obtained in solution. In the case of spheroidene, the S_1 energy extracted from the S_1-S_2 spectra is the same as that determined by this method in solution (Polívka et al., 2001). For rhodopin glucoside the S_1 energy is 250 cm^{-1} lower than that in solution. This difference was explained by confinement of rhodopin glucoside in the LH2 structure, which narrows the distribution of conformers compared to that in solution. This was also supported by modeling CD and absorption spectra of carotenoids in LH2 complexes (Georgakopoulou et al., 2004). Another direct measurement of S_1 energies in the LH2 complex from *Rba. sphaeroides* was carried out by means of two-photon fluorescence excitation (Krueger et al., 1999). Using this technique the S_1 state can be excited directly because the S_0-S_1 transition is allowed for a two-photon transition. By measuring B850 emission after two-photon excitation of the S_1 state achieved by exciting in the 1200–1500 nm spectral range, the two-photon excitation spectrum placed the 0-0 energy of spheroidene in LH2 at 13900 cm^{-1}, confirming the similarity of the S_1 energies in LH2 and in solution.

Together with measurements of S_1 energies, calculations of the couplings between the S_0-S_1 transition and Q_y transitions of both B800 and B850 demonstrated that the S_1 transfer rates can be explained in terms of the same energy transfer mechanism as for the S_2 route. Although the very small transition dipole moment of the S_0-S_1 transition led initially to a suggestion that the Dexter mechanism had to be invoked (Naqvi, 1980; van Grondelle, 1985; Cogdell, 1987), more detailed calculations later showed that the Dexter contribution is negligible and that higher-order Coulombic and polarization interactions dominate the S_1-mediated energy transfer (Nagae et al., 1993; Scholes et al., 1997; Krueger et al., 1998; Damjanovic et al., 1999; Tretiak et al., 2000). In some studies (Damjanovic et al., 1999; Zhang et al., 2000), the Coulombic couplings for the S_1 state were obtained by scaling down the couplings calculated for the S_2 state and using an estimate that the transition dipole moment of the S_0-S_1 transition is about 4–6% of the S_0-S_2 transition. Although the results of this approach were promising, the calculated rates did not reproduce the measured energy transfer rates. To resolve this problem, an increase of S_0-S_1 Coulombic coupling via intensity borrowing from the allowed S_2 state due to S_2-S_1 mixing was proposed (Damjanovic et al., 1999; Zhang et al., 2000). Under the assumption that the degree of mixing is inversely proportional to the square of the S_1-S_2 energy gap, Zhang et al. (2000) calculated S_1 energy transfer rates for LH2 from *Rsp. molischianum* that were reasonably close to the measured values.

Further improvement of the agreement between experiment and theory was obtained by calculations of Coulombic couplings by means of time-dependent density functional theory (TDDFT) (Walla et al., 2000; Hsu et al., 2001). This method, which allows *ab initio* calculations of the S_1 couplings with Q_y states of BChl, confirmed that small mixing between S_2 and S_1 states plays an important role in the Coulombic coupling. S_1 energy transfer rates for LH2 complexes from three different species of purple bacteria were

calculated using the Coulombic couplings obtained from TDDFT, and the obtained values $(9\ ps)^{-1}$ (*Rsp. molischianum*), $(1.2\ ps)^{-1}$ (*Rba. sphaeroides*) and $(3\ ps)^{-1}$ (*Rba. sphaeroides* G1C) (Hsu et al., 2001) are very close to the experimental ones of $(12\ ps)^{-1}$, $(2.4\ ps)^{-1}$ and $(1.4\ ps)^{-1}$ (Zhang et al., 2001). For LH2 from *Rps. acidophila*, an S_1 energy transfer time >25 ps was calculated (Hsu et al., 2001), also in agreement with the very low efficiency obtained experimentally (Macpherson, 2001; Polívka et al., 2002; Wohlleben, 2003).

Although the trend of decreasing efficiency of the S_1-mediated energy transfer was confirmed by experiments using LH2 complexes from *Rba. sphaeroides* having the different carotenoids, neurosporene, spheroidene, and spheroidenone, the drop in efficiency when going from spheroidene to spheroidenone was less than expected on the basis of a change in spectral overlap. The S_1 lifetimes of carotenoids in LH2 complexes of 1.4, 1.5 and 1.4 ps for neurosporene, spheroidene, and spheroidenone were compared with their lifetimes in solution (24, 8.5 and 6 ps), resulting in energy transfer efficiencies of 94, 82 and 76% (Polívka et al., 2004). Knowing that the S_1 energy of spheroidenone is around 13000 cm^{-1} (Zigmantas et al., 2004), the efficiency of energy transfer from spheroidenone does not match that expected from calculations by Hsu et al. (2001). Also, comparison of 76% with the nearly zero efficiency of the S_1-mediated energy transfer obtained for rhodopin glucoside that has a LH2 S_1 energy of 12550 ± 150 cm^{-1} (Polívka et al., 2002) would imply that ~500 cm^{-1} decrease in energy is enough to decrease efficiency from 76 to 5%. Taking into account that S_1 emission of carotenoids is quite broad (Fujii et al., 1998; Frank et al., 2000a), such a drop can be hardly explained as due solely to change in the spectral overlap (Ritz et al., 2000). Thus, it indicates that other factors besides conjugation length may play a role.

One possibility is that the high efficiency of the S_1-mediated energy transfer of spheroidenone is related to the fact that it belongs to the family of carbonyl carotenoids, which possess an excited state with charge-transfer character (Frank et al., 2000b; Zigmantas et al., 2004) that may enhance the spheroidenone-BChl coupling. Another explanation was offered by Ritz et al. (2000) who noted that the conjugated systems of neurosporene and spheroidene, which systematically exhibit highly-efficient energy transfer via the S_1 state, have a non C_{2h}-symmetrical arrangement of their methyl side groups. This is, however, not the case for lycopene and rhodopin glucoside, both exhibiting very low efficiencies of the S_1-mediated energy transfer. Consequently, Ritz et al. (2000) suggested that symmetry breaking of neurosporene and spheroidene may be an important factor in explaining why energy transfer via the S_1 state is much more efficient for these carotenoids compared to lycopene and rhodopin glucoside. Since the conjugated system of spheroidenone has also asymmetric arrangement of methyl groups (Fig. 2), the observation of efficient S_1-mediated energy transfer, despite its low S_1 energy, provides further support for the conjecture proposed by Ritz et al. (2000).

In addition to the S_1-mediated energy transfer occurring from a thermalized S_1 state discussed above, it has been proposed that a portion of the pathway may involve a vibrationally hot S_1 state. Using global analysis, Papagiannakis et al. (2002) reported the decay of a species associated spectrum corresponding to the vibrationally hot S_1 state, suggesting the presence of an energy transfer channel via this route. However, the contribution of this pathway to the total energy transfer efficiency was only 5%. Indirect evidence for the presence of this channel also could be found in experiments carried out by Krueger et al. (1999) who measured the spectral profile of the S_0-S_1 transition in the LH2 complex by two-photon excitation techniques that detect emission from BChl *a*. The 0-0 band of the S_0-S_1 transition was very weak but the intensity of higher vibrational bands was high which may be due to energy transfer occurring from the hot vibrational states. In any case, this energy transfer channel plays only a minor role in the overall carotenoid-BChl energy transfer in LH2. An upper limit of 3% was found in the LH2 complex from *Rps. acidophila* for this channel (Wohlleben et al., 2003). Moreover, as no evidence for energy transfer via a hot S_1 state was provided by a number of other experiments using various LH2 complexes (Papagiannakis et al., 2003a; Koyama et al., 2004; Rondonuwu et al., 2004; Polli et al., 2006), further experiments using both advanced experimental approaches and sophisticated data analysis will be needed to verify the presence of this energy transfer channel.

C. The S State*

Reports of other carotenoid excited states located between the S_2 and S_1 states (see Polívka and Sundström, 2004, for a review) initiated lively debates regarding whether these states act as energy donors

in carotenoid-BChl energy transfer. New ways of data analysis allowed for a more rigorous assignment of various excited state species (van Stokkum et al., 2004), and it was shown in a number of cases that the two-state (S_2 and S_1) model is not sufficient to describe all features revealed in experimental data. The so-called S* state is the best studied in this respect; it was first reported in the excited state manifold of the carotenoid spirilloxanthin in both solution and the LH1 complex of *Rsp. rubrum* (Gradinaru et al., 2001). Using global analysis of data in the 470–720 nm spectral region, it was shown that the S_1-S_n band of spirilloxanthin in solution (peaking at 590 nm) possessed a distinct shoulder at ~540 nm. While the 590 nm band decayed with 1.4 ps corresponding to the S_1 lifetime of spirilloxanthin, the 540 nm shoulder exhibited a much longer decay time of ~6 ps (Gradinaru et al., 2001). This result was explained in terms of two parallel pathways of S_2 depopulation; a major part (70%) decaying to form the S_1 state, while a minor pathway (30%) leads to population of the S* state which then decays to the ground state with a 6 ps lifetime.

Interestingly, as shown in subsequent studies (Papagiannakis et al., 2002; Papagiannakis et al., 2003a; Wohlleben et al., 2003), the S* state is formed with much higher yield when carotenoids are incorporated into purple bacterial LH complexes. Moreover, in LH1 and LH2 complexes the S* state was found to be a precursor of fast carotenoid triplet state formation. A relatively high triplet yield of 25–30% was explained to be a result of a conformational distortion of spirilloxanthin in the LH1 complex, promoting triplet formation via singlet homofission from the S* state (Gradinaru et al., 2001). Further studies confirmed the triplet state formation via the S* state for other complexes containing different carotenoids. The yields of triplet formation varied from nearly 40% for rhodopin glucoside in LH2 from *Rps. acidophila* (Wohlleben et al., 2003) to less than 10% for spheroidene in both native LH2 from *Rba. sphaeroides* (Papagiannakis et al., 2002) and incorporated into the *Rb. sphaeroides* R26.1 mutant (Papagiannakis et al., 2003a). The fact that no triplet formation is observed in solution suggests that the protein environment is a crucial factor governing the formation of the triplet via singlet homofission, and it strongly supports the conclusion that the deviation from planar conformation in antenna complexes of purple bacteria is a necessary condition for efficient triplet formation (Papagiannakis et al., 2003a). However, it turns out that the decay to the S_0 state and triplet formation are not the only possible fates of the S* state in LH2 complexes. In LH2 complexes containing either spheroidene or rhodopin glucoside, the S* state also contributes to carotenoid-BChl energy transfer. For spheroidene in the LH2 of *Rba. sphaeroides*, S*-mediated energy transfer contributes 10–15% to the total spheroidene-BChl energy transfer (Papagiannakis et al., 2002). For rhodopin glucoside in *Rps. acidophila* LH2, the S*-mediated channel was found to be ~10% efficient; i.e., even higher than the energy transfer efficiency from the S_1 state (Wohlleben et al., 2003).

The possibility of carotenoid-BChl energy transfer via the S* state provided important information for determining the origin of the S* state, which is still a matter of debate. The presence of the S*-mediated energy transfer puts the S* energy above the Q_y bands of BChl *a*, eliminating one of the proposed origins, a vibrationally hot ground state (Wohlleben et al., 2004). Instead, it seems that S* is indeed a separate excited state as originally suggested by Gradinaru et al. (2001), but its symmetry and relation to other states remains unknown. It also must be noted that although only a few carotenoids were subject to studies focusing on the S* state so far, it seems obvious that the lifetime of the S* state does not follow any clear dependence on conjugation length. While the S_1 lifetime is changed systematically from ~9 ps (spheroidene) to 4.1 ps (rhodopin glucoside) and 1.5 ps (spirilloxanthin) as a result of increased conjugation length from 10 to 13 (Polívka and Sundström, 2004), the intrinsic S* lifetimes (in the absence of energy transfer) in LH2 and LH1 complexes are scattered in the 6 to 30 ps range without any obvious relation to the conjugation length (Gradinaru et al., 2001; Papagiannakis et al., 2002, 2003a, 2006a; Wohlleben et al., 2003). The lack of correlation between lifetime and conjugation length also casts doubts on assignment of the S* state to the $1B_u^-$ state proposed by some authors (Papagiannakis et al., 2002; Wohlleben et al., 2003).

A recent thorough study of the S* state in LH2 complexes from *Rps. acidophila* and *Rba. sphaeroides* revealed another aspect of the spectroscopic properties of the S* state in LH2 complexes. Population of the S* state exhibits a dependence on intensity of excitation pulses that differs from that observed for the S_1 state (Papagiannakis et al., 2006a), indicating that the S* and S_1 state cannot have a common precursor, the S_2 state, as previously thought. These authors proposed

two models to explain their data. One assumes two different ground-state populations each leading to population of either S_1 or S* state, the other involves higher excited states. These upper excited states are populated via excited state absorption from the S_2 state that is resonant with the excitation pulse, thus the S_2 population created by the front of the excitation pulse can be re-excited into higher excited states by photons arriving in the tail of the pulse. These higher excited states exhibit a relaxation pattern that favors population of the S* state. Because increasing excitation intensity increases the probability of the re-excitation, the S* state gets more populated with higher excitation intensities (Papagiannakis et al., 2006a). This model involving higher excited states has gained support from another study showing that direct excitation of high-lying excited states enhances population of the S* state (Billsten et al., 2005). These authors also suggested that the S* state in solution may be related to a conformational change of the carotenoid molecule. This possibility was later supported by a study on a series of carotenoids with different structures, indicating that the S* state may be a minimum in the S_1 potential surface corresponding to a conformational change (Niedzwiedzki et al., 2006). This conclusion also explains why the S* state is preferentially populated in LH2 complexes where such distortions have been confirmed by X-ray crystallography.

D. Other Pathways

Another excited state widely discussed as a potential energy donor in carotenoid-BChl energy transfer in LH2 complexes is the $1B_u^-$ state. Its presence in the excited state manifold was predicted two decades ago by calculations on polyenes carried out by Tavan and Schulten (1987), and it was shown that for conjugation lengths corresponding to naturally-occurring carotenoids (N = 9-13) it may be located below the strongly absorbing S_2 state. Due to the forbidden nature of the $1B_u^-$ state (it is forbidden for both one- and two-photon transitions from the ground state), experimental verification of the presence of the $1B_u^-$ state between the S_2 and S_1 states is very difficult. In the late nineties Sashima et al. (1999) detected this state using measurements of resonance Raman profiles. Later, the $1B_u^-$ state was also proposed to be active in energy transfer between carotenoids and BChl in LH2 complexes (Rondonuwu et al., 2004; Koyama et al., 2004). Based on previous assignments of spectral signatures thought to be associated with the $1B_u^-$ state in transient absorption spectra recorded in the near-infrared and visible ranges (Koyama et al., 2004), Rondonuwu et al. (2004) used global analysis of data taken on a few LH2 complexes containing different carotenoids and concluded that the $1B_u^-$ state transfers energy to BChl with ~20% efficiency. This corresponds to an energy transfer time of ~0.6 ps for neurosporene and spheroidene in the LH2 complexes from *Rba. sphaeroides*. This pathway was proposed to be inactive for LH2 complexes accommodating the longer carotenoids lycopene and rhodopin glucoside. The absence of this channel in these complexes was explained by the $1B_u^-$ state lying below the Q_x state of BChl *a*, which was assumed to be the energy acceptor. For these carotenoids, however, it was hypothesized that another dark excited state, the $3A_g^-$ state, may be active in carotenoid-BChl energy transfer because the expected $3A_g^-$ energies may be favorable for this state to act as an energy donor. However, no experimental evidence for such a pathway was given (Rondonuwu et al., 2004). It must be noted that both $1B_u^-$ lifetimes and spectral signatures in the visible region obtained by Rondonuwu et al. (2003) are essentially identical to those assigned earlier to the hot S_1 state (Billsten et al., 2002b; de Weerd et al., 2002). Consequently, the data by Rondonuwu et al. (2004) may also be interpreted in terms of the hot S_1 state being the energy donor instead of the $1B_u^-$ state.

Another issue that awaits further clarification is the relationship between the $1B_u^-$ and S* states. It has been proposed that the $1B_u^-$ state is identical with the S* state (Papagiannakis et al., 2002, Wohlleben et al., 2003; Rondonuwu et al., 2003). This assignment may seem correct as the symmetry and origin of the S* state are still unknown, and the $1B_u^-$ state was predicted to be a precursor of ultrafast triplet formation (Rondonuwu et al., 2004; Koyama et al., 2004), the same process reported for the S* state (Gradinaru et al., 2001; Papagiannakis et al., 2002). On the other hand, for the S* state it was shown that there is no $S_1 \leftrightarrow S^*$ conversion (Gradinaru et al., 2001; Papagiannakis et al., 2002; Wohlleben et al., 2003), which is in contradiction with the $1B_u^-$ state being an intermediate state in S_2-S_1 internal conversion (Koyama et al., 2004). Similarly, the $1B_u^-$ lifetimes (see Koyama et al., 2004, for a review) are about an order of magnitude shorter than those measured for the S* state, which argues that the $1B_u^-$ and S* states are not equivalent.

E. The Role of B800

For the LH2 and LH3 complexes, B850 (B820 in LH3) and B800 BChl *a* molecules may be acceptors in carotenoid-BChl energy transfer. The question of partitioning between these two possible acceptors has been addressed in a few studies. Macpherson et al. (2001) investigated S_2-mediated energy transfer by fluorescence up-conversion in B800-B850 and B850-only LH2 complexes from *Rps. acidophila*. Upon combining the results for these two LH2 complexes, they concluded that the S_2 state of rhodopin glucoside transfers energy with 20% efficiency to B800 and with 31% efficiency to B850, leading to the total efficiency of 51% (Macpherson et al., 2001). Similar analysis was carried out by Papagiannakis et al. (2003a) who used transient absorption spectroscopy to study LH2 from a carotenoidless *Rba. sphaeroides* R26.1 mutant lacking the B800 BChl but incorporated with spheroidene. It was reported that the S_2 pathway operates with 25% efficiency, which, when compared with the 57% efficiency of this pathway known for the wild type B800-B850 complex, allowed them to conclude that approximately half of the S_2 population transfers energy to B800 (Papagiannakis et al., 2003a). It should be noted that although this work clearly showed that B800 accepts up to 50% of energy from the S_2 state, calculations based on the X-ray structure of the LH2 complex from *Rps. acidophila* gave appreciable couplings only for the S_2-B850 Q_x channel (Krueger et al., 1998). On the other hand, similar calculations using full Coulombic couplings performed on the basis of the LH2 structure of *Rsp. molischianum*, yielded appreciable couplings of the S_0-S_2 transition with both the B850 and B800 BChls, resulting in close to a 1:1 branching ratio between the B800 and B850 acceptors (Damjanovic et al., 1999). Essentially the same results were obtained by calculations of the lycopene-BChl couplings by means of the collective electronic oscillators algorithm (Tretiak et al., 2000).

Regarding energy acceptors in the S_1-mediated energy transfer route, both B800 and B850 BChls are capable of accepting energy from carotenoids, but the S_1-B800 channel seems to dominate. In experiments employing samples having spheroidene incorporated into LH2 from the carotenoidless *Rba. sphaeroides* R26.1 mutant lacking B800 BChl, the efficiency of energy transfer via the S_1 pathway reached only 35% (Papagiannakis et al., 2003a). This is significantly less than the ~80% observed for the LH2 containing both B800 and B850 (Walla et al., 2000; Zhang et al., 2000; Polívka et al., 2002), signaling that the main pathway involves B800 as an acceptor. The same conclusion was reached for LH2 from *Rps. acidophila*. Although the S_1 efficiency is only 4–5% in the wild type complex, selective removal of the B800 BChls led to a complete absence of S_1 energy transfer (Macpherson et al., 2001). Polívka et al. (2007) used lithium dodecyl sulfate (LDS) which selectively perturbs the B800 site (Chadwick et al., 1987). The LDS-treated complexes lack the B800 band and can be therefore used to investigate the role of B800 in energy transfer (Fig. 6). By measuring the S_1 lifetimes of neurosporene, spheroidene and spheroidenone in LDS-treated LH2 complexes and comparing them with data taken on untreated complexes (Fig. 6), Polívka et al. (2007) showed that the carotenoid S_1 lifetime has values in the range 1.4–1.8 ps for the untreated LH2 complexes, but it is prolonged to 2.7–3.5 ps for LDS-treated complexes. This is consistent with slower energy transfer via the S_1 state. The S_1 state branching ratios for energy transfer to the B850 and B800 BChls were calculated using the S_1 lifetimes of the carotenoids in solution and in untreated and LDS-treated LH2 complexes. The calculations showed that the B800:B850 branching ratio remained essentially the same regardless of the conjugation length of the carotenoid. The values were 65:35 for LH2 with spheroidene and 60:40 for the complexes containing neurosporene

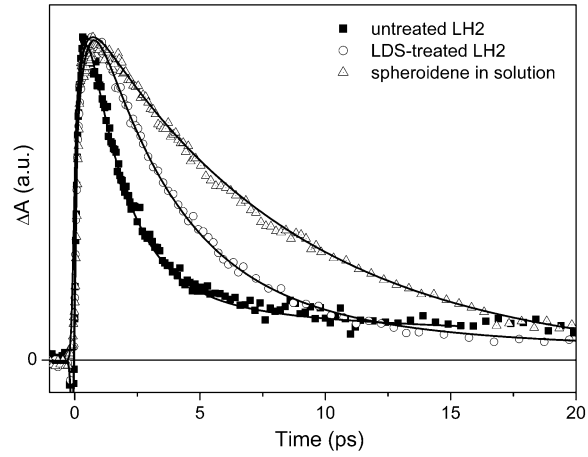

Fig. 6. Kinetics recorded at the maximum of the S_1-S_n band for LH2 complexes from *Rba. sphaeroides* containing spheroidene. Kinetics are shown for spheroidene in solution (open triangles), LDS-treated LH2 complexes (open circles) and untreated LH2 complexes (full squares). The LH2 complexes were excited in the 0-0 band of the carotenoid S_2 state at 515 nm. All kinetics are normalized to maximum. Solid lines represent fits.

or spheroidenone. This confirmed further that the B800 BChl *a* represents the dominant acceptor in carotenoid-BChl energy transfer via the S_1 state. Interestingly, the B800:B850 branching ratio was not affected by conjugation length, indicating that even for spheroidenone having an S_1 energy of ~13000 cm^{-1}, the S_1-B800 pathway remains dominant. The significance of the B800 BChl *a* molecule is further underlined by reports that it is likely the only acceptor in the minor energy transfer routes via the hot S_1 state or the S* state (Papagiannakis et al., 2003a).

IV. Energy Transfer in Light-Harvesting 1 Complexes and Reaction Centers

Carotenoid-BChl energy transfer in LH1 complexes has been much less studied, and most of our current knowledge is limited to information provided by time-resolved studies of the LH1 complex from *Rsp. rubrum*. First experiments, however, were carried out by Ricci et al. (1996) who demonstrated efficient energy transfer via the S_2 state of spheroidene in the LH1 complex from *Rba. sphaeroides*. Based on the comparison between fluorescence up-conversion data on spheroidene in solution and in LH1 they concluded that S_2-mediated energy transfer operates with 65% efficiency (energy transfer time of 90 fs), which is even more efficient than for the spheroidene-containing LH2 complex (Ricci et al., 1996). Later experiments on LH1 complexes from *Rsp. rubrum* containing the long (N=13) carotenoid, spirilloxanthin, revealed only 35% efficient S_2-mediated energy transfer (Gradinaru et al., 2001). Moreover, it was shown that no energy transfer proceeds via the S_1 state of spirilloxanthin. Thus, 35% is the total efficiency of the spirilloxanthin-BChl energy transfer, in agreement with earlier results based on measurements of fluorescence excitation spectra (Rademaker et al., 1980). The absence of the S_1-mediated energy transfer pathway for spirilloxanthin likely results from its very long conjugated system which pushes its S_1 energy too low to transfer energy to BChl *a*. Measurements of the S_1-S_2 spectra of spirilloxanthin in the LH1 complex determined the S_1 energy of the carotenoid to be 11500 cm^{-1}, which is below the energy of both B800 and B850 Q_y transitions (Papagiannakis et al., 2003b).

An investigation of LH1 complexes reconstituted with carotenoids with conjugation lengths in the range 9–13 showed that carotenoid-BChl energy transfer in LH1 obeys the same trend as shown earlier for LH2. The overall carotenoid-BChl energy transfer decreases with increasing conjugation length, dropping from 78% for LH1 with neurosporene (N=9) to 36% for the spirilloxanthin-containing LH1 complex (Akahane et al., 2004). These are slightly less than values obtained for LH2. These authors found efficiencies for the S_1-mediated energy transfer of 20 and 19% for LH1 complexes reconstituted with neurosporene or spheroidene, respectively. No transfer via the S_1 state (efficiency <3%) was observed for carotenoids with N≥11, in agreement with previous studies (Akahane et al., 2004). The values of 20 and 19% for neurosporene and spheroidene are much lower than 94 and 82% obtained for LH2 complexes (Zhang et al., 2000; Polívka et al., 2004). However, it should be noted that the low efficiency of the S_1-mediated energy transfer obtained by Akahane et al. (2004) may be attributed to involvement of the $1B_u^-$ state that, according to the data analysis by Akahane et al. (2004), both transfers energy to BChl *a* and forms a triplet state with appreciable efficiency. Thus, as the $1B_u^-$ state was not included in the analysis carried out by Zhang et al. (2000) or Polívka et al. (2004), the values are not directly comparable. Besides the $1B_u^-$ pathway suggested by Akahane et al. (2004), the LH1 complex of *Rsp. rubrum* is also a system in which the S* state yield is pronounced. In this case, S* does not transfer energy to BChl *a*, but it is a precursor for the fast (~12 ps) formation of a spirilloxanthin triplet state. A relatively high triplet yield of 25–30% was explained on the basis of a conformational distortion of spirilloxanthin in the LH1 complex, promoting triplet formation via singlet homofission from the S* state (Gradinaru et al., 2001).

Carotenoid-BChl energy transfer has also been studied in reaction centers (RC) of purple bacteria. Unlike the antenna complexes, RCs bind carotenoid in a 15,15′-cis configuration. Each RC has one carotenoid that is located in proximity to the BChl *a* monomer (B_B) in the B branch (Allen et al., 1987). Earlier studies of RC from *Rba. sphaeroides* employing fluorescence excitation measurements reported ~75% efficiency of energy transfer from spheroidene to the primary donor (P), and B_B BChl *a* was suggested to play a role of a mediator in this process (Frank et al., 1993). Later, these findings were elaborated by means of femtosecond time-resolved spectroscopy. Lin et al. (2003) studied the RC from aerobically grown *Rba. sphaeroides* that contains the carotenoid spheroidenone. By comparing the spheroidenone S_1

lifetime in RC (1.6 ps) with that in solution (6 ps), they concluded that energy transfer occurs preferentially from the S_1 state of the carotenoid with an efficiency of 75%. This work was recently extended to RCs accommodating neurosporene and spheroidene (Lin et al., 2006). For these two carotenoids the S_1-mediated energy transfer operated with 96 and 84% efficiencies, respectively, matching perfectly the values obtained for the S_1-mediated energy transfer in LH2 complexes (Zhang et al., 2000, Polívka et al., 2004). Thus, regardless of whether the carotenoid is in *all-trans* (LH2) or 15,15′-*cis* (RC) configuration, the S_1-mediated energy transfer proceeds with the same efficiency. Lin et al. (2006) also found that at least 30% of energy must proceed via the S_2 channel. This group also studied a mutant where the B_B pigment was replaced by bacteriopheophytin which has a higher S_1 energy than B_B. In this mutant, the efficiency of total energy transfer from spheroidenone to P decreased substantially, supporting the earlier notion that the B_B BChl *a* is a mediator for carotenoid to P energy transfer in RCs (Lin et al., 2006).

V. Outlook

Structural information in combination with advances in time-resolved spectroscopic techniques and data analysis during the past few years has vastly improved our knowledge of energy transfer processes between carotenoids and BChl in LH2 and LH1 complexes. On the other hand, these improvements brought a number of yet unanswered questions regarding origin of the involved states, routes and efficiencies of energy transfer, and effects of protein environment. An important issue is understanding the roles of the S* and $1B_u^-$ states proposed as energy donors in carotenoid-BChl energy transfer, because the results obtained by the various research groups are often contradictory (see Polívka and Sundström, 2004, for review). The recent finding that the excited state dynamics of carotenoids in LH2 complexes depend on the intensity of excitation pulses adds a new dimension to the problem (Papagiannakis et al., 2006a). Clearly, intensity dependencies should be included in all future experiments. Although Papagiannakis et al. (2006) explored only the intensity dependence of S* state formation, it is likely that similar patterns may also be expected for the $1B_u^-$ state. Ultrafast spectral features assigned earlier to a short-lived intermediate state (Cerullo et al., 2002) were subsequently identified as being due to nonlinear effects caused by high-intensity femtosecond pulses (Kosumi et al., 2005). Another issue that deserves attention in future experiments is the role of vibrational relaxation in energy transfer. Although it was included in some data analyses, most studies attempting to disentangle the complex pattern of carotenoid-BChl energy transfer do not include vibrational levels (Koyama et al., 2004). To resolve these issues, new experimental approaches will be particularly interesting, for example the recently introduced ultrafast methods to control energy flow in LH2 complexes (Wohlleben et al., 2005). Pump-dump-probe (Papagiannakis, 2006c), femtosecond resonance Raman (McCamant et al., 2003) and two-dimensional electronic spectroscopy (Zigmantas, 2006) all hold the promise for elucidating the controlling features of energy transfer pathways in these systems.

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