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Carotenoids in photosynthesis: structure and photochemistry

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Abstract - Carotenoids from phototrophic bacteria carry out light-harvesting in antenna proteins via carotenoid-to-bacteriochlorophyll singlet-singlet energy transfer and photoprotection in the reaction center via bacteriochlorophyll-to-carotenoid triplet-triplet energy transfer. Spectroscopic studies have permitted elucidation of the explicit routes of these transfers in pigment-protein complexes obtained from the bacterium *Rhodobacter sphaeroides*. The molecular details of these mechanisms are presented and discussed in conjunction with studies revealing the structural features of the complexes.

INTRODUCTION

It has long been known that carotenoids are essential for the survival of photosynthetic organisms (ref. 1 & 2). Carotenoids act as protective devices against irreversible photodestruction of the photosynthetic apparatus (ref. 3). The mechanism of photoprotection involves the quenching of chlorophyll triplet states which prevents the chlorophyll-sensitized formation of singlet state oxygen - a major oxidizing agent of chlorophyll (ref. 4-6). Also, carotenoids may scavenge singlet oxygen directly (ref. 7 & 8). Besides functioning as photoprotectors of the photosynthetic apparatus, carotenoids act as light-harvesting agents, supplementing the light-capturing ability of chlorophyll by absorbing light in regions of the visible spectrum where chlorophyll is not a very efficient absorber (ref. 9-12). Carotenoids transfer this energy with high efficiency to other pigments, and the energy is ultimately trapped in the reaction center. The mechanism of this process involves the transfer of energy from an excited singlet state of the carotenoid to an excited singlet state of chlorophyll (ref. 12). The carotenoid energy state complexion is ideal for both the light-harvesting and photoprotective roles: carotenoids have their singlet states higher in energy and their triplet states lower in energy than the corresponding states of chlorophyll. This allows both light-harvesting and photoprotection to be energetically favorable (ref. 2).

Despite this general knowledge of carotenoid properties gleaned from years of investigations, much remains to be learned about the structures of photosynthetic carotenoids *in vivo* and the molecular features which control their photochemical properties. Significant progress has been made by spectroscopic investigations. In particular, absorption (ref. 6 & 13), resonance Raman (rR) (ref. 14-17), nuclear magnetic resonance (NMR) (ref. 18 & 19) and electron paramagnetic resonance (EPR) (ref. 20) spectroscopies have been used to elucidate the structures of the protein-bound carotenoids and, now, these investigations have been confronted with direct structural determinations by X-ray diffraction of crystalline photosynthetic pigment-protein complexes.

STRUCTURAL DETERMINATIONS OF CAROTENOIDS BOUND TO REACTION CENTERS

The crystallization and X-ray analysis of the photosynthetic bacterial reaction centers of *Rhodopseudomonas viridis* (ref. 21-26), the carotenoidless mutant *Rhodobacter sphaeroides* R-26 (ref. 27-38) and the carotenoid-containing *Rhodobacter sphaeroides* wild type strain 2.4.1 (ref. 27-30 & 39) are landmark achievements providing a structural basis from which to understand the mechanism of the photosynthetic primary electron transfer reaction. These structural determinations are also extremely important in elucidating the various roles carotenoids play in photosynthesis. The arrangement of the reaction center pigments is shown in these studies to be very similar for all the species (ref. 30). (See *e.g.* Fig. 1.) There is an approximate two-fold rotation symmetry which relates the bound bacteriochlorophylls, bacteriopheophytins and quinones. Also, both *Rps. viridis* and *Rb. sphaeroides* 2.4.1 have a bound carotenoid molecule which occurs in a 1:1 stoichiometric ratio with the primary donor (ref. 15 & 20). This molecule is the only chromophore in the reaction center that does not adhere to the approximate two-fold rotation symmetry. The data indicate that the reaction center carotenoid is located near the monomeric accessory bacteriochlorophyll which lies between the carotenoid and the primary donor (ref. 29 & 30). (See Fig. 1.) However, several uncertainties persist concerning the carotenoid structures deduced from the X-ray diffraction studies on *Rps. viridis* and *Rb. sphaeroides* 2.4.1. These include: (1) The electron density map generated by the X-ray diffraction patterns for 1,2-dihydroneurosporene in *Rps. viridis* and spheroidene in *Rb. sphaeroides* 2.4.1 contained only parts of the carotenoid structure. The parts that were revealed were exclusively those involved in the π-electron conjugation. For 1,2-dihydroneurosporene in the reaction center of *Rps. viridis* this was not complete. Thus, the complete structures of the reaction center-bound carotenoids are not known. (2) The temperature factors for the fit to the electron density are extremely high (~50) indicating a large amount of uncertainty in the atomic coordinates for the carotenoid. (3) The structure of 1,2-dihydroneurosporene in the reaction center of *Rps. viridis* was fitted to a chain having every carbon-carbon bond distance equal. It is well known that the bond lengths alternate with bond order in

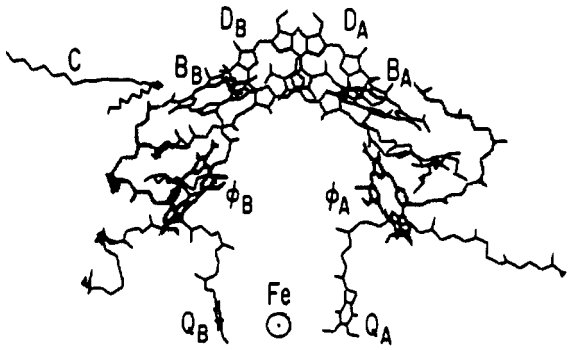

Figure 1. Structure of the reaction center from *Rb. sphaeroides* wild type 2.4.1. This figure was adapted from Allen *et al.* (ref. 29). D_A and D_B comprise the primary donor, B_A and B_B are monomeric bacteriochlorophylls, ϕ_A and ϕ_B are bacteriopheophytins, Q_A and Q_B quinones, Fe is a non-heme iron, and C is the carotenoid, spheroidene.

conjugated polyenes. (4) Molecular orbital calculations (INDO-PSDCI) carried out on the carotenoid X-ray-determined structures predict ground state energies for spheroidene and 1,2-dihydroneurosporene that are unreasonably high (>100kcal/mol above the predicted ground state energies of the all-*trans* isomers). Furthermore, the transition energies and oscillator strengths did not match those experimentally observed for the carotenoid in the reaction center (ref. 40). (5) rR studies carried out on the reaction center carotenoids (ref. 14-18) suggest 15-cis stereoisomer structures. These assignments are not the same as those deduced from the X-ray analyses. (6) NMR studies of spheroidene extracted from the reaction center (ref. 18) and EPR studies of carotenoid-reconstituted reaction centers (ref. 20) suggest a twisted-cis structure. Again this is different from the structure deduced from the X-ray analysis of *Rb. sphaeroides* wild type strain 2.4.1. (7) Arnoux *et al.*(ref. 41) have shown that the carotenoid in crystallized reaction centers of the Y strain of *Rb. sphaeroides* can be fit as a 15-cis isomer. Once again, however, the electron density corresponding to the two extremities of the spheroidene molecule was not well-defined. Additional X-ray diffraction experiments are now being carried out on higher quality *Rb. sphaeroides* reaction center crystals, and new techniques such as solid-state magic-angle sample-spinning (MASS) NMR on specifically-^{13}C-labelled spheroidene reconstituted into *Rb. sphaeroides* R-26 reaction centers (ref. 10) are being brought to bear on this problem of detailing the precise structure of the reaction center-bound carotenoid. Undoubtedly, these new investigations will lead to a refined structure of the molecule that is consistent with all the spectroscopic and structural data.

PHOTOCHEMISTRY OF CAROTENOIDS BOUND TO REACTION CENTER PROTEINS

The reaction centers of phototrophic bacteria contain a primary electron donor consisting of a bacteriochlorophyll dimer ($BChl_2$), an initial electron acceptor known to be a bacteriopheophytin molecule (ϕ_A) with some unspecified involvement of the nearby bacteriochlorophyll (B_A) monomer in the electron transfer , and a subsequent electron acceptor comprising a quinone (Q_A) interacting with a non-heme iron (Fe) atom (ref. 42). Another quinone (Q_B) acts as a terminal acceptor. After absorption of light, the primary donor is promoted to an excited singlet state. The donor becomes oxidized and the acceptors reduced in rapid sequence. Under chemically reducing conditions (~–300mV) or in reaction center preparations devoid of quinones, the primary photochemistry is blocked, and the photoinduced charge separated state of $BChl_2^+\phi_A^-$ undergoes a rapid (~10ns) back reaction. Not all of the reaction centers which back react in this manner return directly to the ground state. Many proceed via a triplet state which develops on the $BChl_2$ dimer. (See Fig. 2.)

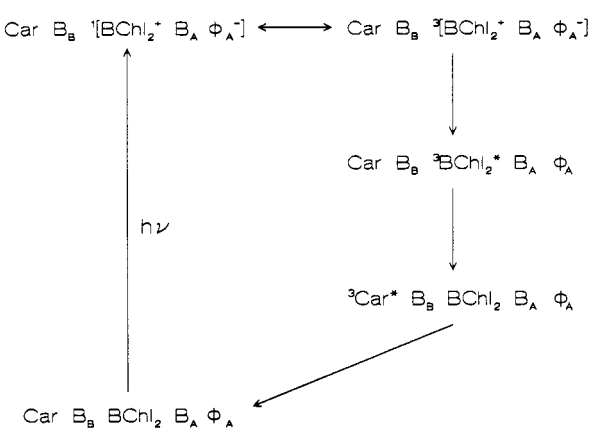

Figure 2. Schematic representation of the participation of the carotenoid in the photosynthetic bacterial reaction center photochemistry under chemically reducing ("blocked") conditions

In the carotenoidless mutant *Rb. sphaeroides* R-26 and in *Rps. viridis* which has a reaction center-bound carotenoid that does not participate in the sequence of back reactions that occur when the primary charge separation is blocked, the $BChl_2$ triplet decays to the ground state. In most carotenoid-containing bacterial

reaction centers, upon charge recombination to form the $BChl_2$ triplet, the state is quenched by the carotenoid triplet. The reaction takes place in ~30ns with approximately 100% efficiency.

Explaining the routes of triplet energy transfer and what energy states are involved in the process has been the focus of several experiments carried out on reaction centers (ref. 43-50), antenna proteins (ref. 51-53), and synthetic model compounds (ref. 51 & 54-58). From the structure presented in Fig. 1 it is tempting to propose that the accessory, monomeric B_B is an intermediate in the triplet energy transfer between the primary donor and the carotenoid. This possibility has been suggested in the literature (ref. 27, 29, 45 & 59). However, there has been no direct experimental evidence to support the hypothesis that the accessory B_B participates in the primary donor-to-carotenoid energy transfer process. Our approach to addressing this problem has been

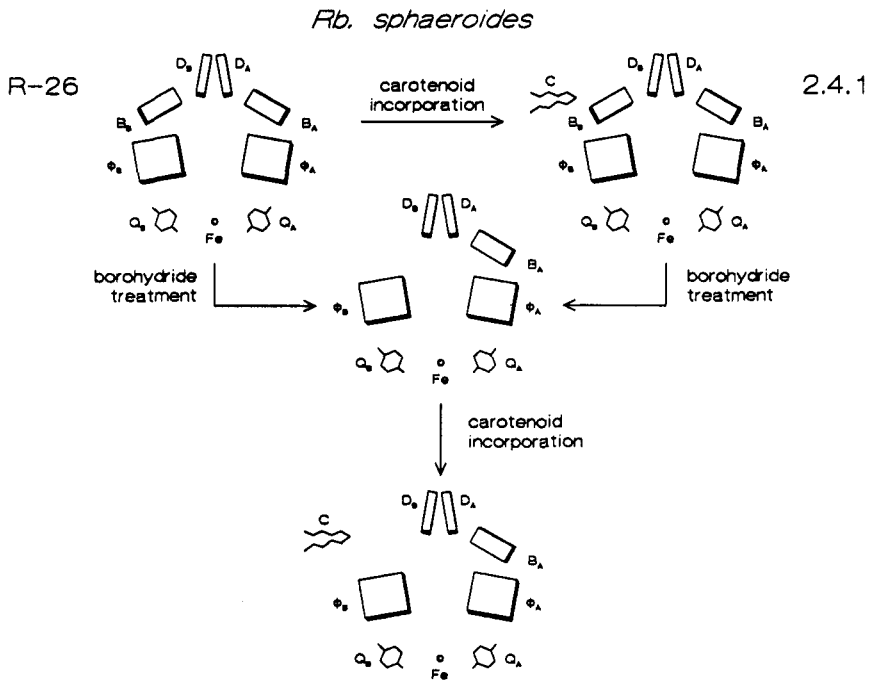

Figure 3. Schematic diagram of carotenoid incorporation into native *Rb. sphaeroides* R-26 reaction centers to form a wild type strain 2.4.1-like complex, and into borohydride-treated *Rb. sphaeroides* R-26 reaction centers.

to use sodium borohydride to extract the monomeric B_B from the reaction centers of the carotenoidless mutant *Rb. sphaeroides* R-26 (ref. 60). The borohydride-treated reaction centers are then reconstituted with the carotenoid, spheroidene (Fig. 3), and the ability of the reaction center to carry out the transfer of triplet energy from the primary donor to the carotenoid is examined by transient optical and EPR spectroscopy.

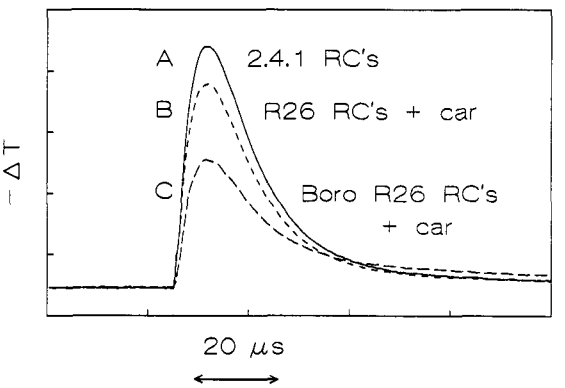

Figure 4. Carotenoid transient optical absorbance changes at 547nm. The signals correspond to the build-up and decay of the carotenoid triplet-triplet absorption. Note that the carotenoid-reconstituted, borohydride-treated reaction centers gave smaller signals indicating a lower yield of carotenoid triplet state formation. Also, note that this decay is biphasic. The longer phase belongs to the primary donor triplet which is not quenched by the carotenoid, but decays to the ground state by intersystem crossing.

Figure 4 shows that the triplet energy transfer from the primary donor to the carotenoid is inhibited in the absence of the accessory B_B. The similarity of the circular dichroism (CD) measurements shown in Fig. 5 demonstrates that spheroidene reconstituted into borohydride-treated *Rb. sphaeroides* R-26 reaction centers is bound in a single site, in the same environment and with the same stereochemical structure as spheroidene reconstituted into native *Rb. sphaeroides* R-26 reaction centers. Hence, the lower yield of carotenoid triplet states in spheroidene-reconstituted, borohydride-treated *Rb. sphaeroides* R-26 reaction centers *versus* spheroidene-reconstituted, native *Rb. sphaeroides* R-26 reaction centers is directly attributable to the absence of the accessory B_B in the former complex. This directly implicates B_B as an intermediate in the primary donor-to-carotenoid triplet energy transfer process.

Therefore, it appears that the construction of the reaction center is ideal for the dual role of rapid electron transfer on the A-subunit side and fast triplet energy transfer to the carotenoid on the B-subunit side.

Structurally isolating these two processes may be the most effective way for nature to accommodate both a stable charge separation *and* photoprotection of the complex.

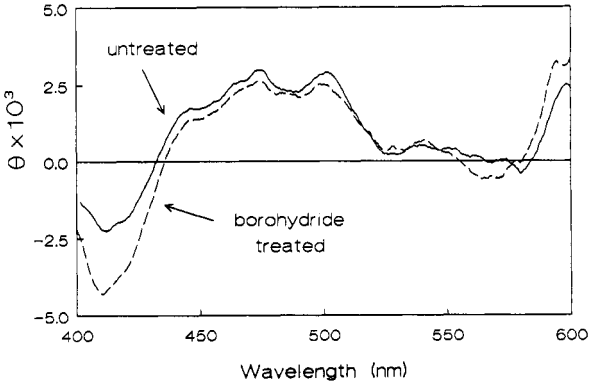

Figure 5. Circular dichroism spectra of spheroidene reconstituted into untreated (native) and borohydride-treated *Rb. sphaeroides* R-26 reaction centers. θ corresponds to ellipticity and is in units of degrees.

PHOTOCHEMISTRY OF CAROTENOIDS BOUND TO ANTENNA PROTEINS

It is known that carotenoids act as light-harvesting agents by transferring singlet energy to bacteriochlorophylls in the antenna of photosynthetic systems. The overall efficiency of this process is variable (ref. 2). The reason for the variability is unknown, but probably depends on several factors such as the structure of the carotenoid, the orientation of the carotenoid with respect to the bacteriochlorophyll, the distance between the carotenoid and bacteriochlorophyll, and the excited state energy complexion of the carotenoid.

The B800-850 light-harvesting complex, so denoted because of its approximate wavelengths of maximum absorption in the near-infrared spectral region, serves as the main antenna complex in several species of phototrophic bacteria (ref. 61). The complex consists of bacteriochlorophyll and carotenoids in a 2:1 stoichiometric ratio bound to two polypeptides (ref. 62 & 63).

Picosecond transient absorption measurements of the dynamics of the bacteriochlorophyll B800-to-B850 singlet energy transfer have revealed transfer times of around 1ps (ref. 64). Measurements of the dynamics of carotenoid-to-bacteriochlorophyll singlet energy transfer yielded ~3-5ps times (ref. 65 & 66). The mechanism of carotenoid-to-bacteriochlorophyll singlet energy transfer is thought to occur via a low-energy ^1Ag -type carotenoid electronic state (ref. 2). The state giving rise to the strong absorption spectra of carotenoids is the so-called ^1Bu state (ref. 2). Internal conversion between these states occurs within ~100fs (ref. 67). Recently, femtosecond transient absorption studies of the energy transfer dynamics in the B800-850 light-harvesting complex of *Rb. sphaeroides* 2.4.1 have been carried out (ref. 68). For complexes solubilized in lauryldimethylamine-N-oxide (LDAO) detergent, the carotenoid-to-bacteriochlorophyll B800 and the carotenoid-to-bacteriochlorophyll B850 energy transfer times were 0.34 and 0.20ps, respectively (ref. 68). The B800-to-B850 energy transfer time was found to be 2.5ps (Fig. 6). For complexes solubilized in lithium dodecyl sulfate (LDS), a detergent which has the effect of greatly diminishing the 800nm bacteriochlorophyll absorption band (Fig. 7) a carotenoid-to-B850 energy transfer time of < 0.2ps was seen, and a portion of the total carotenoid population became decoupled from bacteriochlorophyll. In both LDAO- and LDS-solubilized complexes, an intensity dependent picosecond decay component of the excited B850 population was ascribed to excitation annihilation within minimal units of the complex.

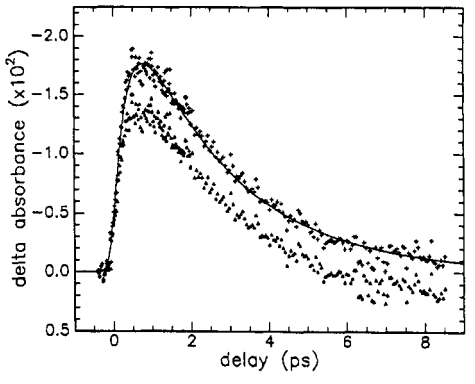

Figure 6. Induced transmission at 800nm after excitation at 510nm in LDAO-solubilized B800-850 complex from *Rb. sphaeroides* wild type strain 2.4.1. The lower curve shows the raw data and the upper curve is corrected for B850 singlet absorption at 800nm. The line through the corrected data is a fit with a rise time of 0.34ps and a fall time of 2.5ps.

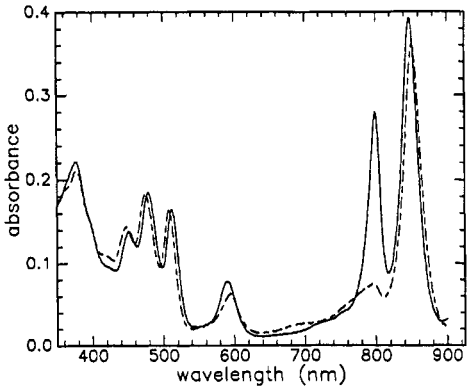

Figure 7. The effect of LDS (dashed line) on the B800-850 complex from *Rb. sphaeroides* wild type strain 2.4.1 purified in LDAO (solid line).

These femtosecond experiments are consistent with a model for the antenna complex where two groups of carotenoids exist. One group is selectively transferring energy to the B800 bacteriochlorophyll. This population of carotenoids becomes decoupled from the transfer process upon solubilization of the complex in LDS. The other group of carotenoids is selectively transferring to the B850 bacteriochlorophylls. The ratio of these two carotenoid populations was not independently determined in this work, but a fit to the data found it to be consistent with previous fluorescence quantum yield results which indicated a Car_{B850}:Car_{B800} population ratio of 3:1 (ref. 69 & 70). The ultrafast nature of these transfers suggests extremely close proximity (essentially van der Waals contact) of the pigments.

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