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THE TRIPLET EXCITON OF CHLOROPHYLL *a* AND CAROTENOID IN SOLUTION AND IN PHOTOSYNTHETIC ANTENNA PROTEINS *

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We have observed the development and decay of triplet excitons formed in the 'antenna' chlorophyll *a*/*b* protein complex by high-intensity laser excitation. The carotenoid triplet (^{3}Car) appeared 5 ns after excitation in the protein isolation, commonly termed CP-II; the risetime in a larger antenna particle, called LHC (light-harvesting complex) was 12 ns. The quantum yield of ^{3}Car in CP-II decreased 11-fold as intensity was increased from 10^{16} to $2 \cdot 10^{17}$ photons/cm^2 per pulse. The effect is attributed to exciton annihilation during the initial period of triplet formation. Above $5 \cdot 10^{16}$ photons/cm^2 per s, the ^{3}Car lifetime decreases substantially from its low intensity value of 8.7 μs. A comparison of the transient absorption spectrum of CP-II with those of chlorophyll and carotenoid in vitro indicates that 'trapped' chlorophyll triplets formed at high intensities. We present a simple model of destructive interaction between ^{3}Car and chlorophyll triplets which is compatible with the observed increased rate of ^{3}Car decay. Indirect evidence suggests similar effects occur in LHC.

Introduction

Both the formation of triplet excitons and the fluorescence of singlet excitons compete with the initial charge-transfer reactions of photosynthesis. In dark-adapted chloroplasts, the amounts of excitation that fluoresce and form triplets are 2.2% and 4.5% respectively [1]. The latter fraction is significant in that the triplet state of chlorophyll, which we denote as ^{3}Chl, is susceptible to photooxidation. The accumulation of its oxidative products would eventually render the photosynthetic unit inoperable. In nature, this breakdown is avoided by the transfer of the triplet exciton from chlorophyll to a neighboring carotenoid. This 'triplet quenching' action of the carotenoid occurs in several nanoseconds and has been observed in photosynthetic bacteria [2], whole chloroplasts, Photosystems I and II, and the associated light-harvesting complex [1,3].

A number of carotenoids have been identified in chloroplasts, β-carotene and lutein being the most common [4]. Though carotenoids are found in all major components of the photosynthetic unit, the amount is only a small fraction of the number of chlorophyll molecules present. The resulting large spatial separation, coupled with an apparently low lying but as yet unmeasured triplet energy level make both carotenoid triplet (^{3}Car) migration and back transfer to chlorophyll improbable. At 294 K, these trapped triplets have been found to persist about 8 μs in degassed

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chloroplasts, but only 4 μs in aerated samples [3] where triplet quenchers such sa oxygen are active.

As excitation intensity of photosynthetic particles is increased, the fluorescence quantum yield is observed to decrease [5–7]. This effect has been attributed to nonlinear effects such as singlet-singlet, singlet-triplet and triplet-triplet exciton fusion [8–10]. These very same processes should likewise reduce the triplet quantum yield at high intensities and this has been observed in whole chloroplasts by Mathis et al. [3]. In a parallel study on subchloroplast particles they found the triplet quantum yield to be independent of excitation intensity.

Using high intensity laser excitation, we have observed a decrease in the triplet quantum yield in subchloroplast particles commonly known as CP-II and LHC. CP-II is a preparation of the light-harvesting chlorophyll *a/b* antenna complex found in higher plants [4,11]. It has been characterized as an apoprotein of about 35 kDa complexed with three molecules of chlorophyll *a*, three of chlorophyll *b* and a carotenoid [22]. Its main absorption bands at 672 and 440 nm are associated with chlorophyll *a*; those at 471 and 653 nm are attributed to chlorophyll *b*, and carotenoid absorption is confined to the Soret region [11]. The fluorescence band profile, which peaks at 681 nm, is insensitive to which of the chlorophylls is excited, reflecting rapid exciton transfer between chromophores [20]. As CP-II degrades, the fluorescence excited at 471 nm shows a relative incresae in chlorophyll *b* emission (665 nm) indicating a breakdown in energy transfer from chlorophyll *b* to *a* [19]. This phenomenon was used to confirm sample integrity before and after kinetic exepriments. The second preparation of the antenna protein, LHC, is inherently more stable than CP-II and contains a larger number of chlorophyll molecules. Approx. 13 chlorophylls are complexed to a 23 kDa apoprotein with a chlorophyll *a/b* ratio of 1.2 [23].

The formation of triplets in chlorophyll solutions was first investigated in 1958 by Linschitz and Sarkanen [13], who measured the triplet spectrum and lifetime of chlorophylls *a* and *b* in vitro. They found a metastable spectrum composed of broad sloping bands which decayed in 1.5 ms. We have found these spectral features persist even under high excitation conditions that produce a sharp decrease in ^{3}Chl yield.

While carotenoids alone in solution do not exhibit transient absorption or fluorescence, they may be sensitized by various donor pigments including chlorophyll [14]. The ^{3}Car is characterized by a strong absorption peak near 515 nm [15,16]. We have traced triplet energy transfer from chlorophyll *a* to β-carotene in vitro by the decay and rise of their respective transient spectra. This energy transfer is found to be virtually 100% efficient in concentrated solutions.

Using these transient spectra we have identified triplet species generated in the antenna complexes CP-II and LHC. In addition to the drop in triplet yield at high intensities, we have discovered an unexpected decrease of ^{3}Car lifetime in CP-II. The destructive interaction of persistent ^{3}Chl with ^{3}Car is discussed as a possible mechanism for this observed lifetime shortening.

Materials and Methods

The basic apparatus used to study triplet kinetics has been previously described [17–19]. A xenon arc lamp provided broad band visible illumination to monitor sample transmission. As each flash stabilized momentarily at maximum intensity, an N_2 laser excited the sample with a single 337.1 nm pulse for 4 ± 1 ns. Both beams passed collinearly through a 1 mm diameter aperture into a 1 cm^2 cuvette, forming a roughly cylindrical region of excitation. The maximum energy provided by the laser we will call I_0; it equalled 130 ± 5 mJ/cm^2, corresponding to $2.2 \cdot 10^{17}$ photons/cm^2 per pulse. A series of neutral density filters was used to attenuate the beam for lower intensity measurements.

The transmitted xenon beam was filtered, focused on the entrance slit of a high-intensity monochromator, and measured at the exit by a photomultiplier sensitive from 300 to 950 nm. The output was connected to a fast storage oscilloscope by a low impedance coaxial line which limited time resolution to 5 ns. Time-resolved transmission data were recorded photographically. All measurements were made at 21°C.

Sample transmission of the excitation source was obtained using a laser power meter. A refer-

ence intensity was measured by passing the beam through a cuvette filled with acetone or water.

Chlorophyll *a* and β-carotene were obtained from Sigma Chemical Co. and dissolved in reagent grade acetone without further purification. Samples were placed in 1 cm^2 cuvettes and exhaustively purged with nitrogen gas before and during kinetic measurements to displace triplet quenching gases and to mix the material. Acetone was added when necessary to maintain a constant chromophore density.

The chlorophyll *a/b* antenna proteins were prepared by established procedures from chloroplast fragments of cultivated peas (*Pisum sativum*). To isolate CP-II, chloroplasts were solubilized in a 1% sodium dodecyl sulfate (SDS) solution for 30 min, centrifuged, and the supernatant applied to polyacrylamide gels [11,20]. The slab gels were electrophoresed until three pigment bands were clearly resolved. The center band containing CP-II was sliced from the gel and homogenized in cold 50 mM Tris-HCl, 0.125% SDS (pH 8.0). After centrifugation, the supernatant was stored in darkness at 0 °C for approx. 2 h before experiments were peorformed. These measures helped preserve the protein which tends to degrade over several hours [21]. Sample integrity was monitored spectrally before and after kinetic experiments.

To prepare LHC, chloroplasts were solubilized with 0.5% Triton X-100, centrifuged, and the supernatant applied to linear sucrose gradients. After centrifugation at $100\,000 \times g$ for 15 h, a highly fluorescent pigment band was removed and treated with $MgCl_2$ to precipitate aggregates of LHC. These were treated with EDTA and dialyzed against a low salt solution overnight to yield lamellar-like sheets of LHC units (LHC_l). The particles were treated with small amounts of digitonin and octyl glucopyranoside to isolate LHC in its monomer form (LHC_m). Details of LHC preparation and its ability to form aggregates reversibly can be found in Refs. 23–25.

The transient difference in sample absorption with and without laser excitation, which we shall call differential absorption, A_D, was determined by digitizing photographically recorded transmission data and applying Beer's Law. Because the photomultiplier was operated in a regime of linear response, voltage deflections were directly proportional to transmitted intensity:

$$A_D(t) = \log \frac{V_0(t)}{V_1(t)} \tag{1}$$

where V_1 and V_0 correspond to sample transmission with and without laser excitation, respectively. Differential absorption is independent of steady-state chromophore and solvent absorption and reflects only absorption changes caused by transient population shifts. Several nanoseconds after excitation, chlorophyll singlets have relaxed leaving longer-lived species such as triplets or ions to contribute to A_D. The differential absorption caused by a single triplet species is easily shown to be:

$$A_D(\epsilon, t) = [\epsilon_T(\lambda) - \epsilon_0(\lambda)] C_T(t) x \tag{2}$$

where ϵ_T and ϵ_0 are the extinction coefficients of the triplet and ground-state species, $C_T(t)$ is the molar concentration of the triplet, and x is the sample path length. A second, similar term must be included when an additional triplet species is present. If the two species possess significantly different spectral bands, their respective behaviors can be resolved.

Transient lifetimes are expressed as the time interval required for a signal to decrease to $1/e$ of its initial value.

Results

Chlorophyll in vitro

The transient absorption of $2.1 \cdot 10^{-5}$ M Chl *a* appeared upon first examination to behave as a biexponential relaxation with lifetimes of 25 and 800 μs. However, the 'fast component's' magnitude was strongly concentration dependent [19] suggesting concentration quenching. Following the analysis of Linschitz and Sarkanen [13], we determined the triplet-ground state quenching parameter to be $2.8 \cdot 10^7$ $M^{-1} \cdot s^{-1}$, which agreed well with their value. We found the triplet-triplet quenching constant to be $9 \cdot 10^9$ $M^{-1} \cdot s^{-1}$. While this is 6-times larger than the Linschitz and Sarkanen value, it is reasonable for an efficient diffusion-controlled process.

The differential absorption of $3.6 \cdot 10^{-5}$ M Chl

a in acetone was measured from 350 to 820 nm. Predominant features included bleaching in the Soret and 660 nm region, and a sizable triplet extinction peaking at 460 nm. The ^{3}Chl *a* was calculated using Eqn. 2, where A_D and ϵ_0 were measured experimentally. The excitation level, 0.8 I_0, was well into the region of nonlinear triplet yield, making an indirect measurement of $C_T(t)$ necessary. An estimated value of C_T was applied in Eqn. 2 and varied to produce a smoothly varying triplet spectrum between 600 and 700 nm. This is in keeping with features found in the pyridine spectra of ^{3}Chl [13,27]. The optimal value of C_T equalled 15 ± 1% of the total Chl *a* concentration, from which the triplet extinction in Fig. 1 was calculated. The placement as well as the magnitudes of the peaks are similar to spectra of Chl *a* in pyridine. Beside the broad peak at 460 nm ($= 5.6 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$), there is substantial absorption between 700 and 800 nm. This latter feature was useful in discriminating between chlorophyll and carotenoid triplet features in the CP-II transient spectrum. The presence of chlorophyll cation radicals was discounted because their characteristic peak at 820 nm [6] was absent.

We observed nonlinear effects as excitation intensity was varied from 0.01 I_0 (corresponding to about one absorbed photon per ten chlorophyll), to I_0. The triplet quantum yield remained constant until the excitation level reached 0.05 I_0 after which it declined steadily reaching one fifth its initial value at I_0. Multi-photon absorption at high intensities is the probable cause for this type of yield decrease [12]. Sample transmission at the laser frequency declined as excitation levels exceeded 0.1 I_0. At I_0 absorption had increased 20%. Chlorophyll *b* in acetone showed a similar absorption increase. This behavior indicated that excited state cross-sections exceeded those of the ground states at 337 nm [19].

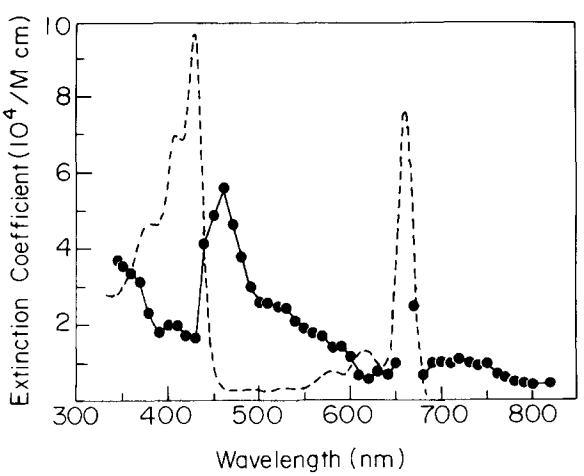

Fig. 1. The ground state (broken line) and triplet state (solid line) absorption of chlorophyll *a* in acetone.

Carotenoid and chlorophyll mixture

The time-resolved transmission of $1.6 \cdot 10^{-4}$ M β-carotene sensitized by $8 \cdot 10^{-5}$ M Chl *a* in acetone was measured at 515 nm, the wavelength of maximum triplet extinction [16]. With curve fitting techniques and standard kinetic equations [19], the chlorophyll-to-carotenoid transfer constant was determined to be $5 \cdot 10^5$ s^{-1}, and the ^{3}Car decay rate was found to be $2.4 \cdot 10^5$ s^{-1}. These parameters are similar to those for Chl-*a* and β-carotene in hexane [16].

The differential absorption of this mixture was measured at two times relative to excitation: at 50 ns when exciton transfer was beginning, and at 2.5 μs when the ^{3}Car transient was a maximum. The transformation of features characteristic of ^{3}Chl to those of ^{3}Car is evident in Fig. 2. Chlorophyll bleaching (610–680 nm) and ^{3}Chl absorption (580 nm and 700–800 nm) decrease with time while the prominent ^{3}Car peak at 515 nm increases. Using Mathis's value [16] of 10^5 $M^{-1} \cdot cm^{-1}$ for the 515 nm ^{3}Car extinction we calculated that during the period between the two spectra, the carotenoid triplet concentration increased from 1.2 to $3.9 \cdot 10^{-6}$ M (±5%), while the ^{3}Chl concentration decreased from 3.6 to $1.1 \cdot 10^{-6}$ M (±10%). This indicates nearly 100% energy-transfer efficiency.

Antenna proteins

The process of carotenoid triplet formation is illustrated in Fig. 3. All pigments in the antenna complex can contribute energy to the photosynthetic process. Singlet excitons formed at chlorophyll-*b* or carotenoid sites migrate to chlorophyll-*a*, whose singlet level is lower in energy. From there the exciton will decay via fluorescence or undergo intersystem crossing to become a triplet. The triplet localized at Chl-*a* will normally transfer to a carotenoid, whose triplet level is believed to be

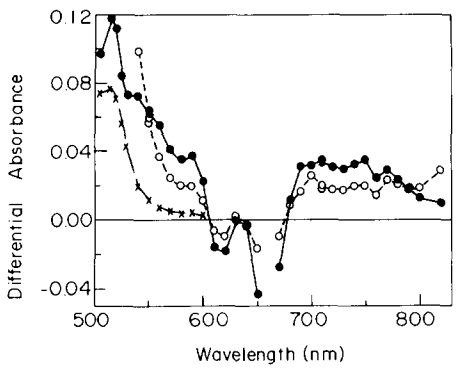

Fig. 2. The differential absorption of β-carotene and chlorophyll *a* in acetone: (●———●) 50 ns after excitation and (○———○) 2.5 μs after excitation when the carotenoid triplet was maximal. The third line (×———×) is a continuation of (○———○) but attenuated 5-fold.

lower in energy – although its exact value has not been determined. It has been suggested that 'trapped' chlorophyll triplets could be formed by high intensity excitation [3,6]. In such an occurrence, transient spectra would contain both chlorophyll and carotenoid triplet-triplet absorption features.

In general, transients generated in the chlorophyll proteins were weaker than those in vitro. Of the three antenna preparations studied, CP-II,

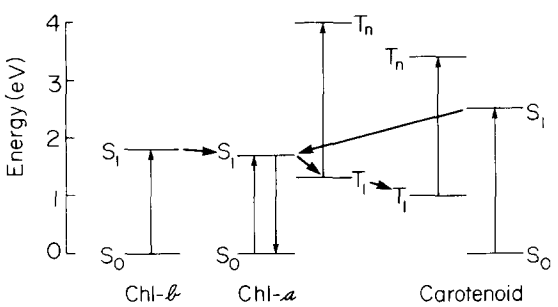

Fig. 3. Exciton formation and transfer among pigments in the antenna protein complex. Visible radiation can be absorbed (vertical arrows) by chlorophyll species *a* and *b*, and by carotenoids. Rapid excitation transfer (horizontal arrows) to chlorophyll *a* occurs before appreciable fluorescence is detected. Intersystem crossing produces a triplet exciton which normally migrates to a neighboring carotenoid. However, any persistant chlorophyll triplet will add its characteristic feature to that of the carotenoid triplet in a transient $T_1 \rightarrow T_n$ absorption spectrum (where T_n designates a higher triplet electron level).

LHC_m and LHC_l, the largest transients were observed in complexes containing the smallest number of chlorophylls – CP-II. LHC_l, which was a lamellar aggregate of monomer LHC and could possibly contain several hundred chlorophyll molecules, had the weakest transients. Similarly, the ^{3}Car lifetime measured under high intensity was longest in the CP-II and shortest in LHC_l. These trends are illustrated by Figs. 4a, d and f.

At lower excitation levels, that is, less than 0.2 I_0, the ^{3}Car lifetime in CP-II ($3.1 \cdot 10^{-5}$ M Chl) was measured as 8.7 ± 0.5 μs. This is similar to triplet lifetimes measured on room temperature chloroplasts [6]. The decay curves could be fit well

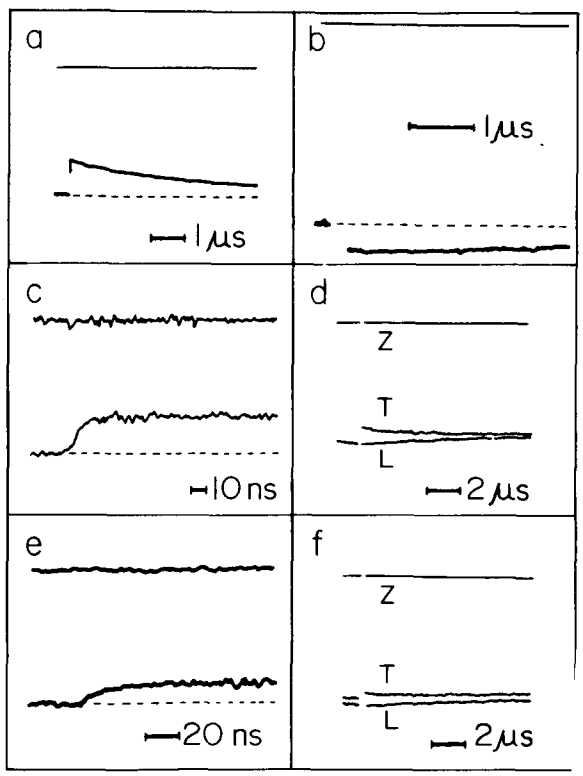

Fig. 4. Experimental data on antenna proteins CP-II and LHC (monomer and lamellar forms): (a) 3.0 μs decay of ^{3}Car transient in CP-II; (b) 5.7 μs bleaching decay at 660 nm in CP-II; (c) 5 ns rise of 515 nm transient in CP-II: (d) 2.2 μs decay and (e) 12 ns rise of ^{3}Car in LHC_m; and (f) 1.0 μs ^{3}Car decay in LHC_l. The upper line in each subfigure (Z) is a baseline; the broken or (L) trace represents the lamp profile (offset slightly in (f) for clarity); while the remaining (T) trace is the transient transmission. All ^{3}Car trnsients were monitored at 515 nm.

with simple exponentials. As seen in Fig. 5, the triplet lifetime decreased as laser intensity was raised. Further, the decays were not fit satisfactorily by a single exponential; better agreement was obtained by allowing the baseline of the exponential decay to assume non-zero values. This procedure was intended to approximate double exponential decay with the second component having a considerably longer decay constant than the first. Under I_0 excitation, the main component of the triplet lifetime had decreased to 3 μs and comprised about 60% of the initial transient signal (Fig. 4a). In a similar manner, the CP-II carotenoid triplet quantum yield, as determined by the magnitude of the 515 nm transient, dropped with increasing intensity. The low intensity yield was 5%. Above excitation intensities of 0.04 I_0 the ^{3}Car yield dropped markedly (Fig. 5), reaching 0.45% at I_0 (corresponding to about 4.8 ^{3}Car per 100 chlorophyll). This intensity threshold for the decrease in quantum yield was 5-times lower than that for the shortening of the ^{3}Car lifetime, suggesting separate causes for the two phenomena.

The risetime of the 515 nm transient reflected the rate at which initially excited chlorophyll singlets produced carotenoid triplets. In CP-II excited at I_0 the risetime of the ^{3}Car was measured as 5 ns (Fig. 4c), a value limited by the response of our system – the actual risetime could have been shorter. This result appears to be in conflict with Kramer and Mathis [1] who report this period to be 13 ns. However, the ^{3}Car risetime, like the quantum yield and decay, may be altered by high-energy illumination. We were unable to resolve the risetime using low intensity excitation, so the difference remains unsettled.

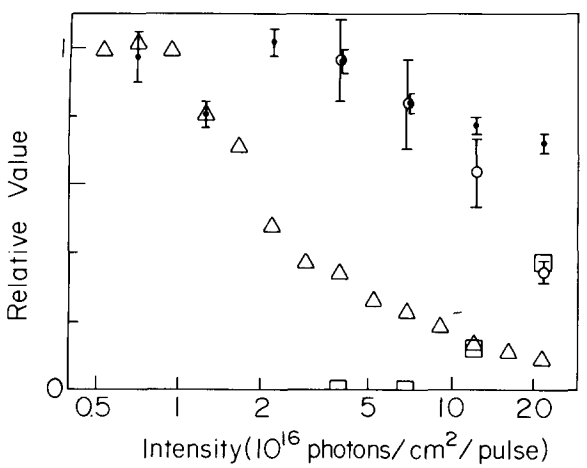

Fig. 5. The relative value of ^{3}Car quantum yield (Δ) and lifetimes (●, ○) as functions of intensity. The lifetimes were determined numerically by fitting exponentials to the decay data, allowing zero (●) and floating (○) baselines. The fractional values of the floating baselines to the initial transient magnitudes are indicated by (□). Full vertical scale corresponds to quantum yield and lifetime values of 8.6% and 8.7 s, respectively.

Unlike that of chlorophyll in vitro, the transmission of CP-II remained constant over the entire range of available excitation energy. With a chlorophyll concentration of $3.1 \cdot 10^{-5}$ M, the CP-II sample had an optical density of 0.95 at the laser frequency. Allowing six chlorophyll molecules per CP-II unit, approx. two out of three CP-II monomers in the excitation zone absorbed a photon at the 0.01 I_0 level. Because absorption of chlorophyll *a* and *b* has been shown to be nonlinear over the same excitation range, the constant absorption of CP-II between 0.01 I_0 and I_0 indicates an effective mechanism for quenching excited states was active in CP-II. Otherwise, some degree of nonlinearity should have been manifest as sizable singlet or triplet chlorophyll populations were generated.

Under high intensity (I_0) the carotenoid triplet lifetimes of LHC_m ($2.5 \cdot 10^{-5}$ M Chl) and LHC_1 ($1.4 \cdot 10^{-5}$ M Chl) were measured as 2.2 and 1.0 (± 0.3) s, respectively (Fig. 4d and f). Although it was not possible to resolve their lifetimes at low intensity levels, it is reasonable that LHC behaves like CP-II, and has a triplet lifetime of several microseconds at low illumination (as observed in other chlorophyll proteins) [3] which shortens as excitation energy is increased. The ^{3}Car quantum yields in LHC may also follow this same trend. They were estimated to be 0.2% and 0.07% for LHC_m and LHC_1 under high-intensity excitation, respectively. The risetime of the 515 nm transient in both forms of LHC's was measured to be about 12 ns. These values are similar to related measurements made by Kramer and Mathis [1].

The differential absorption of CP-II measured 50 ns after high intensity excitation (I_0) is shown in Fig. 6. The dominant feature at 515 nm can be attributed to ^{3}Car. Other features, particularly the shoulder at 580 nm, the bleaching at 660 nm (Fig.

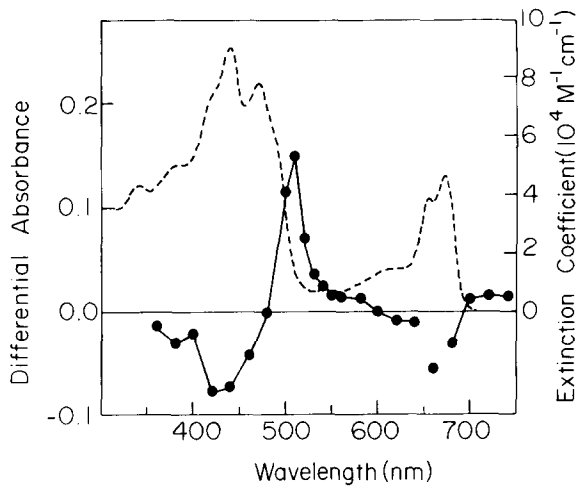

Fig. 6. The ground state extinction coefficient (broken line) and differential absorbance (solid line) of CP-II. The ground state extinction is based on total chlorophyll concentration and the differential absorption determined immediately after exciting 0.028 mg chl/ml CP-II with $2.2 \cdot 10^{17}$ photons/cm^2 per pulse.

4b), and the positive signal between 700 and 800 nm indicate the presence of ^{3}Chl. These triplets amounted to $4.5 \pm 1.5\%$ of the total chlorophyll concentration which corresponds to a quantum yield of 0.4%. Evidence for persistent ^{3}Chl in chloroplasts and photosynthetic proteins has been observed previously [3,6]. The measured lifetime of the 600 nm transient was 5.7 ± 0.4 μs. No measurable concentration of chlorophyll ions was observed as measured by their 820 nm absorbance.

Discussion and Conclusions

The principal conclusion of these experiments concerns changes in the kinetics of triplets generated by increasingly intense irradiance of CP-II, the smallest isolated particle of the light-harvesting chlorophyll-protein complex of higher plants. Under high excitation intensity, there is a decrease in the carotenoid-triplet quantum yield; at even higher intensities the ^{3}Car lifetime shortens and a second, longer-lived component appears.

Decreases in the ^{3}Car yield have been observed in chloroplasts [3,25] under moderately high intensities. At similar excitation levels, however, the ^{3}Car yield in subchloroplast particles have shown no clear evidence of falling [3]. We found a threshold for this expected decrease at 10^{16} photons/cm^2 per pulse (0.05 I_0), which corresponds to about three photons absorbed per CP-II unit. The profile of this decrease as a function of intensity (Fig. 5) is similar to that of fluorescence quenching observed in spinach chloroplasts [12] and *Chlorella* [5].

As in fluorescence quenching [5–10,12], the probable cause for ^{3}Car quenching is exciton annihilation. Any process depleting a singlet population will likewise reduce the resulting triplet population. Because the period of excitation, 4 ns, was of the order of the 3 ns fluorescent lifetime of CP-II [7], annihilation mechanisms involving singlet-triplet, triplet-triplet as well as singlet-singlet interactions could have been involved. Although the fluorescent lifetime of CP-II has been shown to be independent of intensity over the range of 10^{14}–10^{18} photons/cm^2 per pulse, singlet-singlet annihilation may still take place at times short relative to the fluorescent lifetime because of the close chlorophyll-chlorophyll proximity within the complex [7]. On the nanosecond time scale, both ^{3}Chl and ^{3}Car have been shown to be efficient exciton quenchers [3,6,9,10,12]. Resolving which annihilation processes are active would require a mixed picosecond and nanosecond experiment similar to that of Geacintov and Breton [12]. Since annihilation would tend to keep the number of electronically excited species in the complex low, its occurrence is supported by our observation that the transmission of CP-II is constant over our entire excitation range. If large singlet or triplet populations were present, the difference between the absorption cross sections [15,19,26] and those of ground state chromophores would substantially alter sample transmission.

A second ^{3}Car phenomenon appeared when excitation levels reached $5 \cdot 10^{16}$ photons/cm^2 per pulse (0.25 I_0). The decay lifetime of ^{3}Car shortened with increasing intensity. Fig. 5 shows that this decrease has its threshold well-separated from that of the quantum yield, suggesting different causes for the two behaviors. At low intensities the decay behaved as a simple expoential with a lifetime of 8.7 ± 0.5 μs. As intensity increased the shortening ^{3}Car decay curve deviated somewhat from a simple exponential fit. Signal resolution was insufficient to employ a biexponential regression; instead, the baseline of our simple exponen-

tial curve fitting routine was allowed to float, approximately imitating a second, longer-lived component. The single exponential regressions using both zero and floating baselines indicate a shortening lifetime with increasing intensity, the latter case showing a more severe decrese.

Although exciton annihilation can account for the fall of the ^{3}Car yield as a function of increasing intensity by reducing the number of triplets formed, once created the ^{3}Car should decay in its normal manner. Initial annihilation activity should not increase triplet decay rates. One possible cause for the shortened lifetimes, suggested by Breton et al. [6], is that trapped chlorophyll triplets may form in chloroplasts under high excitation conditions. These triplets may slowly detrap and interact with neighboring carotenoids. The presence of persistent ^{3}Chl in our experiments is indicated by chlorophyll triplet features present in the differential absorption of CP-II. By comparing ^{3}Chl and ^{3}Car spectra in Figs. 1 and 2 with Fig. 6 we find evidence of ground-state chlorophyll bleaching and ^{3}Chl absorption in the region between 570 and 750 nm. The chlorophyll bleaching signal at 660 nm (Fig. 4b) suggests that these trapped ^{3}Chl persisted 5.7 ± 0.4 μs. If these chlorophyll triplets interacted with ^{3}Car to cause mutual destruction, the result would be an observed decrease in the ^{3}Car lifetime.

We have examined theoretically several types of ^{3}Chl/^{3}Car interactions, including: ^{3}Chl, in a CP-II monomer independently detrapping and generating ^{3}Car; ^{3}Chl and ^{3}Car annihilating to form a new triplet; and both ^{3}Chl and ^{3}Car mutually annihilating one another. Of these, only the last type of interaction could shorten the observed ^{3}Car lifetime significantly. This increase in decay rate can be illustrated by a simple case where a single trapped ^{3}Chl occupies a CP-II monomer containing ^{3}Car. For simplicity the spontaneous decay rate of ^{3}Chl is assumed slow in comparison with its 'detrapping rate', α, which includes the rate at which ^{3}Chl detraps, migrates to ^{3}Car and annihilates. The relaxation rate of the chlorophyll bleaching at 660 nm (Fig. 4b) would provide a good approximation of α. At a given time, t, ^{3}Car will have relaxed naturally according to its inherent decay rate κ or, it will continue to exist with a probability $\exp(-\kappa t)$. The probability that ^{3}Chl will detrap during the time t and $t + \mathrm{d}t$ is $\alpha \exp(-\alpha t)\mathrm{d}t$. It follows that the probability, at time t', that ^{3}Chl has been responsible for annihilating ^{3}Car, is:

$$P_{\mathrm{ann}}(t') = \int_0^{t'} \alpha \, \mathrm{e}^{-\alpha t} \mathrm{e}^{-\kappa t} \, \mathrm{d}t \tag{3}$$

From this, the probability that ^{3}Chl has not annihilated ^{3}Car is:

$$P(t') = 1 - P_{\mathrm{ann}}(t') \tag{4}$$

or

$$P(t') = \frac{\kappa}{\kappa + \alpha} + \frac{\alpha \mathrm{e}^{-(\kappa+\alpha)t'}}{\kappa + \alpha}$$

The first term on the right hand side of Eqn. 4 is the fraction of the ^{3}Car population which decayed spontaneously before ^{3}Chl detrapped. This portion has its own set of decay kinetics involving the natural relaxation of ^{3}Car, its possible reformation by detrapped ^{3}Chl, followed by the decay of the new ^{3}Car. Its lifetime will not be shortened significantly. The second term in Eqn. 4 represents that portion of the ^{3}Car population which decays rapidly. This $\alpha/(\kappa + \alpha)$ fraction of the total decays at the rate $\kappa + \alpha$. Using 8.7 and 5.7 μs for κ^{-1} and α^{-1}, respectively, the lifetime of this portion would be 3.4 μs, substantially less than the low-intensity ^{3}Car lifetime. An increase in the average number of trapped ^{3}Chl per monomer would cause a further increase in the ^{3}Car decay rate.

The above discussion supports the feasibility that trapped ^{3}Chl interacting destructively with ^{3}Car can significantly reduce the observed carotenoid triplet lifetime. Further, the inevitable heterogenous distribution of ^{3}Car and ^{3}Chl among isolated antenna-protein monomers makes multiphase ^{3}Car decays probable. Some units would contain ^{3}Car by itself, which would relax in its normal manner; others would also contain one or more trapped ^{3}Chl, which would hasten the decay of ^{3}Car. It is also possible that trapped ^{3}Chl could reside by itself, eventually detrapping and forming ^{3}Car – causing an increase rather than a decrease in carotenoid triplet population. This latter behavior has been suggested by Breton et al. [6] as a possible cause for ^{3}Car increases in chloroplasts after excitation has ceased. Their observations

make an interesting contrast to ours suggesting that 'lake model' exciton kinetics appropriate for chloroplasts produce different behavior than those of our 'puddle model' situation with isolated antenna protein monomers. It is possible that trapped ^{3}Chl, once detrapped in a photosynthetic unit, is more likely to transfer its exciton to an available ground-state carotenoid than annihilate with a carotenoid triplet. A second possibility is that exciton energies used by Breton et al. were not sufficiently high to pass the threshold of ^{3}Car lifetime shortening for chloroplasts.

The transients generated in both forms of LHC under high intensity (I_0) were shorter in duration and lower in magnitude than those in CP-II. Low intensity transients in these complexes could not be adequately resolved. However, unless these preparations of the antenna protein occupy a special category, we expect their low intensity ^{3}Car lifetimes to be in the range of 8 μs following the trend of CP-II, chloroplasts and other chlorophyll proteins [3]. Under high intensity illumination, LHC_m and LHC_l exhibited carotenoid triplet lifetimes of 2.2 and 1.0 $\pm$ 0.3 μs, respectively. Comparing these with the 3 μs lifetime in CP-II, it appears that ^{3}Car lifetimes are more severely attenuated in the large antenna complexes (i.e., those containing more chlorophyll per unit). Extending the trapped ^{3}Chl hypothesis, larger chlorophyll arrays may provide more potential self-trapping sites for chlorophyll triplets, or provide a faster detrapping mechanism. This ^{3}Car quenching mechanism may not function in chloroplasts or reaction-center proteins where other triplet-quenching processes are active.

The triplet yield in the complexes also appear to vary according to their size. In CP II, LHC_m and LHC_l solutions with similar chlorophyll concentrations, the ^{3}Car yields (measured by the 515 nm transient) were 4.5, 2.5 and 1.6%, respectively, when excited with intensity I_0. This is expected, because, for a given photon flux, larger complexes will absorb more quanta per unit, enhancing the probability of exciton annihilation. The difference in ^{3}Car yield for LHC_m and LHC_l suggests that once LHC monomers are linked in lamellar form [23–25], excitons can migrate readily between monomer units.

The formation period of ^{3}Car in the two forms of LHC was about 12 ns, which is similar to risetimes measured for other chlorophyll proteins at 20 °C [1]. The risetime in CP-II, however, was much shorter – less than or equal to 5 ns. This apparent contradiction of the Kramer and Mathis measurement of 13 ns [1], is perplexing; one possibility is that the highly energetic condition generated in the small CP-II unit by intense radiation promoted the fast formation of ^{3}Car. Considering that our excitation source was 4 ns in duration, the risetime measurements of the carotenoid triplet are indicative of the time required for ^{3}Chl (formed during or immediately after excitation) to migrate to a carotenoid site, coupled with the probability of exciton transfer from chlorophyll to carotenoid.

In summary, we have found the behavior of triplets formed in the antenna protein complex under high level illumination to be complicated. Exciton interactions not only occur during the initial period following excitation but also may have important effects during the entire lifetime of triplet species. Our hypothesis concerning chlorophyll triplets and their interaction with carotenoid triplets is tentative and in need of further testing. An experiment correlating the population and lifetime of chlorophyll triplets with those of carotenoid triplets at low as well as at high intensities would provide valuable information in this regard.

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