β-Carotene Quenches Singlet Oxygen Formed by Isolated Photosystem II Reaction Centers†

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ABSTRACT: By measuring time-resolved luminescence emission at 1270 nm, we have detected singlet oxygen formation by illuminated, reaction centers of photosystem II isolated from *Pisum sativum*, which is in agreement with earlier work (Macpherson, A. N., Telfer, A., Barber, J., & Truscott, T. G. (1993) *Biochim. Biophys. Acta 1143*, 301−309). In this paper we show that the yield of singlet oxygen is significantly increased if the number of β-carotene molecules bound per isolated complex is reduced from two to one. We conclude, therefore, that β-carotene can act as an effective quencher of singlet oxygen in the photosystem II reaction center. This conclusion is supported by the finding that the rate of light-induced irreversible bleaching of chlorins in the reaction center is increased with decreasing β-carotene levels. The results demonstrate the direct intermediacy of singlet oxygen in causing photooxidative damage within a biological environment and are discussed, specifically, in terms of the role of β-carotene in protecting photosystem II against photoinhibition.

It is generally presumed that one of the roles of carotenoids in photosynthetic tissue is to quench chlorophyll triplets before they react with ground state oxygen to form the highly toxic species, singlet oxygen ($^1O_2$)[1] (Cogdell & Frank, 1987). The extreme sensitivity of carotenoidless mutants to light under aerobic conditions confirms this view (Anderson & Robertson, 1960; Griffith et al., 1955). Indeed, where the structure of chlorophyll protein complexes is known at atomic resolution, carotenoids have been shown to be bound within van der Waals contact of at least some of the chlorophyll(s), i.e., in the purple bacterial reaction center (RC) (Deisenhofer & Michel, 1991) and the chlorophyll *a/b* light harvesting complex of photosystem II (PSII) (Kühlbrandt et al., 1994). This close proximity is required for electron exchange (Dexter, 1953).

Isolated PSII RC (consisting of the D1, D2, cytochrome b_{559}, and I proteins) normally bind two β-carotene molecules per center (Kobayashi et al., 1990; Gounaris et al., 1990). However, when this complex is illuminated, it forms very little carotenoid triplet (Takahashi et al., 1987; Durrant et al., 1990). Instead, charge recombination gives rise to a significant level of the triplet state of the primary electron donor, P680. In the absence of oxygen the P680 triplet lifetime is about 1 ms, but it shortens dramatically when oxygen is present (Durrant et al., 1990; Mathis et al., 1989). This suggests that the β-carotenes are not bound sufficiently close to the primary electron donor of PSII, P680, for them to trap the triplet state. Hence ^{3}P680 is quenched by ground state molecular oxygen ($^3O_2$). The $^1O_2$, formed by this process, should cause oxidative damage. Indeed, the isolated PSII RC is found to be readily inactivated and degraded when illuminated under aerobic conditions (McTavish et al., 1989; Telfer & Barber, 1989; Shipton & Barber, 1991). Recently, isolated PSII RC have been shown, by direct methods, to have a high yield of $^1O_2$ formation, using both near-infrared detection of its luminescence (1270 nm) (Macpherson et al., 1993) and chemical trapping techniques (Telfer et al., 1994). In these papers, we concluded that some of the $^1O_2$ formed by PSII RC is rapidly quenched by components of the pigment-protein complex and that only a proportion, which diffuses out of the RC complex into the suspension medium, is detected by its 1270 nm luminescence or chemical trapping.

Many of the components of biological material are damaged by light and oxygen (Foote, 1990). This is known as the photodynamic effect. It is thought that the damage is caused by $^1O_2$, "sensitized" by added chemicals or natural compounds, which oxidizes amino and nucleic acids, phospholipids, and certain pigments. However, there are also many compounds that can quench $^1O_2$ without chemical reaction, for example, the azide anion (Hasty et al., 1972) and β-carotene (Garner & Wilkinson, 1978), which act by charge transfer and energy transfer mechanisms, respectively. Foote and Denny (1968) showed that β-carotene in solution quenches dye-sensitized $^1O_2$. Therefore, it seems likely that in some biological systems β-carotene might well have an

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[1] Abbreviations: $^1O_2$, singlet oxygen molecule; Φ_Δ, singlet oxygen quantum yield; τ_Δ, lifetime of singlet oxygen luminescence; $AlPcS_2$, aluminium phthalocyanine disulfonate; Car, carotenoid; H_2TPPS_4, *meso*-tetra(4-sulfonatophenyl)porphine; RC, reaction center(s); P680, primary electron donor of PSII; Pheo, pheophytin; PSII, photosystem II.

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important role as a direct quencher of $^1O_2$, and hence protect against photodynamic action (Gorman & Rodgers, 1992).

In this work we have investigated the possibility that β-carotene, bound to the PSII RC, may give protection against the damaging effect of $^1O_2$, by quenching this toxic species before it can attack the amino acids and pigments of the complex. We have found that, when the β-carotene content of PSII RC is decreased, the relative yield of $^1O_2$, detected by time-resolved luminescence measurements at 1270 nm, is considerably increased. There was also a similar increase in the rate of photodamage to the complex. Therefore, we conclude that if $^1O_2$ is formed by isolated PSII RC, β-carotene, bound to the D1 and D2 proteins of the complex, can quench this toxic species, providing protection against oxidative damage.

MATERIALS AND METHODS

Isolation of PSII Reaction Centers with Different Levels of Bound β-Carotene. Isolation of PSII RC from pea, binding 2 β-carotenes per 6 chlorophyll *a* molecules (2-Car RC), was carried out as described previously (De Las Rivas et al., 1993). The β-carotene level could be gradually decreased if the anion-exchange column was washed for increasing lengths of time with low salt buffer containing Triton X-100 as described by De Las Rivas et al. (1993) or by a slight modification of the technique of Yruela et al. (1994) with a Q-Sepharose anion-exchange column. In the latter case, the procedure we used lowered the β-carotene level but did not change the chlorophyll to pheophytin ratio, although Yruela et al. (1994) reported a decrease from 6 to 4 chlorophylls bound per 2 pheophytin molecules. The pigment composition of some preparations was measured by HPLC (De Las Rivas et al., 1993), but the relative level of chlorophyll and β-carotene was also assessed, simply, by comparing the A_{417}/A_{484} ratio of the different preparations (see Results). Irreversible bleaching of absorbance by PSII RC was carried out as described previously (De Las Rivas et al., 1993).

Measurement of Transient Luminescence at 1270 nm. Time-resolved $^1O_2$ luminescence studies were carried out using a nanosecond laser flash photolysis apparatus at the Rutherford Appleton Laboratories (Bishop, 1994; Beeby et al., 1991). Samples were excited at 428 nm by an excimer pumped dye laser system (using the dye stilbene 30 in methanol) by pulses within the energy range 0.05−0.5 mJ/pulse with a pulse width of 10 ns. The emitted $^1O_2$ luminescence was detected at right angles to the excitation beam with a North Coast E0-817P germanium photodiode/amplifier combination. This apparatus contains a 5 × 5 mm Ge device which is cooled to 77 K with liquid nitrogen to reduce noise. The speed of the device was achieved by the application of a high voltage bias of −300 V. The sample was held in a 10 × 10 mm quartz cuvette close to the Ge device and irradiated by the laser source via a 5 mm diameter liquid light guide. The $^1O_2$ luminescence from the sample was passed through a 1270 nm silicon interference filter of bandpass equal to 1%, prior to reaching the device. Time-resolved traces were obtained on a digital storage oscilloscope from the average of at least 64 shots and analyzed using custom designed software.

For the luminescence measurements, PSII RC were suspended in 50 mM Tris-HCl buffer and 2 mM *n*-dodecyl

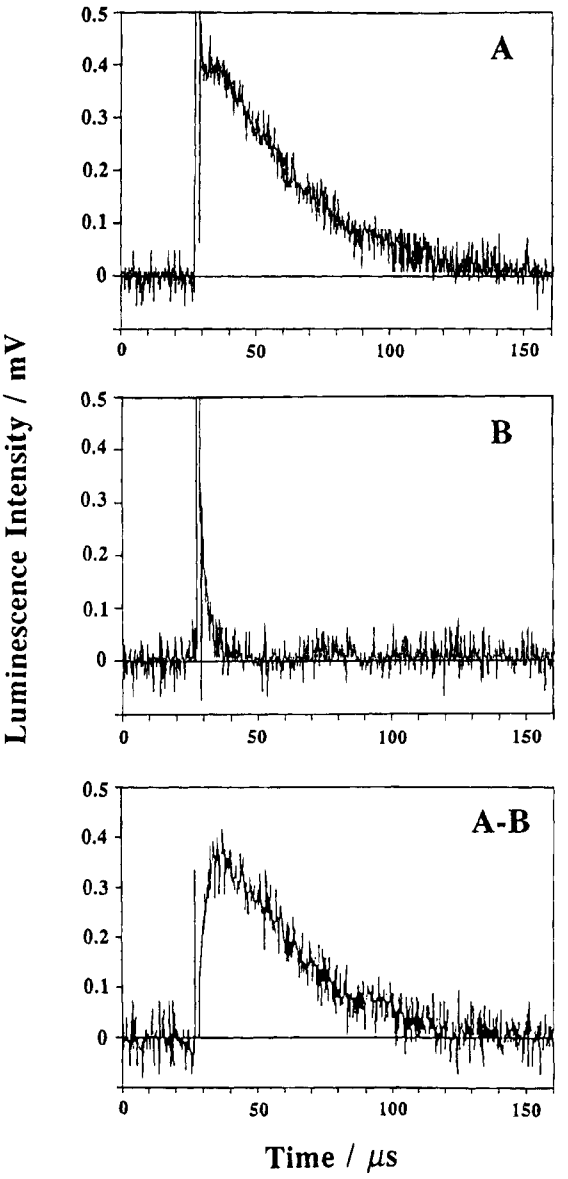

FIGURE 1: Flash-induced luminescence (1270 nm) emitted by PSII RC binding approximately two β-carotenes per RC. (A) In the absence and (B) the presence of 10 mM azide, and the difference between the signals in the absence and presence of azide (A-B). RC were diluted to 7.6 μg/mL chlorophyll into buffer made up in D_2O. The H_2O content was ≈6%. Laser power for excitation was 0.42 mJ/pulse.

β-D-maltoside under air saturated conditions. The buffer was usually made up in D_2O at a pD of 7.2. Stock concentrations of PSII RC, isolated in H_2O medium, were diluted into the deuterated buffer such that the final H_2O content was <2%, unless otherwise stated. Experiments were conducted at ambient temperature.

The quantum yield of $^1O_2$ formation by PSII RC was measured by chemical trapping with histidine as described by Telfer et al. (1994).

Chemicals. Disulfonated aluminium phthalocyanine ($AlPcS_2$) was synthesized as described by Ambroz et al. (1991), and *meso*-tetra-(4-sulfonatophenyl)porphine (H_2TPPS_4) tetrasodium salt was obtained from Porphyrin Products.

RESULTS

Figure 1A shows the flash-induced luminescence at 1270 nm emitted by isolated PSII RC suspended in deuterated

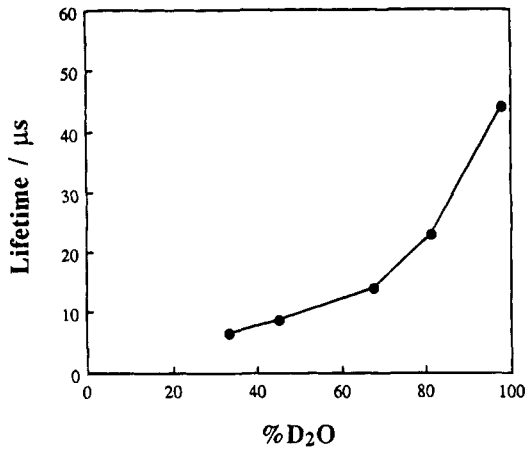

FIGURE 2: Lifetime of $^1O_2$ luminescence as a function of the relative concentration of D_2O in the suspending medium. 2-Car RC were diluted to 2.4 μg/mL chlorophyll into different relative % of D_2O and H_2O buffer, as described in Materials and Methods. The lifetime measurement error was estimated to be ±5 μs.

buffer plus detergent under air saturated conditions. Submicrosecond emission is due to chlorophyll fluorescence, but the microsecond component is characteristic of emission from $^1O_2$. The latter signal is sensitive to azide, a known $^1O_2$ quencher (Figure 1B), and subtraction of the azide plus (chlorophyll fluorescence signal) from the azide minus signals gives panel A-B. The difference decay signal has an ≈5 μs rise time, and the decay is monoexponential. A very similar signal is seen if the transient observed in the presence of an enzymic oxygen trap is subtracted from the aerobic signal (data not shown).

The average lifetime of the decay of $^1O_2$ luminescence (τ_Δ) from PSII RC was 48 μs (±10 μs), which is shorter than the reported τ_Δ of 68 μs generated by sensitizing dyes (Rose Bengal and thionine hydrochloride) dissolved in pure D_2O (Ogilby & Foote, 1982). However, we found that although the τ_Δ sensitized by the dye, H_2TPPS_4, dissolved in deuterated buffer was 65 μs, on addition of detergent (2 mM *n*-dodecyl β-D-maltoside) the τ_Δ decreased to ≈38 μs. This decrease in τ_Δ in the presence of detergent micelles has been observed previously (Rodgers, 1983). Therefore, this effect could account for the shorter lifetime observed in the experiments with the PSII RC, where detergent must be present to avoid aggregation.

Figure 2 shows clearly that H_2O quenches $^1O_2$ luminescence emitted by PSII RC. Gradual replacement of D_2O with H_2O in the suspending medium decreases the τ_Δ from 44 μs to an extrapolated value of ≈2 μs for 100% H_2O. This correlates with the reported lifetimes for luminescence from $^1O_2$ in H_2O, of 3−4 μs (Rodgers & Snowden, 1982; Egorov et al., 1989).

The luminescence emission, seen in Figure 1, was from an RC preparation binding approximately two β-carotenes per center. Figure 3A shows the effect of lowering the level of carotenoid bound to RC on the luminescence signals. The upper trace is emission from an ≈2-Car RC preparation as compared to the lower trace, which shows the signal from a preparation binding approximately one Car per center. Lowering the Car content increases the $^1O_2$ luminescence intensity without affecting the lifetime of the decay. Figure 3B shows the initial extent (exponential decay component extrapolated to $t = 0$) of the luminescence emitted by the two preparations as a function of the laser excitation power.

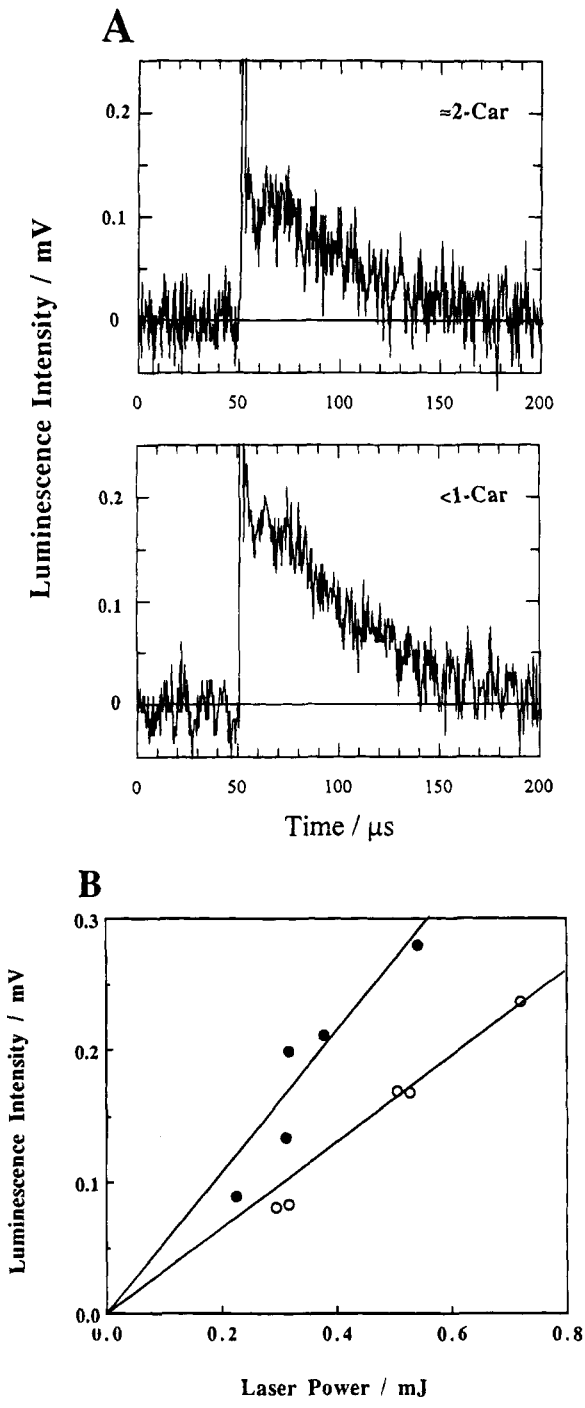

FIGURE 3: (A) Comparison of the flash-induced luminescence from $^1O_2$ seen with an ≈2-Car RC (upper trace) and a <1-Car RC (lower trace). The chlorophyll concentration of the two samples was approximately 2.5 μg/mL. Conditions were as described in Figure 1 except that the excitation power was 0.48 mJ/pulse and the H_2O content was <2%. (B) Microsecond emission at 1270 nm from RC with different levels of β-carotene as a function of laser pulse power. The initial extent of the exponential decay component was extrapolated to $t = 0$, and the estimated error in the measurement was ±0.03 mV. Luminescence intensity values were corrected for a 6% difference in absorbance by chlorophyll between the two samples. Carotenoid level of RC was judged by the ratio of their absorbance at 417 and 484 nm (A_{417}/A_{484}), which for the ≈2-Car RC was 3.30 (open circles) and for the <1-Car RC was 6.02 (closed circles).

It is clear that, on lowering the β-carotene content of the RC, the yield of $^1O_2$ luminescence is greatly increased. Comparison of various RC preparations with different levels

of bound β-carotene showed a consistent increase in relative yield of $^1O_2$, measured by its luminescence at 1270 nm, as the carotenoid level was decreased (see Table 2).

Figure 4A shows the absorption spectra of two preparations of RC with different β-carotene levels. The absorbance at 417 nm is predominantly due to chlorophyll, pheophytin, and oxidized cytochrome b_{559}, whereas at 484 nm it is mainly due to β-carotene. Consequently, we found that the normal 2-Car RC has an A_{417}/A_{484} ratio of about 3.0, and an increase in this ratio indicated a decrease in the relative level of β-carotene. This was confirmed by HPLC measurements (Table 1). However, it should be noted that the comparison of A_{417}/A_{484} ratios is only valid if the Chl to pheophytin (Pheo) proportions are unchanged, as is the case in these experiments.

The absorption difference spectra of Figure 4B show that the rate of irreversible bleaching of pigments, by red light, in an RC preparation binding less than two β-carotenes per center (lower traces) is higher than that seen with a normal, 2-Car, preparation (upper traces). The main pigments bleached are the chlorins, with much less effect on the absorption in the carotenoid absorption region (≈484 nm). Figure 4C shows the integrated area of the bleaching between 600 and 720 nm (due to loss of Chl and Pheo ground state absorption only) plotted as a function of photodamage time. This figure shows that the rate of bleaching is greater in the PSII RC binding low as compared to high levels of β-carotene. This difference is emphasized by the plot of the ratio of these two rates (Figure 4C). During the initial illumination period, the low Car/RC preparation was irreversibly bleached, at approximately 1.6 times the rate of the 2-Car preparation.

Table 2 presents the effect of carotenoid level on the relative quantum yield of $^1O_2$ luminescence (Φ_Δ) emitted by PSII RC. The levels of β-carotene bound per RC were monitored by their A_{417}/A_{484} ratios. Luminescence emission intensity was found to saturate at about 3 μg/mL chlorophyll; thus for relative Φ_Δ measurements the chlorophyll concentration used was well below this (≈1 μg/mL). Table 2 shows that as the carotenoid level is decreased, there is a significant increase in the relative yield of $^1O_2$ luminescence [Φ_Δ(rel)]. It also shows, for two of the RC preparations, the $^1O_2$ yields measured by a chemical trapping technique (Φ_Δ) and the relative initial rates of photodamage induced by red light treatment. Both methods for measuring the relative Φ_Δ, of these two PSII RC preparations, give a similar increase in yield when the carotenoid level is lowered. This increased yield of $^1O_2$ correlates well with the increase in rate of photodamage.

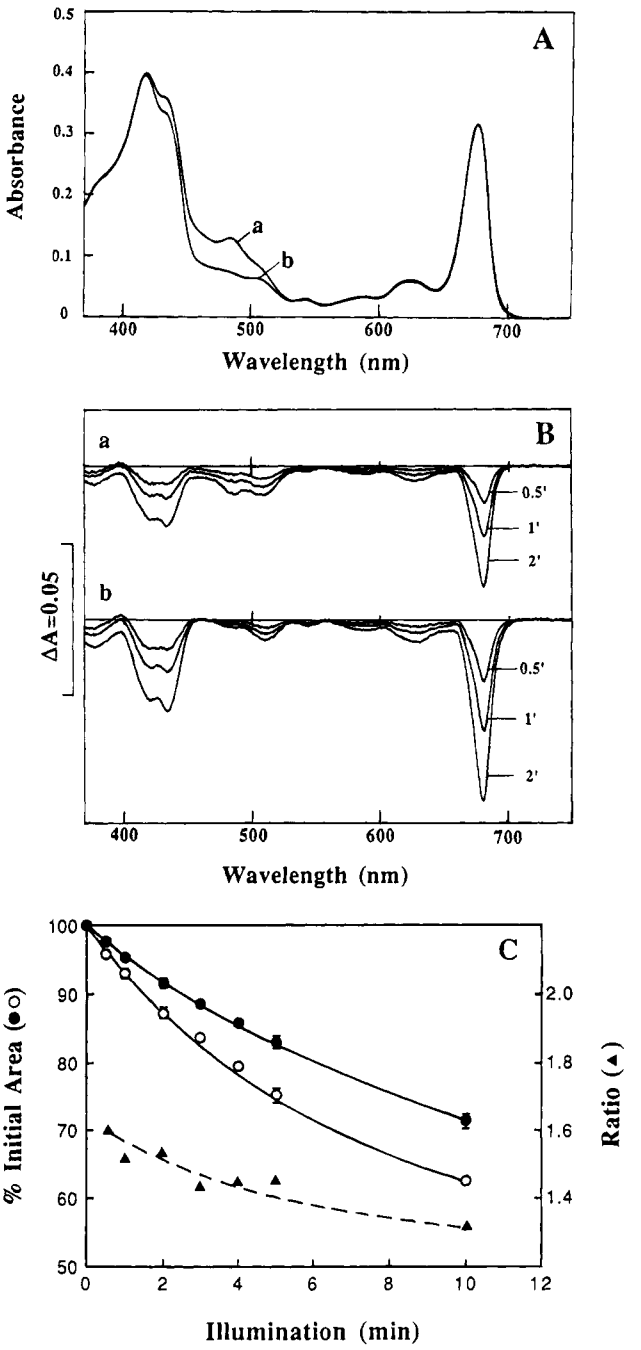

FIGURE 4: Comparison of the irreversible decrease in absorption of PSII reaction centers, binding different levels of β-carotene, induced by light treatment under aerobic conditions. (A) Absorption spectra of dark controls. (B) Difference spectra of irreversible absorption changes in samples illuminated for 0.5, 1, and 2 min. Spectra of light-treated samples minus the initial dark spectrum. (C) Rate of photodamage as measured by the percentage decrease in integrated area of the absorption of the dark control, between 600 and 720 nm (open and closed circles), and ratio of rates of absorption decrease (1-Car RC/2-Car RC) (closed triangles). Traces a and closed circles, ≈2-Car, and traces b and open circles, ≈1-Car per RC: A_{417}/A_{484} 3.06 and 5.25, respectively. The chlorophyll concentration of the two samples (approximately 4 μg/mL) was matched so that the integrated area of absorption between 600 and 720 nm was the same. Photodamage was induced by 600 μE m^{-2} s^{-1} red light (Schott glass cutoff filter, RG665). Error bars indicate standard deviation, $n = 3$.

DISCUSSION

This paper shows that a decrease in the level of β-carotene, bound to PSII RC, significantly increases the yield of $^1O_2$ formed on excitation of this complex both with pulsed light (1270 nm luminescence technique) and continuous light (histidine trapping technique). The rate of light-induced damage to the complex, as estimated by the irreversible bleaching of chlorophyll and pheophytin, was also dependent on the level of β-carotene bound to the complex.

We propose that when isolated PSII RC are illuminated and $^1O_2$ is formed, this active oxygen species has three possible fates. Within the protein matrix of the complex a proportion will interact with endogenous oxidizable targets, amino acids and certain pigments, while some will be quenched by the bound quencher, β-carotene. Both of these

Table 1: β-Carotene Level of Isolated PSII RC Preparations As Determined by HPLC and A_{417}/A_{484} Ratio

RC preparation	HPLC			absorbance data
	Pheo	Chl	Car	A_{417}/A_{484}
i	2	6.5 ± 0.2[a]	2.2 ± 0.3	3.07 ± 0.16
ii	2	6.6	1.6	4.66
iii	2	6.4	1.2	5.53

[a] Standard deviation, $n = 5$.

Table 2: Evidence That β-Carotene Scavenges Singlet Oxygen in Isolated PSII Reaction Centers[a]

approx Car/RC	A_{417}/A_{484}	Φ_Δ(rel)[b]	Φ_Δ[c]	initial rate bleaching (rel)[d]
≈2	3.05	1.00 ± 0.10	0.172 (1)[e]	1
≥1	4.66	1.50 ± 0.22	0.271 (1.58)	1.5
≈1	6.02	1.67 ± 0.29	nd[f]	nd

[a] Relative quantum yield of $^1O_2$ formation (Φ_Δ) measured by its luminescence at 1270 nm and histidine-catalyzed oxygen uptake as compared to the relative rate of photodamage induced by red light treatment under aerobic conditions. Absorbance of all PSII RC preparations and the standard $^1O_2$ sensitizing dye were matched at the wavelength of excitation in each case. [b] Relative $^1O_2$ quantum yield as measured by emission at 1270 nm. Excitation 428 nm. [c] $^1O_2$ quantum yield (Φ_Δ) as measured by light-induced oxygen uptake in the presence of histidine, assuming Φ_Δ for $AlPcS_2$ in H_2O of 0.225, as determined by Telfer et al. (1994). Excitation 666 nm. [d] Initial relative rate of irreversible bleaching of PSII RC of the red absorbance region (decrease in integrated area of absorbance between 600 and 720 nm) of PSII RC. Details as in Figure 4. [e] Relative values in parentheses. [f] Not determined.

processes are very rapid. It has been suggested that the quenching of $^1O_2$ within a biological system is much faster (200 ns range) than its rate of formation (25−100 μs) and that therefore only infinitesimally weak luminescence signals will arise from the $^1O_2$ within the protein environment (Gorman & Rodgers, 1992). However, any $^1O_2$ which diffuses into the surrounding medium will emit luminescence which should decay with a "solvent" dependent lifetime (Ogilby & Foote, 1983). This is what we observed when D_2O was replaced by H_2O (Figure 2) in the resuspension buffer.

The yield of oxidative damage and internal quenching should be controlled by the relative concentration of the endogenous oxidative targets and $^1O_2$ quenchers. If the concentration of quenchers is reduced, both the rate of photodamage and consequently the yield of $^1O_2$ in the external medium would be expected to increase but the luminescence decay lifetime should be unchanged. Our results show this to be the case. We have found that the lower the level of β-carotene bound per PSII RC, the higher the yield of $^1O_2$ (measured in the external medium) and the greater the rate of photobleaching of the pigments (indicative of the internal oxidative damage).

The yield of the triplet state of P680 formed on illumination of PSII RC is about 0.3 (Takahashi et al., 1987; Durrant et al., 1990). The dramatic effect of oxygen on the lifetime of ^{3}P680 (1 ms reduced to 33 μs) suggests that the conversion to $^1O_2$ is very efficient, i.e., the yield of $^1O_2$, at its site of formation, may be 0.3. The chemical trapping method gives a Φ_Δ, for the RC binding two β-carotenes, of about 0.17 [see Table 2 and Telfer et al. (1994)], indicating that a proportion of the $^1O_2$ formed is quenched within the 2-Car RC complex before it diffuses out into the medium and is detected by the chemical trap.

Using the standard value for a reference sensitizer, determined by Verlhac et al. (1984), we calculate a Φ_Δ of about 0.13 from the luminescence data for PSII RC (Table 2). However, as the data for the standard were not obtained under exactly the same solvent conditions as those for the RC, this estimated yield may not be strictly correct. This is because in a different solvent the nonradiative decay of $^1O_2$ could have altered relative to the radiative decay, so that the luminescence yield is not directly proportional to the $^1O_2$ yield (Scurlock & Ogilby, 1987). Notwithstanding this, the values for Φ_Δ obtained by the chemical trapping and luminescence methods are quite similar, which indicates that the latter is a valid method for detecting $^1O_2$ formation by PSII RC.

On lowering the level of β-carotene bound to the PSII RC, from 2 to ≥1 per RC, the relative yield of $^1O_2$ detected in the medium increases ≈60% (Table 2) as does the rate of photodamage; further reduction in Car level increased the yield of $^1O_2$ (Table 2) and the rate of pigment bleaching (data not shown) even more. This suggests that, within the normal 2-Car RC complex, the two β-carotene molecules quench a considerable amount of $^1O_2$, before it escapes to the medium. This quenching competes efficiently with consumption of $^1O_2$ by oxidative damage, thus providing significant protection to the pigments (Figure 4 and Table 2) and presumably also to the proteins. The greatest protection of the pigments in the 2-Car RC is seen during the initial bleaching period. This suggests that β-carotene is bound in such a way that it preferentially protects the primary electron donor, P680, which is bleached before the other chlorins (Macpherson et al., 1993).

We conclude that although β-carotene does not protect the PSII RC from photoinduced damage by quenching the triplet state of P680, it is able to play a protective role as a singlet oxygen scavenger. Its apparent lack of function as a quencher of ^{3}P680 in the isolated PSII RC (Takahashi et al., 1987; Durrant et al., 1990), and also in larger PSII complexes (Satoh & Mathis, 1981), probably stems from the fact that the high oxidation potential of $P680^+$ (Klimov et al., 1979) is such that the normal protective mechanism of triplet energy transfer to carotenoid is not possible. This is presumably because the location of β-carotene (or any other pigment) close to P680 would simply result in its rapid oxidation and, subsequently, its inactivation as a triplet quencher. Such a situation contrasts with all other RC where the oxidation potential of the primary electron donor is insufficient to oxidize other pigments [see Thompson and Brudvig (1988)]. Nevertheless, it seems that it has been advantageous for PSII to contain carotenoids in its RC, where they can play an important protective role which is needed because the evolution of oxygen is an inevitable consequence of normal PSII function.

It is highly likely that the formation of $^1O_2$ is one route by which PSII is damaged *in vivo* and that this may underlie the phenomenon of photoinhibition (Barber & Andersson, 1992; Prasil et al., 1992). The possibility of this happening will arise if the primary quinone acceptor, Q_A, becomes overreduced and thus primary radical pair recombination is allowed to occur. This route of damage has been termed "acceptor" side photoinhibition, and there is increasing experimental evidence that it occurs *in vivo* (Vass et al., 1992; Hideg et al., 1994a,b).

As discussed by Foote (1990) and Gorman and Rodgers (1992), in reviews on photodynamic action, it is very difficult to show that $^1O_2$, sensitized by dyes, actually mediates oxidative damage and finally kills tumor cells. The experiments with isolated PSII RC, reported here, clearly show a correlation between the degree of photooxidative damage to the complex, the level of β-carotene (a known $^1O_2$ quencher) bound within the protein environment, and the yield of $^1O_2$ which escapes to the surrounding medium. Thus, we present strong evidence for the intermediacy of $^1O_2$ in causing oxidative damage within a biological environment.

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REFERENCES

Ambroz, M., Beeby, A., McRobert, A. J., Simpson, M. S. C., Svenson, R. K., & Phillips, D. (1991) *J. Photochem. Photobiol., B 9*, 87−95.

Anderson, I. C., & Robertson, D. A. (1960) *Plant Physiol. 35*, 531−534.

Barber, J., & Andersson, B. (1992) *Trends Biochem. Sci. 17*, 61−66.

Beeby, A., Parker, A. W., & Phillips, D. (1991) in *Rutherford Appleton Central Laser Facility Annual Report, RAL-91−025*, pp 193−195, Chilton, Oxfordshire, U.K.

Bishop, S. M. (1994) Preparation and Properties of Phthalocyanine Sensitisers for Photodynamic Therapy, Ph.D. Thesis, University of London.

Cogdell, R. J., & Frank, H. A. (1987) *Biochim. Biophys. Acta 895*, 63−79.

Deisenhofer, J., & Michel, H. (1991) *Annu. Rev. Biophys. Biophys. Chem. 20*, 247−266.

De Las Rivas, J., Telfer, A., & Barber, J. (1993) *Biochim. Biophys. Acta 1142*, 155−164.

Dexter, D. L. (1953) *J. Phys. Chem. 21*, 836−850.

Durrant, J. R., Giorgi, L. B., Barber, J., Klug, D. R., & Porter, G. (1990) *Biochim. Biophys. Acta 1017*, 167−175.

Egorov, S. Y., Kamalov, V. F., Koroteev, N. I., Krasnovsky, A. A., Tolutaev, B. N., & Zinukov, S. V. (1989) *Chem. Phys. Lett. 163*, 412−424.

Foote, C. S. (1990) *Future Directions and Applications in Photodynamic Therapy*, Proc. SPIE, Vol. IS 6, pp 115−126, SPIE—The International Society for Optical Engineering.

Foote, C. S., & Denny, R. W. (1968) *J. Am. Chem. Soc. 90*, 6233−6235.

Garner, A., & Wilkinson, F. (1978) in *Singlet Oxygen Reactions with Organic Compounds and Polymers* (Ranby, B., & Rabek, J. F., Eds.) pp 48−53, Wiley, New York.

Gorman, A. A., & Rodgers, M. A. J. (1992) *J. Photochem. Photobiol., B 14*, 159−176.

Gounaris, K., Chapman, D. J., Booth, P., Crystall, B., Giorgi, L. B., Klug, D. R., Porter, G., & Barber, J. (1990) *FEBS Lett. 265*, 88−92.

Griffith, M., Sistrom, W. R., Cohen-Bazire, G., & Stanier, R. Y. (1955) *Nature 176*, 1211−1214.

Hasty, N., Merkel, P. B., Radlick, P., & Kearns, D. R. (1972) *Tetrahedron Lett. 13*, 49−52.

Hideg, E., Spetea, C., & Vass, I. (1994a) *Photosynth. Res. 39*, 191−199.

Hideg, E., Spetea, C., & Vass, I. (1994b) *Biochim. Biophys. Acta 1186*, 143−152.

Klimov, V. V., Allakhverdiev, S. I., Demeter, S., & Krasnovsky, A. A. (1979) *Dokl. Akad. Nauk SSSR 249*, 227−230.

Kobayashi, M., Maeda, H., Watanabe, T., Nakane, H., & Satoh, K. (1990) *FEBS Lett. 260*, 138−140.

Kühlbrandt, W., Wang, D. N., & Fujiyoshi, Y. (1994) *Nature 367*, 614−621.

Macpherson, A. N., Telfer, A., Barber, J., & Truscott, T. G. (1993) *Biochim. Biophys. Acta 1143*, 301−309.

Mathis, P., Satoh, K., & Hansson, Ö. (1989) *FEBS Lett. 251*, 241−244.

McTavish, H., Picorel, R., & Seibert, M. (1989) *Plant Physiol. 89*, 452−456.

Ogilby, P. R., & Foote, C. S. (1982) *J. Am. Chem. Soc. 104*, 2069−2070.

Ogilby, P. R., & Foote, C. S. (1983) *J. Am. Chem. Soc. 105*, 3423−3430.

Prasil, O., Adir, N., & Ohad, I. (1992) in *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., Ed.) Topics in Photosynthesis 11, pp 295−348, Elsevier, Amsterdam.

Rodgers, M. A. J. (1983) *Photochem. Photobiol. 37*, 99−103.

Rodgers, M. A. J., & Snowden, P. T. (1982) *J. Am. Chem. Soc. 104*, 5541−5543.

Satoh, K., & Mathis, P. (1981) *Photobiochem. Photobiophys. 2*, 189−198.

Scurlock, R. D., & Ogilby, P. R. (1987) *J. Phys. Chem. 91*, 4599−4602.

Shipton, C. A., & Barber, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6653−6658.

Takahashi, Y., Hansson, Ö., Mathis, P., & Satoh, K. (1987) *Biochim. Biophys. Acta 893*, 49−59.

Telfer, A., & Barber, J. (1989) *FEBS Lett. 246*, 223−228.

Telfer, A., Bishop, S. M., Phillips, D., & Barber, J. (1994) *J. Biol. Chem. 269*, 13244−13253.

Thompson, L. K., & Brudvig, G. W. (1988) *Biochemistry 27*, 6653−6658.

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.-M., & Andersson, B. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1408−1412.

Verlhac, J. B., Gaudemer, A., & Kraljic, I. (1984) *Nouv. J. Chim. 8*, 401−406.

Yruela, I., van Kan, P. J. M., Müller, M. G., & Holzwarth, A. R. (1994) *FEBS Lett. 339*, 25−30.