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Redox Functions of Carotenoids in Photosynthesis†

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ABSTRACT: Carotenoids are well-known as light-harvesting pigments. They also play important roles in protecting the photosynthetic apparatus from damaging reactions of chlorophyll triplet states and singlet oxygen in both plant and bacterial photosynthesis. Recently, it has been found that β-carotene functions as a redox intermediate in the secondary pathways of electron transfer within photosystem II and that carotenoid cation radicals are transiently formed after photoexcitation of bacterial light-harvesting complexes. The redox role of β-carotene in photosystem II is unique among photosynthetic reaction centers and stems from the very strongly oxidizing intermediates that form in the process of water oxidation. Because of the extended π-electron-conjugated system of carotenoid molecules, the cation radical is delocalized. This enables β-carotene to function as a "molecular wire", whereby the centrally located oxidizing species is shuttled to peripheral redox centers of photosystem II where it can be dissipated without damaging the system. The physiological significance of carotenoid cation radical formation in bacterial light-harvesting complexes is not yet clear, but may provide a novel mechanism for excitation energy dissipation as a means of photoprotection. In this paper, the redox reactions of carotenoids in photosystem II and bacterial light-harvesting complexes are presented and the possible roles of carotenoid cation radicals in photoprotection are discussed.

Functions of Carotenoids in Photosynthesis

Carotenoids perform an enormously diverse set of functions in photosynthetic organisms. They protect the photosynthetic apparatus by quenching chlorophyll triplet states which inhibits the formation of singlet state oxygen (*1*−*3*). They scavenge singlet oxygen directly (*4*, *5*). They deactivate excess chlorophyll (Chl)[1] excitation energy beyond that which is required for photosynthesis (*6*, *7*). They act as antenna pigments and capture light energy in the visible spectrum where chlorophyll is not a very efficient absorber

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[1] Abbreviations: BChl, bacteriochlorophyll; B800, BChl species in the LH2 complex that absorbs at 800 nm; B850, BChl species in the LH2 complex that absorbs at 850 nm; Car, carotenoid; Chl, chlorophyll; CP43, 43 kDa chlorophyll-binding subunit of PSII; CP47, 47 kDa chlorophyll-binding subunit of PSII; CT, charge transfer; Cyt, cytochrome; D1, D1 polypeptide of PSII; D2, D2 polypeptide of PSII; EPR, electron paramagnetic resonance; HPLC, high-pressure liquid chromatography; LH2, light-harvesting complex 2 from purple photosynthetic bacteria; P680, primary donor chlorophyll of PSII; PSII, photosystem II; Y_Z and Y_D, redox-active tyrosines in the D1 and D2 polypeptides of PSII, respectively.

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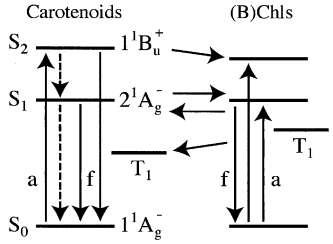

FIGURE 1: Low-lying energy states of carotenoids and (B)Chls and the various photochemical processes that can occur between them. The dashed lines represent nonradiative processes. a is absorption. f is fluorescence. B_u^+ and A_g^- are the symmetry representations of the electronic states of carotenoids in the idealized C_{2h} point group.

(*8*−*11*). They stabilize light-harvesting protein structures (*12*−*14*). They are involved in the regulation of energy flow to and from Chl (*6*, *7*, *14*−*20*). Most of these functions are made possible by the fact that many naturally occurring carotenoids have excited singlet states higher in energy and triplet states lower in energy than the corresponding excited states of Chl (Figure 1) and molecular oxygen. This allows light harvesting and photoprotection to be thermodynamically favorable (*21*).

The simple carotenoid state diagram presented in Figure 1, belies the complexity of the photophysics of these molecules. As the arrows in the figure indicate, carotenoids have many different avenues of deactivation and energy transfer to and from Chl and bacteriochlorophyll (BChl). For example, in light-harvesting complexes, carotenoids are able to transfer energy via their S_1 and S_2 states. The extent to which the overall transfer efficiency is partitioned between these donor states depends on the structure of the molecule. In general, carotenoids with lower ($N < 10$, where N is the number of π-electron-conjugated double bonds) extents of π-electron conjugation transfer energy primarily from S_1, whereas those having higher ($N > 10$) extents of π-electron conjugation transfer energy primarily from S_2 (*22*−*24*). The idea of this two-donor state energy transfer scheme has persisted for some time as an adequate model for carotenoid-to-(B)Chl energy transfer. However, the situation has recently become more complicated because of several reports of additional excited states in the vicinity of S_1 and S_2 that may play a role in controlling the dynamics and mechanism of energy transfer (*25*−*29*). In particular, a low-lying $1^1B_u^-$ state has been suggested to control nonradiative relaxation from the strongly allowed S_2 ($1^1B_u^+$) state (*30*, *31*). Also, recent investigations have suggested the presence of low-lying $3^1A_g^-$ states in carotenoids having chain lengths of N >10 (*32*), and ultrafast spectroscopic experiments (*33*) have postulated that an intermediate singlet state denoted S_x controls the dynamics of nonradiative relaxation between the S_2 ($1^1B_u^+$) and S_1 ($2^1A_g^-$) states. Another singlet state lying between S_1 and S_2 and denoted S* is thought to be an intermediate in the depopulation of S_2 and has been suggested to play a role in carotenoid triplet state formation in several light-harvesting complexes (*34*−*36*), and new data from various combinations of pump−probe transient absorption spectroscopic techniques have been interpreted in terms of yet another carotenoid excited state denoted $S^‡$ (*37*). Experiments aimed at elucidating the molecular origins of these various spectroscopic observations are ongoing in several laboratories.

While carotenoids are generally thought to function as energy donors in light-harvesting complexes, their role as electron donors has only recently emerged as being potentially important in the photosynthetic process. Carotenoid radical cation formation has been well-documented by electrochemistry experiments carried out on molecules dissolved in solvent solutions (*38*−*45*). In addition, Moore and co-workers routinely employ carotenoid derivatives as electron donors in synthetic carotenoporphyrin molecules which serve as models for understanding the photochemistry and photophysics of photosynthetic reaction center and antenna complexes (*46*−*52*). The extended π-electron-conjugated chain of carotenoid molecules makes them susceptible to oxidation by other molecules that are in proximity, provided the reduction potential of the electron acceptor is sufficiently accommodating to render the redox chemistry thermodynamically favored. As will be discussed in this paper, this is apparently the case in photosystem II (PSII) in higher plants, algae, and cyanobacteria and in some light-harvesting pigment−protein complexes from photosynthetic bacteria. The mechanism of radical cation formation in these systems has been the subject of a number of steady state and dynamic optical spectroscopic experiments, electron paramagnetic resonance studies, and quantum mechanical computations. The goal of the investigations is to understand the molecular factors controlling carotenoid radical cation formation and its physiological role in the photosynthetic reaction center and light-harvesting pigment−protein complexes.

Carotenoid Radicals in Photosystem II

The normal function of PSII under physiological conditions is to use light energy to catalyze the oxidation of water to molecular oxygen and the reduction of plastoquinone to plastoquinol (*53*). These reactions occur via a series of light-induced electron transfer reactions that involve chlorophyll, tyrosine, and manganese redox centers on the electron donor side of the system, and pheophytin and plastoquinone on the electron acceptor side. The recently determined X-ray crystal structures of PSII (*54*−*56*) reveal the locations of these redox centers buried within the structure of the transmembrane protein complex (Figure 2).

PSII core complexes (*57*) and D1−D2−Cyt b_{559} reaction center preparations (*58*) have been used to study the roles of carotenoids in PSII. PSII core complexes contain the D1 and D2 subunits, two tightly associated light-harvesting proteins called CP43 and CP47, Cyt b_{559}, several extrinsic proteins, and a number of other small transmembrane protein subunits. The D1−D2 heterodimer forms the reaction center that contains all of the redox cofactors involved in the primary electron transfer steps (Figure 2B). The recently determined crystal structures of PSII have been obtained for cyanobacterial PSII core complexes. On the basis of extraction and HPLC analysis, it was found that the PSII core complex from *Synechocystis* PCC 6803 contains ∼17 Car molecules, of which ∼14 are β-carotene and the rest are mainly zeaxanthin (*59*). The more resolved D1−D2−Cyt b_{559} reaction center complex has been found to contain two β-carotenes (*60*, *61*).

The positions of some of the Car molecules have been modeled in the PSII crystal structures of Kamiya and Shen

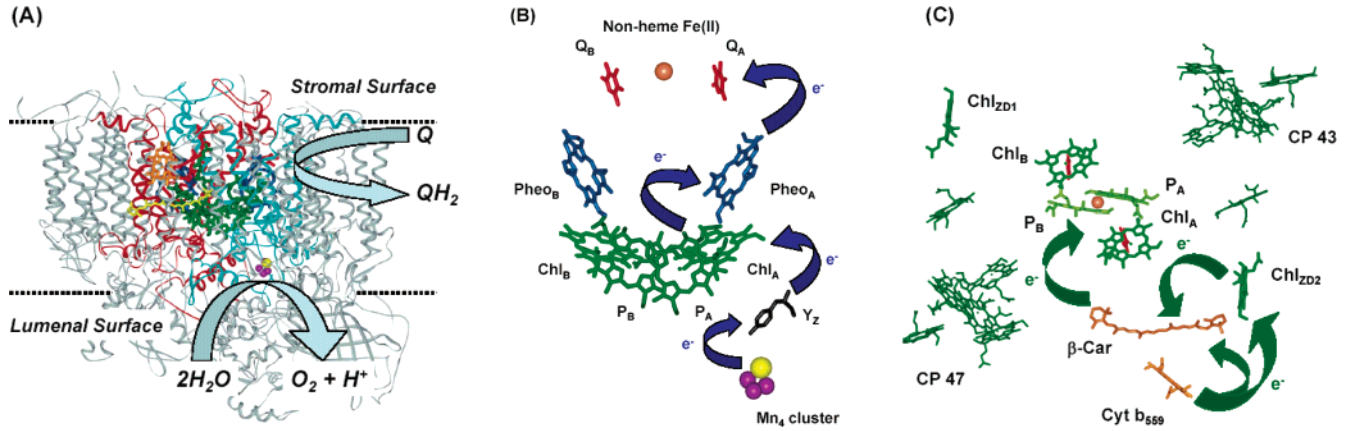

FIGURE 2: Structure of the PSII complex. (A) View along the membrane plane of the overall structure of the PSII complex showing the D1 and D2 proteins in blue and red, respectively, and the remaining proteins in gray. (B) View from the same perspective as in panel A of the redox cofactors involved in the primary electron transfer reactions with arrows denoting the primary electron transfer steps. (C) View along the membrane normal of the redox cofactors involved in the secondary electron transfer reactions with arrows denoting the secondary electron transfer steps involving Chl_Z, Cyt b_{559}, and β-carotene. Coordinates were from Ferreira et al. (*56*).

(*55*) and Ferreira et al. (*56*). Kamiya and Shen (*55*) modeled two carotenoids in the D2 subunit, one of which is in an all-*trans* configuration and the other of which has a bent structure. Ferreira et al. (*56*) modeled seven all-*trans* β-carotenes in the PSII core complex. One of these is in a position in the D2 subunit similar to that of the all-*trans* carotenoid in the Kamiya and Shen (*55*) structure (Figure 2), but the bent carotenoid was not confirmed. The other six β-carotenes modeled in the Ferreira et al. (*56*) structure are located near the periphery of the PSII complex at the interface between CP43 or CP47 and small transmembrane subunits.

To oxidize water, PSII generates a very strong oxidant, $P680^+$ [the cation is localized on the chlorophyll *a* species called P_A (Figure 2)], with an estimated E_m of ~1.3 V (*62*). If not transferred rapidly to the oxygen-evolving complex, the oxidizing equivalent on $P680^+$ can cause oxidative damage to PSII. This is a unique problem for PSII among the various types of photosynthetic reaction centers because only PSII generates an oxidant that is sufficiently strong to oxidize its own chlorophyll and carotenoid light-harvesting pigments [Figure 3 (*62*−*64*)]. As a result of this propensity for oxidative damage, PSII is unique among photosynthetic reaction center complexes in having secondary electron donors. Under conditions when the primary electron donors of PSII are inhibited, such as in Mn-depleted PSII, or at low temperatures when turnover of the oxygen-evolving complex is blocked, alternate electron donors, including Cyt b_{559}, accessory chlorophylls, and β-carotenes, can be photooxidized (*59*). It is known that Cyt b_{559} can be photoreduced by accepting electrons from Q_B (*65*) and photooxidized by donating electrons to $P680^+$ (*66*). Therefore, Cyt b_{559} can participate in a cyclic electron transfer pathway within PSII. In addition, the secondary electron transfer reactions generate Chl cation radicals that are potent quenchers of excitation energy (*67*). It has been proposed that cyclic electron transfer processes and/or quenching by Chl cation radicals play a role under high-light or other stress conditions to protect the PSII reaction center from oxidative damage (*68*).

Electron transfer from Cyt b_{559} to $P680^+$ is thought to occur via a redox intermediate because the edge-to-edge distance separating the heme and P680 is more than 35 Å, which is too large for rapid electron transfer in a single step. Redox-

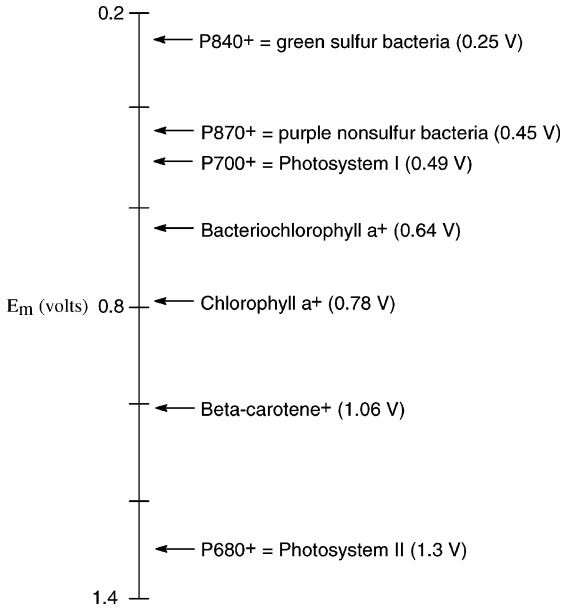

FIGURE 3: Reduction potentials of the primary electron donors in several types of photosynthetic reaction center complexes in comparison to solution values for β-carotene, BChl *a*, and Chl *a* (*62*−*64*).

active Car and/or Chl species appear to be intermediates in the photooxidation of Cyt b_{559}; both Car and Chl photooxidation have been demonstrated in intact PSII core complexes from *Synechocystis* and in PSII-enriched spinach membranes when assessed at low temperatures. However, the pathway(s) of electron transfer involving Cyt b_{559}, Chl, and Car remains unclear, and both linear and branched pathways from Cyt b_{559} to $P680^+$ have been proposed (*69*−*75*).

The first report of Car photooxidation in PSII was demonstrated by flash absorption spectroscopy studies (*76*). A near-IR absorption at 990 nm was observed that is characteristic of Car^+ (Figure 4). However, formation of Car^+ in significant yield required the addition of ferricyanide and a redox uncoupler such as carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene (ANT-2p), or tetraphenylboron (TPB). Phenolic herbicides also have been found to facilitate Car^+ formation (*77*). Because of the requirement for lipophilic anions or phenolic herbicides to facilitate Car^+

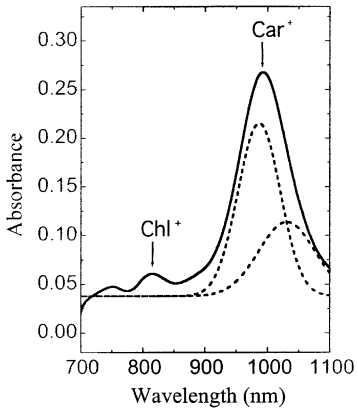

FIGURE 4: Near-IR absorbance spectrum of the β-carotene (Car^+) and chlorophyll *a* (Chl^+) cation radicals generated by illumination at 20 K of PSII core complexes from *Synechocystis* PCC 6803. The dashed lines show the deconvoluted spectra of two distinct β-carotene cation radicals that were identified by kinetic and temperature dependence measurements [adapted from Tracewell and Brudvig (*73*)].

formation in PSII, the prevailing view until recently was that Car photooxidation was not intrinsic to untreated PSII. It is still not clear how the lipophilic anions and phenolic herbicides facilitate Car^+ formation in PSII, but they appear to mediate nonphysiological electron transfer processes (*78*, *79*).

It has long been known that Chl can be photooxidized by illumination of ferricyanide-treated PSII at low temperatures (*80*, *81*). A light-induced EPR signal is formed under these conditions, which was assigned to a Chl cation radical based on the *g* value of 2.0025 and the observation of absorbance changes in the Q_y band of Chl at 670−680 nm. The Chl cation radical can also be identified by its near-IR absorption at 820 nm (see Figure 4) and by high-field EPR spectroscopy (*82*). This redox-active Chl was shown to be a secondary electron donor and was designated Chl_Z^+ (*63*). There are two peripheral Chl molecules that are symmetrically located in the D1 and D2 subunits that have been denoted Chl_{ZD1} and Chl_{ZD2}, respectively. A study of site-directed mutations of the axial histidine ligands to Chl_{ZD1} and Chl_{ZD2}, in conjunction with EPR and resonance Raman studies, identified Chl_{ZD1} as the location of the Chl cation radical in PSII core complexes from *Synechocystis* PCC 6803 (*83*). However, this assignment has been controversial (*84*), and it is likely that both Chl_{ZD1} and Chl_{ZD2} can be photooxidized with species-dependent yields (*72*). Although low-temperature illumination of ferricyanide-treated PSII was also found to form Car^+ (*76*), the predominant opinion until recently was that Chl^+ cations were the physiologically relevant species because of the finding that lipophilic anions or phenolic herbicides are needed to facilitate Car^+ formation in PSII.

More recent studies have shown both Car and Chl_Z can be photooxidized in high yield, both in D1−D2−Cyt b_{559} preparations (*85*, *86*) and, more recently, in PSII core complexes (*69*, *70*, *87*). Photooxidation of Car in D1−D2−Cyt b_{559} preparations requires the addition of an exogenous electron acceptor such as silicomolybdate because this preparation lacks the native plastoquinone electron acceptors and the charge separation is not reversible. In PSII core complexes, which contain a functional Q_A electron acceptor, Car and Chl are reversibly photooxidized in high yield in samples that have been treated with only ferricyanide. Ferricyanide is known to oxidize the heme of Cyt b_{559}, but not the Car or Chl_Z cofactors of PSII. When Cyt b_{559} is preoxidized, Car^+ or Chl_Z^+ is formed by illumination of PSII core complexes at low temperatures, and the charge-separated state is stable for hours at 20 K. If the sample is warmed, the Car^+ and Chl_Z^+ species decay by charge recombination. The reaction is completely reversible when the sample is reilluminated at low temperatures.

At low temperatures, PSII can form only a single stable charge-separated state because the electron acceptor Q_A can accept only one electron (*88*). However, it is found that both Car^+ and Chl_Z^+ are formed in substoichiometric amounts, with the total yield never exceeding 1 equiv per PSII (*72*). Moreover, recent studies have shown that two spectroscopically distinct Car^+ molecules (*73*) and at least two spectroscopically distinct Chl^+ molecules are formed upon low-temperature illumination (*70*, *72*). These observations have been explained by a hole-hopping model (*73*) in which rapid hopping of the oxidizing equivalent among the secondary electron donors allows the hole to migrate to the center with the lowest reduction potential. This explains why Cyt b_{559} is the preferential donor when the heme is reduced because, in this case, the heme is the lowest-potential donor. To explain the observation that a heterogeneous population of Car^+ and Chl^+ is formed in samples in which Cyt b_{559} is oxidized, it was proposed that freezing the sample traps a distribution of conformers, yielding a distribution of redox potentials of Car^+ and Chl^+ (*73*). If the reduction potentials of the Car^+ and Chl^+ molecules among the secondary electron donors are all relatively close, then different PSII complexes may have different Chl or Car species which have the lowest reduction potential. In this case, illumination of a frozen ferricyanide-treated sample will generate a charge separation with a heterogeneous population of Car^+ and Chl^+ species.

The sequence of reactions involving Cyt b_{559}, Car, and Chl in the secondary electron transfer pathways of PSII is not established, but a branched pathway seems to be most likely based on both kinetic studies (*71*) and the locations of the redox centers in the crystal structure (*54*−*56*). There is general agreement that β-carotene is the initial electron donor to $P680^+$ based on the observations that Car^+ is formed in highest yield at the lowest temperatures (4 K) and that the charge-separated state $Car^+Q_A^-$ decays by recombination at a faster rate than $Chl_Z^+Q_A^-$. Moreover, the all-*trans* β-carotene identified in the D2 subunit of PSII by Ferreira et al. (*56*) is well positioned to act as a "molecular wire" in the transfer of electrons from Cyt b_{559} and/or Chl_{ZD2} to $P680^+$. Following oxidation of β-carotene(D2), transfer of the hole to other secondary donors can occur. The closest redox partners to β-carotene(D2) are Cyt b_{559} and Chl_{ZD2}, and it is likely that these centers are direct donors to β-carotene$(D2)^+$. However, to account for the observation that several other spectroscopically distinct Chl and Car species can be photooxidized, there must be a series of subsequent hole-hopping reactions. It is possible that the secondary electron transfer pathways include Chl molecules in the light-harvesting protein CP43, which could provide a novel pathway for hole migration to Chl_{ZD1} (*74*).

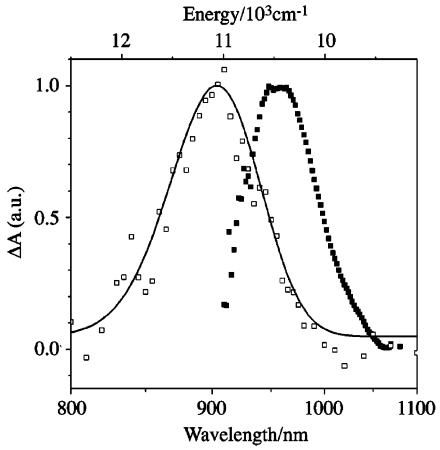

FIGURE 5: Comparison of the differential transient absorption spectrum of the LH2 complex from *Rb. sphaeroides* (■) with the spectrum of the spheroidene radical generated in a methanol solution (□). The spectra were recorded at room temperature. This figure was adapted from Polivka et al. (*89*).

Carotenoid Radicals in Light-Harvesting Complexes

Until recently, no observations of carotenoid radical formation in intact photosynthetic antenna pigment−protein complexes had been reported. Thus, it was unexpected when Polivka et al. (*89*) recently observed a signal associated with a carotenoid radical cation which was formed after excitation of the S_2 state of spheroidene in the LH2 complex of *Rhodobacter sphaeroides*. The assignment of the signal to the carotenoid cation radical was made from its transient absorption in the near-infrared region which showed a clear signal at 960 nm that was very similar to that observed from the spheroidene radical cation generated in a methanol solution (Figure 5) (*89*).

The spheroidene radical signal in the LH2 complex from *Rb. sphaeroides* was produced ~200 fs after light absorption with a yield of 5−8%. The signal decayed in 8 ps. The spectrum of the transient signal was obtained from the wavelength dependence of the amplitude of the 8 ps component extracted from a multiexponential fit of the kinetics after excitation of spheroidene at 515 nm. The spectrum of the spheroidene radical in methanol was obtained over delay times of 18−24 μs using 8 ns duration laser pulses at 355 nm (*89*). These two spectra are similar, but differences in their positions and linewidths are evident (Figure 5). The radical cation spectrum of spheroidene in a methanol solution has a maximum at 905 nm and is shifted by approximately 55 nm (650 cm^{-1}) to higher energy from the maximum of the 960 nm band observed for spheroidene in the LH2 complex. This shift can be explained by the interaction of the spheroidene molecule with the protein environment. Spectral shifts due to the protein environment have been observed for the β-carotene radical in PSII (e.g., Figure 4 and refs *59*, *69*, *70*, *72*, *73*). The differences in linewidth may be due to contributions from competing signals associated with the B850 BChl in the LH2 spectrum which are not present in the spectrum of spheroidene in solution.

Although the assignment of the signal at 960 nm to a spheroidene radical cation was compelling, the experiments did not reveal the mechanism of its formation in the LH2 complex. The simple fact that the radical cation was formed in ~200 fs suggested that an electron acceptor must reside in the proximity. The most likely candidates in the LH2 complex for this role are the B800 or B850 BChl molecules. The atomic resolution structures of the LH2 complexes from *Rhodopseudomonas acidophila* (Figure 6; *90*) and *Rhodospirillum molischianum* (*91*) show that van der Waals contact exists between the bound carotenoid and both the B800 and B850 pigments. It was observed that carotenoid radical cation formation was significantly more pronounced for spheroidene ($N = 10$) in the LH2 complex of *Rb. sphaeroides* than for rhodopsin glucoside ($N = 11$) in the LH2 complex of *Rps. acidophila* (*89*). This suggests that either structural differences in the two LH2 complexes exist that affect the efficiency of radical formation or the reaction depends on the chain length of the carotenoid.

The origin of carotenoid radical formation in LH2 complexes was investigated computationally using the coordinates of the crystal structure of *Rps. acidophila* (*89*). Polivka et al. (*89*) discuss the details of this approach which reveal electronic transitions from occupied valence orbitals of rhodopin glucoside to a p* orbital of a neighboring B800 molecule. It was postulated that these intermolecular charge

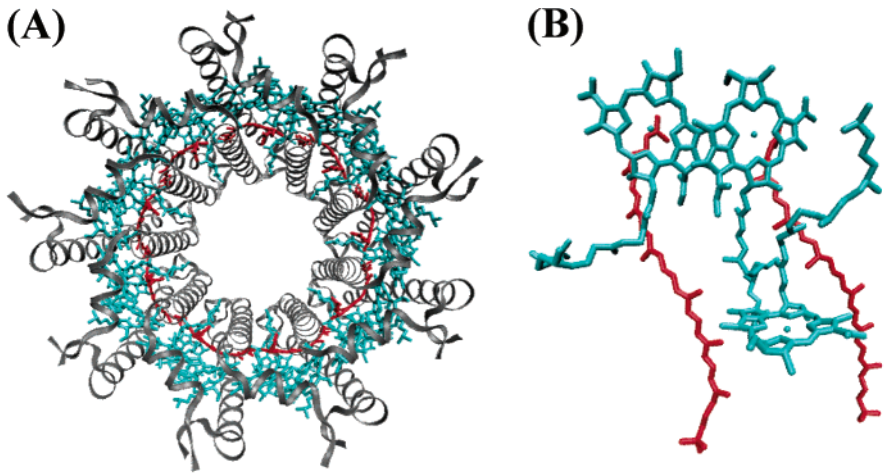

FIGURE 6: Two views of the structure of the LH2 complex from *Rps. acidophila*. (A) Complete LH2 pigment−protein complex. The carotenoid, rhodopsin glucoside, is shown in red. The BChls are shown in blue. (B) Selected pigments from the full ring rotated approximately 90° from the view shown in panel A. The top of the figure shows two of the 18 B850 BChl pigments. At the bottom is one of the nine B800 BChl pigments. Two of the nine rhodopsin glucoside molecules are shown to be in van der Waals contact with both sets of BChl pigments. The coordinates were taken from the structure published by McDermott et al. (*90*).

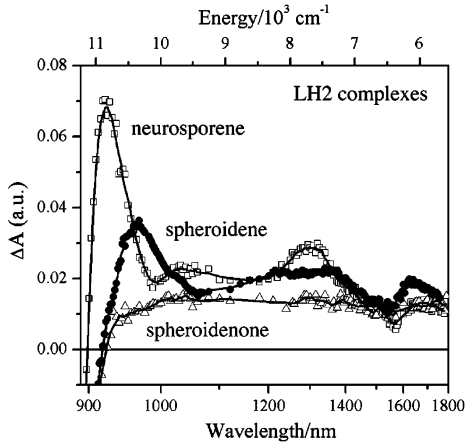

FIGURE 7: Comparison of the differential transient absorption spectra of the LH2 complexes from *Rb. sphaeroides* G1C containing neurosporene (□), Rb. sphaeroides wild type containing spheroidene (●), and an LH2 complex isolated from wild-type *Rb. sphaeroides* grown under aerobic conditions in which spheroidenone selectively accumulates (△). See Polivka et al. (*95*) for additional experimental details.

transfer (CT) states could be formed after fast relaxation from the initially excited carotenoid S_2 state. The limitations in the accuracy of the computations notwithstanding, they show that CT states are indeed present in the excited state manifold of the carotenoid−BChl complex in LH2. Support for this idea has also emerged from quantum calculations performed on carotenoid−Chl *a* (*92*, *93*) and carotenoid−BChl (*94*) complexes where the CT states also appear. These authors propose that electron transfer between the carotenoid and Chl *a* could be a mechanism of nonphotochemical quenching in higher plants (*92*, *93*). At the very least, the presence of energetically low-lying CT states means that they need to be carefully considered when issues of energy transfer among carotenoids and (B)Chl pigments are being considered.

A recent ultrafast optical spectroscopic investigation comparing three different LH2 pigment−protein complexes sought to discover the origin of carotenoid radical formation in these systems (*95*). LH2 complexes from *Rb. sphaeroides* G1C containing neurosporene ($N = 9$, where N is the number of π-electron-conjugated double bonds) and wild-type *Rb. sphaeroides* containing spheroidene ($N = 10$, discussed above) and an LH2 complex isolated from wild-type *Rb. sphaeroides* grown under aerobic conditions in which spheroidenone selectively accumulates ($N = 11$) were investigated. The transient absorption data in the near-IR region show (Figure 7) the yield of the radical formation is highest (10−15%) for the LH2 containing neurosporene and lower for the LH2 containing spheroidene (5−8%), and for the LH2 complex containing spheroidenone, no radical signal was found. The behavior of this systematic series of LH2 complexes from *Rb. sphaeroides* clearly indicates that the efficiency of radical cation formation is dependent on the conjugation length of the carotenoid. The investigation also revealed that the carotenoid radical cation could only be formed upon carotenoid excitation. Therefore, the mechanism of formation is not associated with the quenching of the excited states of BChl. Also, the effect of lithium dodecyl sulfate (LDS) which alters selectively the intensity of the 800 nm-absorbing band in the LH2 complex (*96*) was investigated. It was found that LDS treatment of the LH2 complex from *Rb. sphaeroides* G1C containing neurosporene substantially inhibited carotenoid radical formation. This is proof that the B800 molecule is obligatory for the formation of the carotenoid radical in these systems. Additional optical signals in the near-IR region could be assigned to the BChl radical anion, indicating that the cation−anion radical pair consists of the carotenoid and B800 BChl.

An important question emerges from these observations. Is carotenoid radical cation formation in light-harvesting complexes physiologically significant in photosynthesis, or does it occur simply as a consequence of ultrafast laser excitation of π-electron-conjugated molecules held in proximity? As mentioned above, the quantum computations suggest electron transfer between the carotenoid and Chl could be a mechanism by which excess excited energy is dissipated from the photosynthetic apparatus as a means of photoprotection (*92*, *93*). Redox quenching of the excited state of a pheophytin molecule in the self-assembled film of a carotenoid−pheophytin system has been reported (*97*). Also, Moore and co-workers have reported quenching of porphyrin excited states in synthetic carotenoporphyrin molecules consistent with an electron transfer mechanism (*98*−*100*). However, the conclusions are equivocal. Only some of the carotenoporphyrin molecules quenched porphyrin fluorescence, consistent with an electron transfer mechanism. Other carotenoporphyrins behaved in a manner more suggestive of an energy transfer mechanism (*98*, *101*, *102*). Clearly, more work will be required to reveal the precise mechanism by which carotenoids quench Chl or porphyrin fluorescence in naturally occurring and synthetic systems and to answer the question regarding the physiological role of carotenoid radical cation formation in photosynthetic pigment−protein complexes.

Perspective

A redox role can be added to the already diverse set of functions that carotenoids perform in photosynthesis. Two types of redox reactions involving carotenoids have been identified. First, carotenoids can serve as electron transfer agents in chemical processes that occur at very high reduction potentials. Carotenoids have been utilized in this manner as electron donors in artificial donor−acceptor complexes. However, it has only recently been found that β-carotene also performs this function in natural photosynthesis as an electron transfer intermediate in PSII. Because of their extended π-electron conjugation, carotenoids may act as molecular wires to shuttle electrons rapidly over large distances. This unique property of carotenoids affords the possibility of dissipation of harmful oxidizing equivalents in PSII and may also be important in other radical scavenging reactions that protect the photosynthetic apparatus. Second, carotenoid cation radicals can be generated by excited state photochemical processes. The formation of a transient carotenoid cation radical has been observed very recently in bacterial LH2 complexes. This is an emerging area of carotenoid photochemistry, and further work will be needed to determine whether such excited state redox processes are prevalent among photosynthetic pigment−protein complexes. On the basis of recent computational studies, it is likely that

similar redox reactions involving carotenoids occur in other light-harvesting complexes from higher plants and algae.

The redox reactions of carotenoids in photosynthesis remain to be fully characterized. The molecular details of the electron transfer pathways involving carotenoids in PSII and the physiological role of these reactions still remain to be determined. Of particular interest is determining the factors that control the electron versus energy transfer functions of carotenoids in PSII. In addition, the physiological role of carotenoid cation radical formation in excited states of photosynthetic pigment protein complexes is not yet clear. An interesting hypothesis is that electron transfer between carotenoids and chlorophylls could be a mechanism for quenching of excited states for regulating the process of light energy utilization under conditions of varying light frequency and intensity. Further research will clarify the types of redox roles that carotenoid molecules play in photosynthetic systems. We are hopeful that these studies will provide a fundamental understanding of the energy and electron transfer functions of carotenoids for elucidating the controlling features of natural photosynthesis, but also for designing novel artificial systems. An exciting possibility for future applications is utilization of the principles gleaned from studies of natural photosynthetic systems to design artificial biomolecular nanoscale devices that incorporate the light harvesting, molecular wire, and photoprotection capacities of carotenoids that have been achieved in photosynthetic systems.

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REFERENCES

1. Foote, C. S. (1968) Mechanisms of photosensitized oxidation, *Science 162*, 963−970.
2. Renger, G., and Wolff, C. (1977) Further evidence for dissipative energy migration via triplet states in photosynthesis. The protective mechanism of carotenoids in *Rhodopseudomonas sphaeroides* chromatophores, *Biochim. Biophys. Acta 460*, 47−57.
3. Boucher, F., van der Rest, M., and Gingras, G. (1977) Structure and function of carotenoids in the photoreaction center from *Rhodospirillum rubrum*, *Biochim. Biophys. Acta 461*, 3349−3357.
4. Foote, C. S., Chang, Y. C., and Denny, R. W. (1970) Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection, *J. Am. Chem. Soc. 92*, 5216−5218.
5. Krinsky, N. I. (1971) Function, in *Carotenoids* (Isler, O., Guttman, G., and Solms, U., Eds.) pp 669−716, Birkhauser Verlag, Basel, Switzerland.
6. Demmig-Adams, B. (1990) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin, *Biochim. Biophys. Acta 1020*, 1−24.
7. Niyogi, K. K. (1999) Photoprotection revisited: Genetic and molecular approaches, *Annu. Rev. Plant Physiol. Plant Mol. Biol. 50*, 333−359.
8. Goedheer, J. C. (1959) Energy transfer between carotenoids and bacteriochlorophyll in chromatophores of purple bacteria, *Biochim. Biophys. Acta 35*, 1−8.
9. Cogdell, R. J. (1978) Carotenoids in photosynthesis, *Philos. Trans. R. Soc. London, Ser. B 284*, 569−579.
10. Govindjee and Govindjee, R. (1975) Bioenergetics of photosynthesis, in *Cell Biology* (Govindjee, Ed.) pp 2−50, Academic Press, New York.
11. Frank, H. A., and Christensen, R. L. (1995) Singlet energy transfer from carotenoids to bacteriochlorophylls, in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 373−384, Kluwer Academic Publishers, Dordrecht, The Netherlands.
12. Lang, H. P., and Hunter, C. N. (1994) The relationship between carotenoid biosynthesis and the assembly of the light-harvesting LH2 complex in *Rhodobacter sphaeroides*, *Biochem. J. 298*, 197−205.
13. Sandonà, D., Croce, R., Pagano, A., Crimi, M., and Bassi, R. (1998) Higher plants light harvesting proteins. Structure and function as revealed by mutation analysis of either protein or chromophore moieties, *Biochim. Biophys. Acta 1365*, 207−214.
14. Yamamoto, H., and Bassi, R. (1996) Carotenoids: Localisation and function, in *Oxygenic Photosynthesis: The light reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 539−563, Kluwer Academic Publishers, Dordrecht, The Netherlands.
15. Horton, P., and Boyer, J. R. (1990) Chlorophyll fluorescence transients, in *Methods in Plant Biochemistry* (Harwood, J. L., and Boyer, J. R., Eds.) pp 259−296, Academic Press, London.
16. Demmig-Adams, B., and Adams, W. W. I. (1992) Photoprotection and other responses of plants to high light stress, *Annu. Rev. Plant Physiol. Mol. Biol. 43*, 599−626.
17. Demmig-Adams, B., and Adams, W. W., III (1993) The xanthophyll cycle, in *Carotenoids in Photosynthesis* (Young, A. J., and Britton, G., Eds.) pp 206−251, Chapman and Hall, London.
18. Demmig-Adams, B., and Adams, W. W. (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis, *Trends Plant Sci. 1*, 21−26.
19. Horton, P., Ruban, A. V., and Walters, R. G. (1996) Regulation of light harvesting in green plants, *Annu. Rev. Plant Physiol. Mol. Biol. 47*, 655−684.
20. Young, A. J., Phillip, D., Ruban, A. V., Horton, P., and Frank, H. A. (1997) The xanthophyll cycle and carotenoid-mediated dissipation of excess excitation energy in photosynthesis, *Pure Appl. Chem. 69*, 2125−2130.
21. Frank, H. A., and Cogdell, R. J. (1996) Carotenoids in photosynthesis, *Photochem. Photobiol. 63*, 257−264.
22. Desamero, R. Z. B., Chynwat, V., van der Hoef, I., Jansen, F. J., Lugtenburg, J., Gosztola, D., Wasielewski, M. R., Cua, A., Bocian, D. F., and Frank, H. A. (1998) The mechanism of energy transfer from carotenoids to bacteriochlorophyll: light harvesting by carotenoids having different extents of π-electron conjugation incorporated into the B850 antenna complex from the carotenoidless bacterium *Rhodobacter sphaeroides* R-26.1, *J. Phys. Chem. B 102*, 8151−8162.
23. Zhang, J.-P., Fujii, R., Qian, P., Inaba, T., Mizoguchi, T., Koyama, Y., Onaka, K., and Watanabe, Y. (2000) Mechanism of the carotenoid-to-bacteriochlorophyll energy transfer via the S_1 state in the LH2 complexes from purple bacteria, *J. Phys. Chem. B 104*, 3683−3691.
24. Macpherson, A. N., Arellano, J. B., Fraser, N. J., Cogdell, R. J., and Gillbro, T. (2001) Efficient energy transfer from the carotenoid S_2 state in a photosynthetic light-harvesting complex, *Biophys. J. 80*, 923−930.
25. Sashima, T., Shiba, M., Hashimoto, H., Nagae, H., and Koyama, Y. (1998) The $2A_g^-$ energy of crystalline all-trans-spheroidene as determined by resonance-Raman excitation profiles, *Chem. Phys. Lett. 290*, 36−42.
26. Sashima, T., Nagae, H., Kuki, M., and Koyama, Y. (1999) A new singlet-excited state of all-trans-spheroidene as detected by resonance-Raman excitation profiles, *Chem. Phys. Lett. 299*, 187−194.
27. Sashima, T., Koyama, Y., Yamada, T., and Hashimoto, H. (2000) The $1B_u^+$, $1B_u^-$, and $2A_g^-$ energies of crystalline lycopene, β-carotene, and mini-9-β-carotene as determined by resonance-Raman excitation profiles: Dependence of the $1B_u^-$ state energy on the conjugation length, *J. Phys. Chem. B 104*, 5011−5019.
28. Fujii, R., Ishikawa, T., Koyama, Y., Taguchi, M., Isobe, Y., Nagae, H., and Watanabe, Y. (2001) Fluorescence spectroscopy of all-trans-anhydrorhodovibrin and spirilloxanthin: detection of the $1B_u^-$ fluorescence, *J. Phys. Chem. A 105*, 5348−5355.
29. Fujii, R., Onaka, K., Nagae, H., Koyama, Y., and Watanabe, Y. (2001) Fluorescence spectroscopy of all-trans-lycopene: comparison of the energy and the potential displacements of its $2A_g^-$ state with those of neurosporene and spheroidene, *J. Lumin. 92*, 213−222.
30. Zhang, J.-P., Inaba, T., Watanabe, Y., and Koyama, Y. (2000) Excited-state dynamics among the $1B_u^+$, $1B_u^-$ and $2A_g^-$ states of all-trans-neurosporene as revealed by near-infrared time-resolved absorption spectroscopy, *Chem. Phys. Lett. 332*, 351−358.

31. Rondonuwu, F. S., Watanabe, Y., Zhang, J.-P., Furuichi, K., and Koyama, Y. (2002) Internal-conversion and radiative-transition processes among the $1B_u^+$, $1B_u^-$ and $2A_g^-$ states of all-trans-neurosporene as revealed by subpicosecond time-resolved Raman spectroscopy, *Chem. Phys. Lett. 357*, 376−384.
32. Fujii, R., Inaba, T., Watanabe, Y., Koyama, Y., and Zhang, J. P. (2003) Two different pathways of internal conversion in carotenoids depending on the length of the conjugated chain, *Chem. Phys. Lett. 369*, 165−172.
33. Cerullo, G., Polli, D., Lanzani, G., De Silvestri, S., Hashimoto, H., and Cogdell, R. J. (2002) Photosynthetic light harvesting by carotenoids: detection of an intermediate excited state, *Science 298*, 2395−2398.
34. Papagiannakis, E., Das, S. K., Gall, A., Stokkum, I. H. M., Robert, B., van Grondelle, R., Frank, H. A., and Kennis, J. T. M. (2003) Light harvesting by carotenoids incorporated into the B850 light-harvesting complex from *Rhodobacter sphaeroides* R-26.1: excited-state relaxation, ultrafast triplet formation, and energy transfer to bacteriochlorophyll, *J. Phys. Chem. B 107*, 5642−5649.
35. Papagiannakis, E., Kennis, J. T. M., van Stokkum, I. H. M., Cogdell, R. J., and van Grondelle, R. (2002) An alternative carotenoid-to-bacteriochlorophyll energy transfer pathway in photosynthetic light harvesting, *Proc. Natl. Acad. Sci. U.S.A. 99*, 6017−6022.
36. Wohlleben, W., Buckup, T., Herek, J. L., Cogdell, R. J., and Motzkus, M. (2003) Multichannel carotenoid deactivation in photosynthetic light harvesting as identified by an evolutionary target analysis, *Biophys. J. 85*, 442−450.
37. Larsen, D. S., Papagiannakis, E., van Stokkum, I. H. M., Vengris, M., Kennis, J. T. M., and van Grondelle, R. (2003) Excited-state dynamics of β-carotene explored with dispersed multi-pulse transient absorption, *Chem. Phys. Lett. 381*, 733−742.
38. Deng, Y., Gao, G., He, Z., and Kispert, L. D. (2000) Effects of polyene chain length and acceptor substituents on the stability of carotenoid radical cations, *J. Phys. Chem. B 104*, 5651−5656.
39. Grant, J. L., Kramer, V. J., Ding, R., and Kispert, L. D. (1988) Carotenoid cation radicals: Electrochemical, optical and EPR study, *J. Am. Chem. Soc. 110*, 2151−2157.
40. Hapiot, P., Kispert, L. D., Konovalov, V. V., and Saveant, J.-M. (2001) Single two-electron transfers vs successive one-electron transfers in polyconjugated systems illustrated by the electrochemical oxidation and reduction of carotenoids, *J. Am. Chem. Soc. 123*, 6669−6677.
41. He, Z., and Kispert, L. D. (1999) Effect of electrolytes and temperature on dications and radical cations of carotenoids: electrochemical, optical absorption, and high-performance liquid chromatography studies, *J. Phys. Chem. B 103*, 10524−10531.
42. He, Z., and Kispert, L. D. (1999) Electrochemical and optical study of carotenoids in triton X100 micelles: electron transfer and a large blue shift, *J. Phys. Chem. B 103*, 9038−9043.
43. Khaled, M., Hadjipetrou, A., and Kispert, L. (1990) Electrochemical and electron paramagnetic resonance studies of carotenoid cation radicals and dications: Effect of deuteration, *J. Phys. Chem. 94*, 5164−5169.
44. Khaled, M., Hadjipetrou, A., Kispert, L., and Allendoerfer, R. D. (1991) Simultaenous electrochemical and electron paramagnetic resonance studies of carotenoid cation radicals and dications, *J. Phys. Chem. 95*, 2438−2442.
45. Liu, D., and Kispert, L. D. (1999) Electrochemical aspects of carotenoids, *Recent Res. Dev. Electrochem. 2*, 139−157.
46. Gust, D., Moore, T. A., and Moore, A. L. (1998) Mimicking bacterial photosynthesis, *Pure Appl. Chem. 70*, 2189−2200.
47. Gust, D., Moore, T. A., Moore, A. L., Kuciauskas, D., Liddell, P. A., and Halbert, B. D. (1998) Mimicry of carotenoid photoprotection in artificial photosynthetic reaction centers: triplet−triplet energy transfer by a relay mechanism, *J. Photochem. Photobiol., B 43*, 209−216.
48. Gust, D., Moore, T. A., and Moore, A. L. (1993) Molecular mimicry of photosynthetic energy and electron transfer, *Acc. Chem. Res. 26*, 198−205.
49. Hermant, R. M., Liddell, P. A., Lin, S., Alden, R. G., Kang, H. K., Moore, A. L., Moore, T. A., and Gust, D. (1993) Mimicking carotenoid quenching of chlorophyll fluorescence, *J. Am. Chem. Soc. 115*, 2080−2081.
50. Liddell, P. A., Nemeth, G. A., Lehman, W. R., Joy, A. M., Moore, A. L., Bensasson, R. V., Moore, T. A., and Gust, D. (1982) Mimicry of carotenoid function in photosynthesis: Synthesis and photophysical properties of a carotenopyropheophorbide, *Photochem. Photobiol. 36*, 641−645.
51. Gust, D., and Moore, T. A. (1991) Mimicking photosynthetic electron and energy transfer, in *Advances in Photochemistry* (Volman, D., Hammond, G., and Neckers, D., Eds.) pp 1−63, John Wiley & Sons, New York.
52. Bensasson, R. V., Land, E. J., Moore, A. L., Crouch, R., Dirks, G., Moore, T. A., and Gust, D. (1981) Mimicry of antenna and photoprotective carotenoid functions by a synthetic carotenoporphyrin, *Nature 290*, 329−332.
53. Diner, B. A., and Babcock, G. T. (1996) Structure, dynamics, and energy conversion efficiency in photosystem II, *Adv. Photosynth. 4*, 213−247.
54. Zouni, A., Witt, H. T., Kern, J., Fromme, P., Krauss, N., Saenger, W., and Orth, P. (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution, *Nature 409*, 739−743.
55. Kamiya, N., and Shen, J. R. (2003) Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7 Å resolution, *Proc. Natl. Acad. Sci. U.S.A. 100*, 98−103.
56. Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J., and Iwata, S. (2004) Architecture of the photosynthetic oxygen-evolving center, *Science 303*, 1831−1838.
57. Tang, X.-S., and Diner, B. A. (1994) Biochemical and spectroscopic characterization of a new oxygen-evolving photosystem II core complex from the cyanobacterium *Synechocystis* PCC 6803, *Biochemistry 33*, 4594−4603.
58. Nanba, O., and Satoh, K. (1987) Isolation of a photosystem II reaction center consisting of D1 and D2 polypeptides and cytochrome b_{559}, *Proc. Natl. Acad. Sci. U.S.A. 84*, 109−112.
59. Tracewell, C. A., Vrettos, J. S., Bautista, J. A., Frank, H. A., and Brudvig, G. W. (2001) Carotenoid photooxidation in Photosystem II, *Arch. Biochem. Biophys. 385*, 61−69.
60. Hashimoto, H., Koyama, Y., Ichimura, K., and Kobayashi, T. (1989) Time-resolved absorption spectroscopy of the triplet state produced from the all-trans, 7-cis, 9-cis, 13-cis, and 15-cis isomers of β-carotene, *Chem. Phys. Lett. 162*, 517−522.
61. Gounaris, K., Chapman, D. J., Booth, P., Crystall, B., Giorgi, L. B., Klug, D. R., Porter, G., and Barber, J. (1990) Comparison of the D1/D2/cytochrome-b_{559} reaction center complex of photosystem 2 isolated by two different methods, *FEBS Lett. 265*, 88−92.
62. Rappaport, F., Guergova-Kuras, M., Nixon, P. J., Diner, B. A., and Lavergne, J. (2002) Kinetics and pathways of charge recombination in photosystem II, *Biochemistry 41*, 8518−8527.
63. Thompson, L. K., and Brudvig, G. W. (1988) Cytochrome b_{559} may function to protect photosystem II from photoinhibition, *Biochemistry 27*, 6653−6658.
64. Edge, R., Land, E. J., McGarvey, D. J., Burke, M., and Truscott, T. G. (2000) The reduction potential of the β-carotene·β-carotene couple in an aqueous micro-heterogeneous environment, *FEBS Lett. 471*, 125−127.
65. Buser, C. A., Thompson, L. K., Diner, B. A., and Brudvig, G. W. (1990) Electron-transfer reactions in manganese-depleted photosystem II, *Biochemistry 29*, 8977−8985.
66. Buser, C. A., Diner, B. A., and Brudvig, G. W. (1992) Photooxidation of cytochrome b_{559} in oxygen-evolving photosystem II, *Biochemistry 31*, 11449−11459.
67. Schweitzer, R. H., and Brudvig, G. W. (1997) Fluorescence quenching by chlorophyll cations in photosystem II, *Biochemistry 36*, 11351−11359.
68. Stewart, D. H., and Brudvig, G. W. (1998) Cytochrome b_{559} of photosystem II, *Biochim. Biophys. Acta 1367*, 63−87.
69. Vrettos, J. S., Stewart, D. H., de Paula, J. C., and Brudvig, G. W. (1999) Low-temperature optical and resonance Raman spectra of a carotenoid cation radical in photosystem II, *J. Phys. Chem. B 103*, 6403−6406.
70. Hanley, J., Deligiannakis, Y., Pascal, A., Faller, P., and Rutherford, A. W. (1999) Carotenoid oxidation in photosystem II, *Biochemistry 38*, 8189−8195.
71. Faller, P., Pascal, A., and Rutherford, A. W. (2001) β-Carotene redox reactions in photosystem II: Electron transfer pathway, *Biochemistry 40*, 6431−6440.
72. Tracewell, C. A., Cua, A., Stewart, D. H., Bocian, D. F., and Brudvig, G. W. (2001) Characterization of carotenoid and chlorophyll photooxidation in photosystem II, *Biochemistry 40*, 193−203.
73. Tracewell, C. A., and Brudvig, G. W. (2003) Two redox-active β-carotene molecules in photosystem II, *Biochemistry 42*, 9127−9136.
74. Vasil′ev, S., Brudvig, G. W., and Bruce, D. (2003) The X-ray structure of photosystem II reveals a novel electron transport

pathway between P680, cytochrome b_{559} and the energy quenching cation, Chl_Z^+. *FEBS Lett. 543*, 159−163.
75. Telfer, A. (2002) What is β-carotene doing in the photosystem II reaction centre? *Philos. Trans. R. Soc. London, Ser. B 357*, 1431−1439.
76. Schenck, C. C., Diner, B. A., Mathis, P., and Satoh, K. (1982) Flash-induced carotenoid radical cation formation in photosystem II, *Biochim. Biophys. Acta 680*, 216−227.
77. Mathis, P., and Rutherford, A. W. (1984) Effect of phenolic herbicides on the oxygen-evolving side of photosystem II: Formation of the carotenoid cation, *Biochim. Biophys. Acta 767*, 217−222.
78. Ghanotakis, D. F., Yerkes, C. T., and Babcock, G. T. (1982) The role of reagents accelerating the deactivation reactions of water-splitting enzyme system-Y (ADRY reagents) in destabilizing high-potential oxidizing equivalents generated in chloroplast photosystem II, *Biochim. Biophys. Acta 682*, 21−31.
79. Yerkes, C. T., and Crofts, A. R. (1984) A mechanism for the ADRY induced photo-oxidation of cytochrome b_{559}, in *Advances in Photosynthesis Research* (Sybesma, C., Ed.) pp 489−492, Martinus Nijhoff/Dr. W. Junk, The Hague, The Netherlands.
80. Knaff, D. B., and Arnon, D. I. (1969) Light-induced oxidation of a chloroplast b-type cytochrome at −189 °C, *Proc. Natl. Acad. Sci. U.S.A. 63*, 956−962.
81. Visser, J. W. M., Rijgersberg, C. P., and Gast, P. (1977) Photooxidation of chlorophyll in spinach chloroplasts between 10 and 180 K, *Biochim. Biophys. Acta 460*, 36−46.
82. Lakshmi, K. V., Reifler, M. J., Brudvig, G. W., Poluektov, O. G., Wagner, A. M., and Thurnauer, M. C. (2000) High-field EPR study of carotenoid and chlorophyll cation radicals in photosystem II, *J. Phys. Chem. B 104*, 10445−10448.
83. Stewart, D. H., Cua, A., Chrisholm, D. A., Diner, B. A., Bocian, D. F., and Brudvig, G. W. (1998) Identification of histidine 118 in the D1 polypeptide of photosystem II as the axial ligand to chlorophyll Z, *Biochemistry 37*, 10040−10046.
84. Wang, J., Gosztola, D., Ruffle, S. V., Hemann, C., Seibert, M., Wasielewski, M. R., Hille, R., Gustafson, T. L., and Sayre, R. T. (2002) Functional asymmetry of photosystem II D1 and D2 peripheral chlorophyll mutants of *Chlamydomonas reinhardtii*, *Proc. Natl. Acad. Sci. U.S.A. 99*, 4091−4096.
85. Telfer, A., De Las Rivas, J., and Barber, J. (1991) β-Carotene within the isolated photosystem II reaction center: Photooxidation and irreversible bleaching of this chromophore by oxidized P_{680}, *Biochim. Biophys. Acta 1060*, 106−114.
86. Telfer, A., Frolov, D., Barber, J., Robert, B., and Pascal, A. (2003) Oxidation of the two β-carotene molecules in the photosystem II reaction center, *Biochemistry 42*, 1008−1015.
87. Noguchi, T., Mitsuka, T., and Inoue, Y. (1994) Fourier-transform infrared spectrum of the radical cation of β-carotene photoinduced in photosystem II, *FEBS Lett. 356*, 179−182.
88. de Paula, J. C., Innes, J. B., and Brudvig, G. W. (1985) Electron transfer in photosystem II at cryogenic temperatures, *Biochemistry 24*, 8114−8120.
89. Polívka, T., Zigmantas, D., Herek, J. L., He, Z., Pullerits, T., Cogdell, R. J., Frank, H. A., and Sundström, V. (2002) The carotenoid S_1 state in LH2 complexes from purple bacteria *Rhodobacter sphaeroides* and *Rhodopseudomonas acidophila*: S1 energies, dynamics, and carotenoid radical formation, *J. Phys. Chem. B 106*, 11016−11025.
90. McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J., and Isaacs, N. W. (1995) Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria, *Nature 374*, 517−521.
91. Koepke, J., Hu, X., Schulten, K., and Michel, H. (1996) The crystal structure of the light harvesting complex II (B800−850) from *Rhodospirillum molischianum*, *Structure 4*, 581−597.
92. Dreuw, A., Fleming, G. R., and Head-Gordon, M. (2003) Charge-transfer state as a possible signature of a zeaxanthin-chlorophyll dimer in the non-photochemical quenching process in green plants, *J. Phys. Chem. B 107*, 6500−6503.
93. Dreuw, A., Fleming, G. R., and Head-Gordon, M. (2003) Chlorophyll fluorescence quenching by xanthophylls, *Phys. Chem. Chem. Phys. 5*, 3247−3256.
94. Dreuw, A., and Head-Gordon, M. (2004) Failure of time-dependent density functional theory for long-range charge-transfer excited states: the zincbacteriochlorin-bacteriochlorin and bacteriochlorophyll-spheroidene complexes, *J. Am. Chem. Soc. 126*, 4007−4016.
95. Polívka, T., Pullerits, T., Frank, H. A., Cogdell, R. J., and Sundström, V. (2004) Ultrafast formation of a carotenoid radical in LH2 antenna complexes of purple bacteria, *J. Phys. Chem. B* (submitted for publication).
96. Chadwick, B. W., Zhang, C., Cogdell, R. J., and Frank, H. A. (1987) The effects of lithium dodecyl sulfate and sodium borohydride on the absorption spectrum of the B800−850 light-harvesting complex from *Rhodopseudomonas acidophila* 7750, *Biochim. Biophys. Acta 893*, 444−451.
97. Pan, J., Xu, Y., Sun, L., Sundstrom, V., and Polivka, T. (2004) Carotenoid and pheophytin on semiconductor surface: self-assembly and photoinduced electron transfer, *J. Am. Chem. Soc. 126*, 3066−3067.
98. Cardoso, S. L., Nicodem, D. E., Moore, T. A., Moore, A. L., and Gust, D. (1996) Synthesis and fluorescence quenching studies of a series of carotenoporphyrins with carotenoids of various lengths, *J. Braz. Chem. Soc. 7*, 19−30.
99. Fungo, F., Otero, L., Durantini, E., Thompson, W. J., Silber, J. J., Moore, T. A., Moore, A. L., Gust, D., and Sereno, L. (2003) Correlation of fluorescence quenching in carotenoporphyrin dyads with the energy of intramolecular charge transfer states. Effect of the number of conjugated double bonds of the carotenoid moiety, *Phys. Chem. Chem. Phys. 5*, 469−475.
100. Kodis, G., Herrero, C., Palacios, R., Mariño-Ochoa, E., Gould, S., de la Garza, L., van Grondelle, R., Gust, D., Moore, T. A., Moore, A. L., and Kennis, J. T. M. (2004) Light harvesting and photoprotective functions of carotenoids in compact artificial photosynthetic antenna designs, *J. Phys. Chem. B 108*, 414−425.
101. Gust, D., Moore, T. A., Moore, A. L., Devadoss, C., Liddell, P. A., Heman, R., Nieman, R. A., Demanche, L. J., Degraziano, J. M., and Gouni, I. (1992) Triplet and singlet energy transfer in carotene-porphyrin dyads: Role of the linkage bonds, *J. Am. Chem. Soc. 114*, 3591−3603.
102. Carbonera, D., Di Valentin, M., Agostini, G., Giacometti, G., Liddell, P. A., Gust, D., Moore, A. L., and Moore, T. A. (1997) Energy transfer and spin polarization of the carotenoid triplet state in synthetic carotenoporphyrin dyads and in natural antenna complexes, *Appl. Magn. Reson. 13*, 487−504.

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