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FURTHER EVIDENCE FOR DISSIPATIVE ENERGY MIGRATION VIA TRIPLET STATES IN PHOTOSYNTHESIS

THE PROTECTIVE MECHANISM OF CAROTENOIDS IN *RHODOPSEUDOMONAS SPHEROIDES* CHROMATOPHORES*

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SUMMARY

The protective action of carotenoids against irreversible photodestruction was discovered in photosynthetic bacteria by Stanieda and coworkers. In green plant material it was found by Wolff and Witt ((1969) Z. Naturforsch. 24b, 1031–1037 and (1972) Proc. 2nd. Int. Congr. Photosynthesis Res. Stresa (Forti, G., Avron, M. and Melandri, A., eds.), Vol. 2, pp. 931–936, Dr. W. Junk, N. V. Publ. The Hague) that the formation of special carotenoid triplet states (via very rapid energy transfer from excited chlorophylls) and their fast radiationless decay in $\tau_{\frac{1}{2}} \approx 3\ \mu s$ is at least one mechanism for the protective action of carotenoids to irreversible photooxidation of the chlorophylls. Hence, it is anticipated that the same mechanism might be realized also in bacteria. The present study gives evidence for such a "triplet valve" to be established also in bacteria. This conclusion was derived from the following observations:

1. The light-induced difference spectrum shows a bleaching of a carotenoid at three characteristic wavelength between 400 and 500 nm. A positive peak around 533 nm indicates the formation of a carotenoid triplet state.
2. The absorption changes can be induced by red light which excites only bacteriochlorophyll. This indicates an energy transfer from bacteriochlorophyll to carotenoids.
3. The light-induced carotenoid triplets decay radiationless in 3 μs in air-saturated aqueous suspensions of the chromatophores.
4. The carotenoid triplet formation occurs only at actinic flash intensities where the photosynthesis becomes saturated.
5. Addition of dithionite, which blocks photosynthesis, markedly increases the extent of carotenoid triplet formation.

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The different types of exciton migration within the photosynthetic unit are discussed, especially the routes leading to the dissipation of excess excitation energy.

INTRODUCTION

The realization with high efficiency of photosynthesis under light intensities varying up to five orders of magnitude (dim to bright light) requires suitable adaption mechanisms. Generally, two problems have to be solved: (1) At low light intensities the few quanta impinging on the photosynthetic apparatus must be completely funneled into the reaction centers. This is achieved by a bulk pigment system acting via singlet-singlet energy transfer. In this way the cross section area of the reaction centers for photon capture is at least 100-fold increased. This mechanism is long known (for review see refs.1 and 2). (2) At high light intensities there exists an excess of excitation energy due to the limitation of the photochemical turnover rate by the secondary electron transfer reactions. Most of this excess energy is rapidly dissipated by radiative (fluorescence) or non-radiative (heat) decay of the excited singlet states. However, these decay routes are not sufficient for a complete dissipation of the excess of electronically excited states, because via intersystem crossing the formation of chlorophyll triplets with lifetimes of the orders of microseconds can occur which are very reactive, especially with molecular oxygen giving rise to singlet oxygen, $O_2(^1\Delta_g)$. Thus, in order to avoid irreversible oxidative photodestructions, an additional "valve" reaction is required for the dissipation of these excited states.

About 20 years ago carotenoids were discovered to function as protective cell components preventing lethal photodestructions under aerobic conditions in photosynthetic bacteria [3, 4] and algae [5]. Generally, this effect can be achieved by two mechanisms: (a) by a rapid quenching of the chlorophyll triplets via triplet-triplet energy transfer to carotenoids and the subsequent radiation-less decay of the generated carotenoid triplets. The mechanism has been referred to as "triplet valve" [6, 7], (b) by a powerful quenching of $O_2(^1\Delta_g)$ (see ref. 8). Investigations of Wolff and Witt [6, 7] and Mathis [27] on green plant material supported ample evidence for the realization of the former mechanism. It was inferred, that a specific structural organization of carotenoids and chlorophyll within the thylakoid membrane establishes a "triplet valve".

As it was reasonable to assume, that in bacteria the carotenoids of the bulk pigment system might operate in a similar way as in chloroplasts, experiments were made in order to prove the existence of a "triplet valve" also in bacteria. The obtained results reported and discussed in this paper (a first short note was given in the Abstract Book of the 5th Int. Biophys. Congr., Copenhagen, p. 57, P-75, August 1975) provide evidence for the realization of the carotenoid "triplet valve" mechanism in bacteria. The same conclusion was reached very recently by Kung and De Vault [9] and Monger et al. [10], which came to our knowledge after the completion of this manuscript.

MATERIALS AND METHODS

Assay conditions. Chromatophores of cells of *Rhodopseudomonas spheroides*, strain 2.4.1., grown anaerobically under constant illumination at 30 °C in the medium of Sistrom [11], were prepared according to the method described in ref. 12. The reaction mixture contained: chromatophores (bacteriochlorophyll content of the cuvette see legends of the figures), 50 mM KCl, 8 mM $MgCl_2$, 50 mM *N*-tris(hydroxymethyl)methylglycin · NaOH, pH 7, 4, and 10 % sucrose. Other additions are indicated in the legends of the figures.

Measurements. The absorption changes were detected by a repetitive flash spectroscopic technique similar to that published in ref. 13. The optical pathlength of the cuvette was 1.2 or 20 mm, respectively (see legends of the figures), optical bandwidth 5 nm. The excitation flashes ($\tau_{\frac{1}{2}} \approx 500$ ns) of an ultra short flash lamp PEK XE 9-3 (see ref. 6) were passed through a Schott filter RG 1/2 mm. (cut off filters, opens above $\lambda_{50} = 600$ nm). For the attenuation of actinic flash intensities grey filters Schott NG 5 of different thickness were used, time t_d between the flashes: 2s. The signals were stored and averaged in a Fabri-Tek, model 1062. The electrical bandwidth ranged from 0 to 300 kHz.

RESULTS

If one presupposes that the protective function of the carotenoids in bacteria is based on the same principle of a "triplet valve" for the dissipation of excess excitation energy as was shown in algae and higher plants [6, 7], then the formation of carotenoid triplets and the characteristic behaviour of these states as a valve reaction should be observable. In algae and chloroplasts the carotenoid "triplet valve" reaction is frequently analyzed via the fast 520 nm absorption change because at this wavelength a pronounced positive peak arises which reflects the formation of the carotenoid triplet state [6, 7, 37]. In aerobic chloroplast suspensions excitation with short flashes leads to an instantaneous rise (< 20 ns) of the 520 nm absorption change followed by a characteristic 3 μs decay [6]. This 520 nm absorption change is easily separable from the contribution due to the electrochromic effect [14–16] because of the much slower decay of the electrical field (0.1–1 s).

A typical absorption change at 522 nm caused by excitation of chromatophores of *Rh. spheroides* with short flashes is depicted in Fig. 1. There occurs an "instantaneous" rise, too fast to be resolvable by our equipment, followed by a fast decay phase with a half time of approx. 3 μs, which overlaps with a relative slow rise phase accomplished after 100–150 μs (see insert Fig. 1). The fast decay phase ($\tau_{\frac{1}{2}} \approx 3$ μs) provides a first hint for the existence of a "triplet valve" reaction in bacteria because of the kinetical coincidence with the corresponding absorption change observed in chloroplasts. The subsequent slow μs rise reflects via the electrochromic effect the second phase of the formation of an electrical potential difference, $\Delta\varphi$, across the chromatophore membrane which is caused by a vectorial electron transfer from cytochrome *c* to oxidized reaction center bacteriochlorophyll P-870^+ [17, 18]. The separation of both phases reflecting different physical processes can be achieved by an exponential extrapolation as is shown in Fig. 1. The obtained amplitude $\Delta A_{\mathrm{Car_T}}$ is assigned to indicate the function of a protective carotenoid triplet valve in bacteria, whereas ΔA_{el} reflects the well known light-induced electrical potential gradient $\Delta\varphi$ across the chromatophore membrane [19]. If this interpretation is correct, then the amplitudes $\Delta A_{\mathrm{Car_T}}$ and A_{el} are expected to show a sharply different

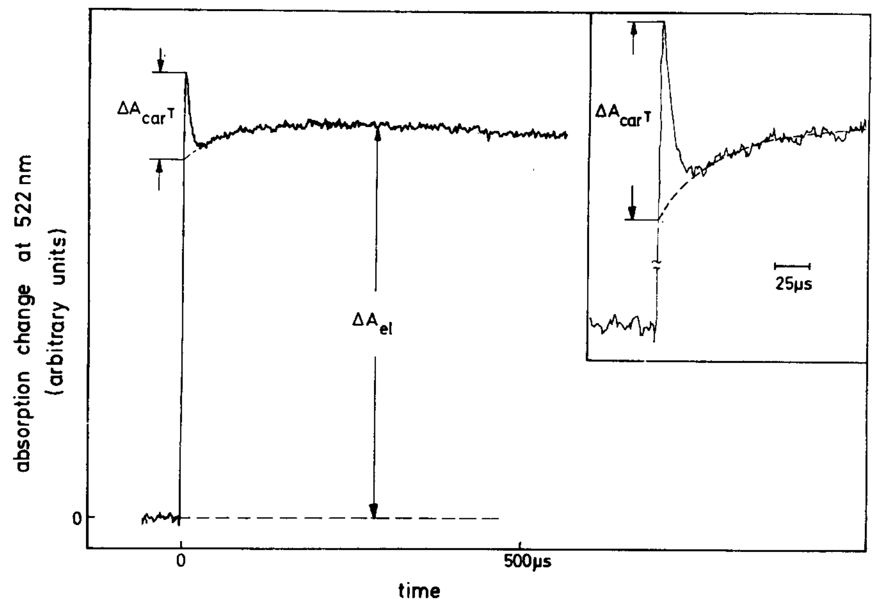

Fig. 1. Kinetics of the flash-induced absorption change at 522 nm in chromatophores of *Rh. spheroides*, strain 2.4.1. Insert: 4-fold magnification of the rapid part ascribed to $\Delta A_{\mathrm{Car_T}}$. 7.5 μM bacteriochlorophyll, optical pathlength 20 mm, 256 signals were averaged. Other experimental details as described in Materials and Methods.

dependency on the light intensity of the actinic flashes. The electrical potential gradient, $\Delta\varphi$, is directly coupled with the primary electron transfer reactions. Therefore, ΔA_{el} as a function of flash intensity has to show the same saturation behaviour as photosynthesis. On the other hand, absorption changes indicating a valve reaction responsible exclusively for the dissipation of excess excitation energy should appear only when the reaction centers become closed thus being unable for exciton trapping. If the photosynthetic units are assumed to be isolated entities with respect to excitation energy transfer, then one obtains theoretically for the dependency on the actinic flash intensity of the amplitudes ΔA_{el} and $\Delta A_{\mathrm{Car_T}}$, respectively, (see Appendix):

$$\Delta A_{\mathrm{el}}(\rho) = (\Delta A_{\mathrm{el}})^{1} \cdot (1 - e^{-\rho}) \tag{1}$$

$$\Delta A_{\mathrm{Car_T}}(\rho) = \Delta\varepsilon \cdot c_0 \cdot d \left[1 - \frac{1}{1-\Phi} \left(e^{-\Phi \cdot \rho} - \Phi e^{-\rho} \right) \right] \tag{2}$$

where ρ = average number of light quanta absorbed per photosynthetic unit, $(\Delta A_{\mathrm{el}})^{1}$ = amplitude of the electrochromic absorption change due to a saturating single turnover flash, $\Delta\varepsilon$ = differential extinction coefficient, d = optical pathlength, c_0 = concentration of carotenoids convertable to $\mathrm{Car_T}^*$ and Φ = quantum yield for $\mathrm{Car_T}^*$ formation (the quantum yield of the photochemical reaction centre reaction is 1, see ref. 20). The experimental data depicted in Fig. 2 fit the theoretical curves sufficiently. Eqn. 1 is applied to calibrate the actinic flash intensity into units of ρ. Introducing suitable values for $\Delta\varepsilon_{522\,\mathrm{nm}}$ ($3 \cdot 10^4$ $\mathrm{M}^{-1} \cdot \mathrm{cm}^{-1}$ as compared to 10^5 $\mathrm{M}^{-1} \cdot \mathrm{cm}^{-1}$ for the peak (see ref. 10) at 533 nm, see Fig. 3) and the size of the photo-

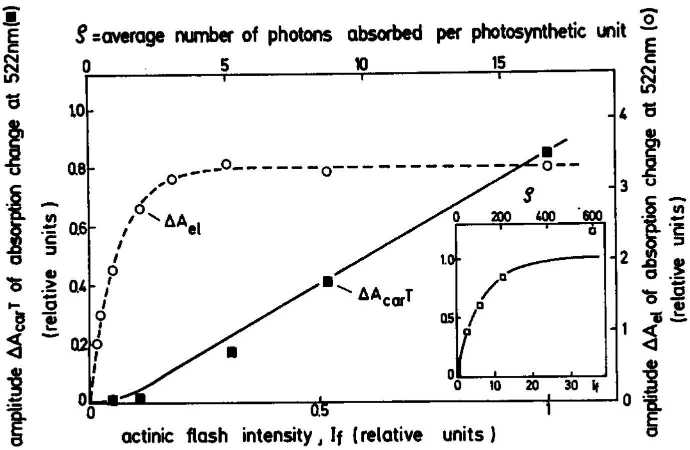

Fig. 2. Dependency of the amplitudes ΔA_{CarT} and ΔA_{el} of the 522 nm absorption change on the intensity of the actinic flashes in *Rh. spheroides*, strain 2.4.1. For experimental details and the separation of the amplitudes ΔA_{CarT} and ΔA_{el} see Fig. 1 and Materials and Methods. Ordinate in units of $\Delta I/I = 10^{-3}$, except for the insert, which gives the number of $\mathrm{Car_T}^*$ per photosynthetic unit. The theoretical curves are evaluated according to Eqn. 1 (dotted line) and Eqn. 2 (full line). The insert gives the theoretical curve (Eqn. 2) for much higher intensities, the open circles are experimental data redrawn from Fig. 4B of ref. 10. The black bar at the origin indicates the dimension of Fig. 2 within the larger scale of the insert.

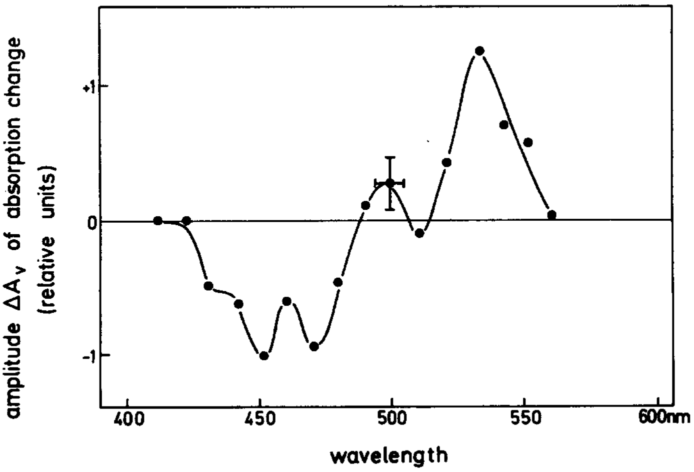

Fig. 3. Transient spectrum of the formation of the carotenoid triplet state in chromatophores of *Rh. spheroides*, strain 2.4.1. 150 μM bacteriochlorophyll, optical pathlength 1.2 mm, 1024 signals were averaged.

synthetic unit (about 200 bacteriochlorophyll) one derives from the measured $\Delta A_{\mathrm{Car_T}}$ that about 0.15 $\mathrm{Car_T}^*$ per photosynthetic unit are generated at $I_f = 1$. Then, according to Eqn. 2, Φ results to approx. 0.01, a value one order of magnitude lower than earlier estimates made for chloroplasts (see ref. 21). By extrapolation one can estimate that at much higher intensities about 1 $\mathrm{Car_T}^*$ should be formed per photosynthetic unit. These results are in excellent agreement with the current data of Monger et al. [10] obtained under very distinct excitation conditions (25-ns flashes of much higher intensity), which are given for comparison in the insert of Fig. 2. At extremely high intensities a $\mathrm{Car_T}^*$ formation with drastically reduced quantum yield takes place [10], which is not described by Eqn. 2.

The above mentioned measurements, in analogy to our earlier results in chloroplasts [6, 7], support the assumption that the $\Delta A_{\mathrm{Car_T}}$ component of the 522 nm absorption change indicates a valve reaction for dissipation of excess excitation energy only.

In order to substantiate that the substance, responsible for the valve reaction, is a carotenoid triplet state, the difference spectrum of $\Delta A_{\mathrm{Car_T}}$ was measured in the range of 400–560 nm (see Fig. 3). The obtained spectrum of $\Delta A_{\mathrm{Car_T}}$ closely resembles to that found in spinach chloroplasts for carotenoid triplet formation [6, 37]. 3 negative peaks at 450, 470 and 510 nm, respectively, and a hump probably indicating a fourth peak at 430 nm are observable. This result is in agreement with the assumption of a light-induced carotenoid bleaching because the absorption spectra due to the carotenoid components spheroidene and spheroidenone of *Rh. sheroides*, strain 2.4.1, are characterized in solution (light petroleum or cyclohexane) by absorption maxima at 430, 453–460, 483–485 and 515 nm, respectively [22]. The strong positive peak at 533 nm supports evidence for the formation of light-induced carotenoid triplet [23–25] though a slight bathochromic shift is observed in vivo. In Fig. 3 appears additionally a small positive peak at 498 nm which has no corresponding counterpart in chloroplasts. Despite uncertainties of the data (see cross at 500 nm) its existence might be realistic, because it was also found by Monger et al. [10]. In toto, the difference spectrum of Fig. 3 confirms the assignment to a carotenoid triplet formation of the light-induced absorption changes $\Delta A_{\mathrm{Car_T}}$.

The decay kinetics of the carotenoid triplets of in vivo and in vitro systems were found to be accelerated by paramagnetic quenchers like O_2 or NO [26, 27]. Therefore, the removal of oxygen from the chromatophore suspension should lead to a slower decay time of $\Delta A_{\mathrm{Car_T}}$. This was observed by the finding that dithionite addition causes an increase of the half decay time of $\Delta A_{\mathrm{Car_T}}$ from 3 to 6 μs. On the other hand, dithionite does not only decrease the oxygen content of the cuvette, but, more important, it also blocks photosynthesis by reduction of the acceptor side. Therefore, the amplitude of $\Delta A_{\mathrm{Car_T}}$ should increase due to a higher degree of excess excitation energy at the same intensity of the actinic flashes. Indeed, in the presence of dithionite the amplitude $\Delta A_{\mathrm{Car_T}}$ is increased up to 5-fold (see Discussion).

It should be mentioned that, in contrast to the effect of dithionite, the addition of $K_3[Fe(CN)_6]$ blocking the photochemistry of the reaction centers by the chemical oxidation of *P*-870 leads to a decrease of the positive $\Delta A_{\mathrm{Car_T}}$-peak rather than to the anticipated increase. It could be possible that $K_3[Fe(CN)_6]$ not only acts on *P*-870 but additionally influences (oxidizes?) the carotenoids. Further experiments are required to clarify this point (see also Discussion).

DISCUSSION

The close similarity of the results presented in this paper for bacteria and those reported earlier for algae and chloroplasts [6, 7] support strong evidence for a common mechanism of protection by carotenoids to radiative damage throughout the different types of photosynthesizing organisms. Accordingly, the bacterial carotenoids are proposed to build up a "triplet valve" analogous to that discovered in algae and chloroplasts.

This assumption is based on the following arguments: (1) The difference spectrum of Fig. 3 indicates the light-induced carotenoid triplet formation. (2) As the singlet-triplet crossover efficiencies are known to be rather low for numerous polyenes including bacterial carotenoids, the triplet states of carotenoids are very poorly excitable intramolecularly [28]. On the other hand, the probability of sensitized carotenoid triplet formation by an energy transfer from excited triplet states of other molecules like chlorophylls was shown to be much higher in vitro [28] as well as in chloroplasts [6, 7, 27]. Therefore, the carotenoid triplets observed in bacteria are generated by triplet-triplet energy transfer. This conclusion is experimentally confirmed by the fact that the difference spectrum, characteristic for the carotenoid triplet formation, is induced by red light which is absorbed by bacteriochlorophyll (BChl) but not by carotenoids. (3) A remarkable carotenoid triplet formation does not occur before the photochemical turnover of the reaction centers becomes saturated (see Fig. 2). Hence, the triplet-triplet energy transfer via carotenoids dissipates only excess excitation energy. (4) Addition of dithionite which blocks photosynthesis markedly increases the extent of $\Delta A_{\mathrm{Car_T}}$.

Very recently, Kung and De Vault [9] as well as Monger et al. [10] came to similar conclusions. However, their excitation conditions significantly differ from ours. At the highest intensities of our actinic flash the average time interval between two photons absorbed by the same photosynthetic unit is of the order of 20 ns. This time is long in comparison to the lifetime of $\mathrm{BChl_S}^*$, of the same order of magnitude as the lifetime of $\mathrm{BChl_T}^*$ in carotenoid-containing chromatophores [10], but short in comparison to the lifetime of $\mathrm{Car_T}^*$. That means, singlet-singlet exciton annihilation can be excluded, triplet-singlet annihilation is rather small and $\mathrm{Car_T}^*$ decay during the flash is negligible. Therefore, our estimated quantum yield Φ for $\mathrm{Car_T}^*$ formation should be equal to the probability of $\mathrm{BChl_T}^*$ generation from $\mathrm{BChl_S}^*$, i.e. of the order of 1–2 %, if $k'_T \ll k'_{\mathrm{Car_T}}$ (see fig. 4). This was experimentally verified [10].

Under natural excitation conditions the average temporal distance between the photons is at least three orders of magnitude larger, thus exceeding markedly the lifetime of $\mathrm{Car_T}^*$. Therefore, in vivo the steady-state triplet concentration within the bulk pigment system is very low (close to zero). On the contrary, even at the lowest intensities which are markedly beyond the saturation level of photosynthesis applied in refs. 9 and 10 the average time interval between two photons per photosynthesic unit is ≤ 2 ns. Most of the measurements were carried out under conditions where singlet-singlet annihilation arises (the faster fluorescence decay times at high intensities are caused by these processes, see ref. 29). In comparison to the in vivo situation these excitation conditions appear to be more artificial than ours. At these intensities it was impossible to show, that the "valve" is "switched on" only, if photosynthesis becomes saturated. In contrast to the above-discussed "triplet valve" mechanism the

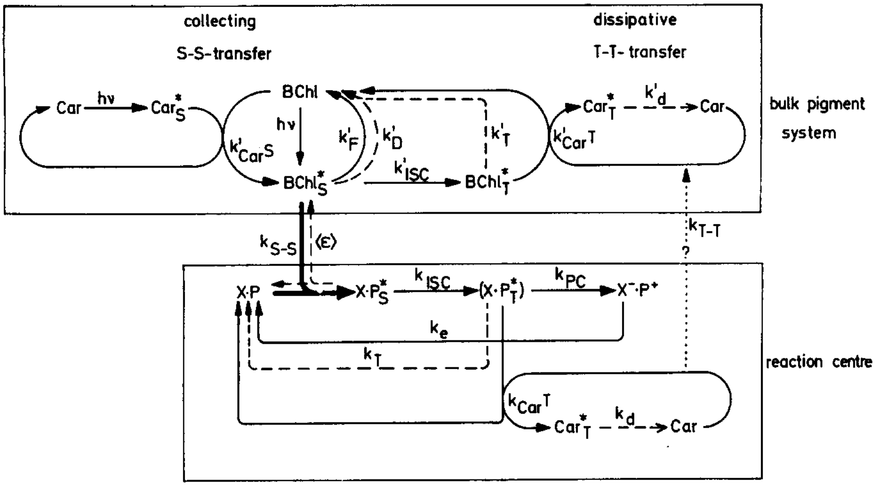

Fig. 4. Scheme of excitation energy fluxes within the photosynthetic unit (for the sake of simplicity energy transfer between different photosynthetic units and exciton fusion at extremely high intensities are omitted). Car = carotenoids, BChl = bacteriochlorophyll, P = primary electron donor (pigment complex), X = primary electron acceptor, S refer to singlet, T to triplet processes, rate constants k labeled by $'$ describe processes in the bulk pigment system, indices indicate: D = non-radiative, F = radiative decay, ISC = intersystem crossing, PC = photochemical charge separation, e = secondary thermal electron transfer reactions, T = triplet decay, Car^S = singlet-singlet transfer from carotenoids to bacteriochlorophyll, Car^T = triplet-triplet transfer of the valve reaction, d = dissipative decay of Car_T^*.

protective function of the carotenoids is sometimes assumed (see ref. 8) to be caused mainly by their ability to prevent a photodynamic action due to singlet oxygen, $O_2(^1\Delta_g)$. If carotenoids would predominantly act as $O_2(^1\Delta_g)$ quenchers, then the carotenoid triplet formation should be caused by the reaction:

$$O_2(^1\Delta_g) + \mathrm{Car} \rightarrow O_2(^3\Sigma_g^-) + \mathrm{Car}_T^* \qquad (2)$$

where $O_2(^1\Delta_g)$ can be generated by interaction of ground state triplet oxygen, $O_2(^3\Sigma_g{}^-)$, with Bchl_T^*. In this way the bacteriochlorophyll triplets would also lead to the generation of carotenoid triplets, but indirectly via $O_2(^1\Delta_g)$ as intermediate. However, this mechanism appears to be of minor probability for the following reasons: (a) The extent of triplet formation Car_T^* is independent of the concentration of O_2 in a wider range and it occurs even at −160 °C in frozen degassed (vacuum) samples [6, 7, 26]. (b) The very fast rise time of the carotenoid triplets ($<20\,\mu s$) points against a mechanism including $O_2(^1\Delta_g)$ as intermediary reactant. Recent data [10] support our assumption. Therefore, we conclude that harmful excess excitation energy (in the form of Bchl_T^*) is predominantly dissipated via the direct "triplet valve". Nevertheless, the ability of carotenoids to quench singlet oxygen, $O_2(^1\Delta_g)$, provides an additional protective mechanism which might become important under special physiological conditions (e.g. $O_2(^1\Delta_g)$ formation by biochemical reactions). Lately it was found, that also by the embedding of chlorophylls in a protein matrix an additional protection to oxidative destruction seems to be caused [30].

The "triplet valve" principle as the unique protective mechanism for chlorophyll (bacteriochlorophyll) should be realized not only in the bulk pigment system but also in the reaction centers, because there is now increasing evidence for a transient triplet formation at the primary donor pigment complexes P in plants [31, 32] and bacteria [33], respectively. Indeed, it was found very recently, that the reaction centers of *Rhodopseudomonas* contain a special carotenoid molecule, (spheroidene) bound to the same protein unit as the bacteriochlorophyll complex [34], which functions as a "triplet valve" [10, 34]. In summary, the pathway of the electronically excited states can be described by the scheme given in Fig. 4. The functional connection between the antennae and the reaction center occurs via the well known singlet exciton coupling ($k_{\text{S-S}}$). Whether there exists a dissipative triplet coupling link via the carotenoid valves ($k_{\text{T-T}}$) remains an open question.

In order to account for the high photochemical quantum yield [20] the triplet valve of the reaction centre has to be "switched off" in the state $\text{X} \cdot \text{P}$, which is achieved kinetically by $k_{\text{PC}} \gg k_{\text{Car}_\text{T}}$ ($k_{\text{PC}} \geq 5 \cdot 10^9\ \text{s}^{-1}$ versus $k_{\text{Car}_\text{T}} \approx 10^8\ \text{s}^{-1}$, see ref. 34). In state $\text{X}^- \cdot \text{P}$ ($k_{\text{PC}} = 0$) the reaction center would act as a dissipative sink funneling excitation energy via the "triplet valve". This is confirmed by the 5–6-fold enhancement of $\Delta A_{\text{Car}_\text{T}}$ in the presence of $\text{Na}_2\text{S}_2\text{O}_4$ (data not shown). The quantum yield for Car_T^* formation at the reaction center is significantly higher than in the bulk, which is in correspondence with the steeper saturation curve reported in ref. 10. However, this quantum yield is too low (≤ 0.1) for a complete energy flux via the carotenoid valve. Hence, in the state $\text{X}^- \cdot \text{P}$ either the formation of $(\text{X}^- \cdot \text{P}_\text{T}^*)$ occurs with lower efficiency than in state $\text{X} \cdot \text{P}$ or an additional dissipative pathway (k_T) becomes predominant when X is reduced. Only the former interpretation is reconcilable with earlier data indicating a close similarity of the saturation curves for P_F (probably P_T^*) and Car_T^* formation in reaction center preparations in the presence of dithionite [34]. That means X^- either reduces k_{ISC} or accelerates the dissipation of P_S^* via other routes.

A completely different situation arises if P is oxidized to P^+. Then due to its bleaching P^+ cannot further act as exciton trap and the triplet valve remains non-functional. Therefore, the extent of Car_T^* should remain uneffected by chemical oxidation of P. However, if one considers that P^+ generally can act as a non-photochemical quencher (as is known for chlorophyll $a_{\text{II}}{}^+$, see refs. 35 and 36) one would expect a decreased quantum yield Φ for Car_T^* formation in the bulk pigment system provided that the quenching efficiency of $\text{X} \cdot \text{P}^+$ exceeds that of $\text{X}^- \cdot \text{P}^+$. This effect which could explain our decrease of $\Delta A_{\text{Car}_\text{T}}$ in the presence of $\text{K}_3[\text{Fe(CN)}_6]$ should be observable only at lower actinic flash intensities and therefore might escaped the detection under excitation conditions as in refs. 9 and 10. Additionally $\text{K}_3[\text{Fe(CN)}_6]$ could attack chemically also the bulk pigments leading to a further decrease of $\Delta A_{\text{Car}_\text{T}}$ (see ref. 10). As according to the above discussion reaction centers closed for photochemistry are assumed to be quenchers of excitation energy, irrespective of the blockage type (quenching in $\text{X}^- \cdot \text{P}$ via the triplet valve, in $\text{X} \cdot \text{P}^+$ and $\text{X}^- \cdot \text{P}^+$ via P^+), the discrepancy between the relatively small variable fluorescence and very high photochemical quantum yield (see ref. 20) becomes easily understandable.

A last point should be mentioned. From the present data it was inferred that the quantum yield for triplet formation of bulk bacteriochlorophyll appears to be rather low (1–2 %). By contrast, if one accepts the triplet state as intermediate of the photochemical charge separation, the intersystem crossing probability of the bacteriochlorophyll in the reaction center complex has to be close to 1 in state X · P, but low in state X^- · P. Therefore, two questions have to be answered for a deeper understanding of the primary steps of light energy conversion: (1) By which mechanism is the intersystem crossing process regulated in photosynthesizing organisms? (2) What is the functional importance of the triplet state during the process of charge separation?

APPENDIX

For the derivation of the Eqns. 1 and 2 four simplifying assumptions are introduced: (1) Photosynthetic units act as isolated entities with respect to excitation energy transfer. (2) The photon distribution over the photosynthetic units occurs statistically according to Poisson. (3) The photochemical quantum yield of the charge separation is 1. (4) During the dark time between the flashes all reaction centers relax to the state X · P (see fig. 4), so that the first photon absorbed at a photosynthetic unit is exclusively consumed for photochemistry, thereby closing the reaction centers. Each of the following photons leads to Car_T^* formation with a quatum yield Φ.

Then one obtains for the absorption changes:

Photochemistry:

$$\Delta A = \Delta A^1 \sum_{m=1}^{\infty} \frac{\rho^m}{m!} \cdot e^{-\rho} \tag{A1}$$

where ΔA is an absorption change, which is linearly related to the photochemical process, ΔA^1 = absorption change caused by a single turnover flash and ρ = average number of photons absorbed per photosynthesic unit.

Car_T formation*:

$$\Delta A_{Car_T} = \Delta\varepsilon \cdot c_0 \cdot d \sum_{m=2}^{\infty} [1-(1-\Phi)^{m-1}] \frac{\rho^m}{m!} \cdot e^{-\rho} \tag{A2}$$

$\Delta\varepsilon$ = differential extinction coefficient for Car_T^* formation, c_0 = carotenoid concentration available and d = optical pathlength. The summation in Eqns. A1 and A2 leads to Eqns. 1 and 2 given in the text.

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