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PICOSECOND KINETICS OF CHLOROPHYLL AND CHLOROPHYLL/QUINONE SOLUTIONS IN ETHANOL

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SUMMARY

The mechanism of quenching by quinones of the lowest excited singlet state of chlorophyll has been investigated using picosecond laser spectroscopy. With chlorophyll alone, laser excitation resulted in immediate (< 10 ps) bleaching of the 665 nm band and production of new absorption bands in the regions 460–550 and 800–830 nm. The lifetimes of these changes were greater than 500 ps. Addition of 2,6-dimethylbenzoquinone caused quenching of these absorbance changes. No indication of chlorophyll cation radical formation was obtained. Thus, the interaction between quinone and the chlorophyll excited singlet state results in energy dissipation without measurable formation of radical species having lifetimes longer than 10 ps. This is in marked contrast to the quenching of the chlorophyll lowest triplet state by quinones, during which easily detectable stable radical formation has been observed.

INTRODUCTION

It is generally accepted that photosynthesis begins with a photo-induced energy transfer resulting in oxidation-reduction reaction. The primary process in Photosystem II is believed to involve the interaction of a photoexcited chlorophyll molecule with an acceptor, possibly a benzoquinone analog (Q) [1, 2] resulting in a one-electron transfer.

The mechanism of electron transfer between chlorophyll and Q has been investigated in vitro by flash photolysis, ESR and other spectroscopic methods (refs. 3–5 and Rizzuto, F., Castelli, F. R. and Tollin, G., unpublished). These results indicate that chlorophyll is oxidized while the quinone acts as the electron acceptor. Although all the mechanistic details are not known, the previous data has shown that the lowest triplet state of chlorophyll is located at a sufficiently high energy level to be able to transfer an electron to a quinone molecule resulting in a chlorophyll cation radical $Chl^{\cdot +}$ and a quinone anion radical, $Q^{\cdot -}$, i.e.

$$\mathrm{Chl}+h\nu \rightarrow {}^{1}\mathrm{Chl}^{*} \rightarrow {}^{3}\mathrm{Chl}^{*}$$

$$^{3}\mathrm{Chl}^{*} \overset{\mathrm{Q}}{\rightarrow} \mathrm{Chl}\cdot^{+}+\mathrm{Q}\cdot^{-} \qquad (1)$$

The identity and lifetime of several of these components has been measured by flash photolysis [3, 4], including such species as ^{3}Chl*, free Chl$\cdot^{+}$, and free Q$\cdot^{-}$.

On the other hand, NMR and ESR studies [2] suggest that quinone radical formation does not proceed via the chlorophyll lowest excited singlet state even though more than enough energy is available and the chlorophyll fluorescence is quenched by quinones. To elucidate the mechanism of interaction between the excited singlet-state chlorophyll and quinone and to ascertain the possible short-time existence of a Chl$\cdot^{+}$ intermediate we have carried out experiments in the picosecond time domain. With a time resolution of $\simeq$10 ps we have found no evidence for the presence of Chl$\cdot^{+}$ during the interaction of photoexcited chlorophyll with quinone.

EXPERIMENTAL

Chlorophyll samples were prepared by previously described methods [6]. In the experiments presented in this paper, dilute solutions of $2 \cdot 10^{-4}$ M chlorophyll *a* in ethanol were used in a 2-mm optical path length cell. Since we were primarily interested in observing the chlorophyll excited singlet state, the samples were not deoxygenated. The excitation pulse at 630 nm was the stimulated Stokes Raman of cyclohexane generated by the 530 nm second harmonic of the laser emission. The absorbance of the sample was $\simeq$ 0.4 at 630 nm which corresponds to an absorbance of 2 at the 665 nm band maximum. The time element was provided by a 20 or 6 ps/step echelon [7], while the presence of transient absorption, in the range of 460–900 nm, was investigated by means of picosecond continua [8, 9] generated in various liquids, including water, ethanol, carbon tetrachloride and cyclohexane.

The optical system is reproduced schematically in Fig. 1. The amplified single pulse at 1060 nm contained an energy of $\simeq$ 75 mJ with a pulse width of about 7 ps. The 530-nm single pulse was generated by passing the 1060 nm pulse through a frequency-converting KDP crystal. Subsequently the 530 nm pulse was collimated into a 10-cm cell (No. 7, Fig. 1) containing cyclohexane resulting in the emission of the 630-nm stimulated Stokes Raman utilized for excitation of the sample, with an energy of 1–2 mJ. The 630-nm Raman line for excitation was generated in a short optical path cell to assure that only stimulated Stokes Raman lines are generated while the self-focusing and phase modulation which result in a continuum are prevented to a large extent.

Partial focus of the fundamental 1060 nm or SHG 530 nm second harmonic pulse into a 20-cm length cell (No. 8, Fig. 1) containing carbon tetrachloride or cyclohexane, resulted in the emission of the picosecond continuum covering the desired region of 460–900 nm.

Arrangement of the optics, especially location of the pellicle beam splitter, Fig. 1, No. 10, provided for the experiment to be performed in two different ways.

(A) To eliminate possible errors arising from any permanent change in the absorption as a result of excitation by an intense picosecond pulse, one of the interrogating beams, I_0, traversed the sample 300 ps before the excitation pulse passed

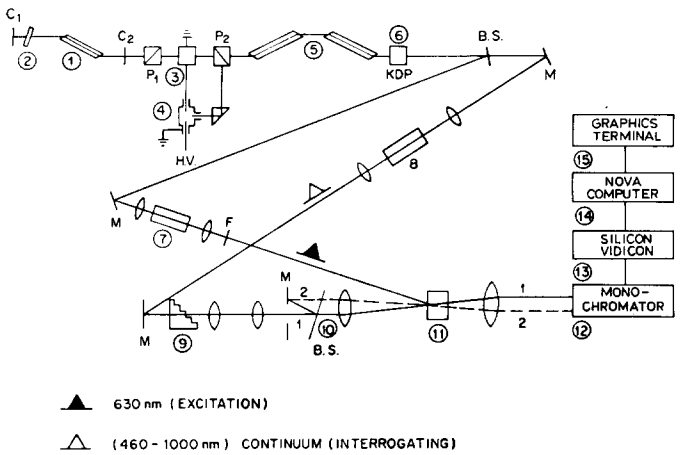

Fig. 1. Schematic representation of the experimental system. The components are: (1) Nd^{3+} glass oscillator with cavity mirrors C_1 and C_2; (2) saturable dye absorber cell; (3) Pockels cell positioned between crossed Glan polarizers P_1 and P_2; (4) spark gap; (5) Nd^{3+} glass amplifiers; (6) second harmonic generator; (7) stimulated Raman Stokes cell containing cyclohexane; (8) broad-band continuum cell containing carbon tetrachloride or cyclohexane; (9) stepped-delay transmission echelon; (10) double-beam splitter; (11) sample cell; (12) monochromator; (13) silicon vidicon optical data digitizer; (14) Nova computer; (15) graphics terminal. Mirrors are denoted by M; beam splitters by B.S.; optical filters by F.

through the same point of the sample. One of the first echelon segments of the second beam, I, was coincident with the excitation pulse while the remaining echelon segments followed at 7- or 20-ps intervals. This optical arrangement allowed the first beam to act as the reference, I_0, beam sampling the unexcited sample just previous to excitation while the second beam, I, probed the induced transient absorption from the time of excitation to several hundred ps later. This arrangement is utilized in every other laser shot to assure the integrity of the sample.

(B) To extend the time interval for probing kinetics of transient species, both beams of A were utilized immediately after excitation. In this experimental situation one of the first echelon segments of the first beam was coincident with the excitation pulse while all the subsequent segments provided time information for the 0–100 ps time region in steps of 20 ps. Then there is a "dead time" of 300 ps and the second echelon becomes operative for the 400–500 ps time range. The intervals can be changed proportionally by means of longer or shorter echelon segments while the distance between the pellicle and reflector can easily alter the dead time from 300 ps to several ns.

The transient absorption spectra were observed by means of a silicon vidicon optical data digitizer system and then analyzed, averaged and displayed by a Nova computer, Tektronix display and hard copier. The details of this diagnostic system can be read in ref. 13. When the experimental system B as described above was in force, alternate shots with and without excitation were taken to insure that fluctuation due to the laser emission or other external sources was appropriately considered. The timing of the pulses was actually measured experimentally rather than relying on simple computations, which due to the possible changes of the index of refraction as a

result of the high laser fields and other effects, could invalidate the well-known simple group velocity calculations.

The absorbance changes, ΔA, for each time period were calculated from the ratio of the measured intensities of the reference (I_0) and probing (I) beams, $\Delta A = \log(I/I_0)$. When the optical arrangement of case B was utilized, I and I_0 refer to the intensity of the beam with and without excitation. Experimental ΔA values for the I beam (with excitation) fell in the range of 0.3–0.7 while the variations for the I_0 beam (without excitation) were at least one order of magnitude less or $\simeq 0.03$.

Chlorophyll *a* was isolated from spinach by methods described previously [6]. The acceptor, 2,6-dimethylbenzoquinone (Eastman grade), was used after it was purified by recrystallization from ethanol/water solution.

RESULTS

Investigation of the absorption changes from the time of excitation to 500 ps was carried out in the wavelength intervals from 460 to 550 and 800 to 830 nm, respectively. Although we measured the kinetics of these regions on consecutive 10-nm steps, for brevity, we show the data at 820, and 480 nm only. We also observed

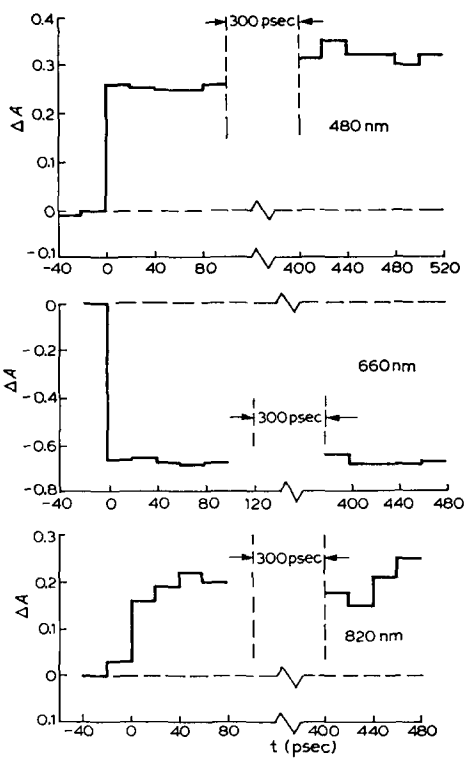

Fig. 2. Kinetics of chlorophyll in ethanol after excitation with a single 530 nm 6-ps pulse, displayed as absorbance changes ΔA vs. time in ps. Top: kinetics at 480 nm; middle: kinetics at 660 nm; bottom: kinetics at 820 nm.

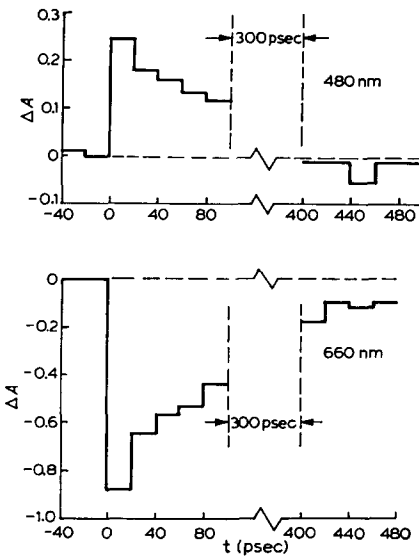

Fig. 3. Kinetics of chlorophyll/ethanol solution with the addition of 0.15 M, 2,6-dimethylbenzoquinone, under the same excitation conditions as in Fig. 2. Top: kinetics at 480 nm; bottom: kinetics at 660 nm.

that the intensity of the absorption band located at 665 nm diminished as a result of excitation at 630 nm. The specific changes observed for each of these three wavelengths regions can be described as follows.

(A) Absorption at 460 nm to 550 nm

The absorption band reached its maximum intensity in less than 10 ps. This is apparent from the histogram of the formation process shown in Fig. 2, upper. The lifetime of this band is much longer than the 0.5 ns time shown in Fig. 2. In this figure, no decay is evident within a period of more than 500 ps after excitation, but rather a small increase in absorption can be seen after 400 ps which is within the experimental uncertainty.

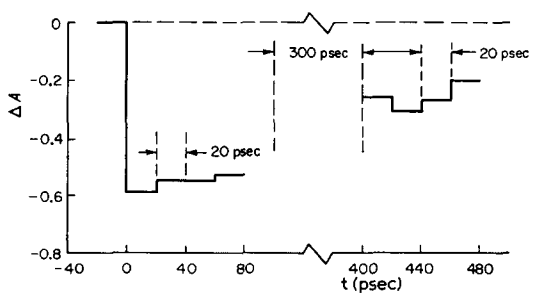

Fig. 4. Rate of recovery of the 660-nm band of chlorophyll/ethanol after the addition of 0.05 M 2,6-dimethylbenzoquinone.

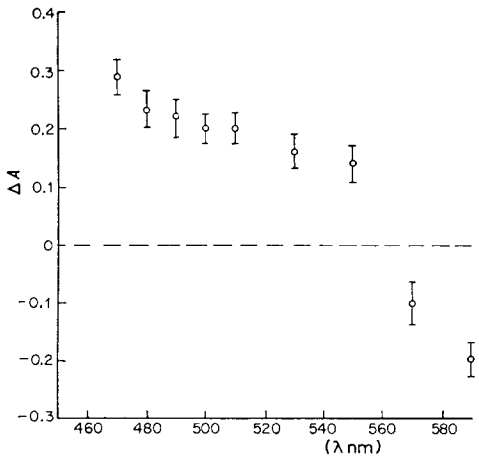

Fig. 5. Absorbance changes (ΔA) of chlorophyll *a* in ethanol as a function of wavelength (λ) after excitation by a 630 nm ps pulse.

Similar experiments performed with the addition of 0.15 M 2,6-dimethylbenzoquinone revealed that while the formation rate of the 460–550 nm band remained the same, the decay lifetime decreased sharply as shown in Fig. 3 (top). Note that the decay is virtually complete at the end of the experiment, indicating that no long lived species absorbing in this region were being produced.

The spectrum between 460 and 550 nm consists of a broad structureless band (see Fig. 5), which exhibits a gradual increase in absorbance from long to shorter wavelengths. The molar absorption coefficient at 480 nm is estimated to be $\varepsilon_{480} \cong 2 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ from the ratio $|\Delta A_{480\,nm}|/|\Delta A_{665\,nm}| \simeq 0.3$ and the value of $\varepsilon_{660\,nm} \simeq 7 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ [11].

(*B*) *The 800–830 nm absorption band*

Immediately after excitation of the chlorophyll/ethanol sample by the 530 nm ps pulse, we observed the formation of another band in the region of 800–830 nm. The increase in absorbance at 820 nm, ΔA, was small ($\Delta A_{820\,nm} \cong 0.12$) in comparison to the 460–550 nm absorption changes. Because of this small $\Delta A \cong 0.12$, the rate at 820 nm may not be as accurately measured. This difficulty arises from the molecular extinction coefficient being lower at 820 nm than at 480 nm, the 0.03 ΔA

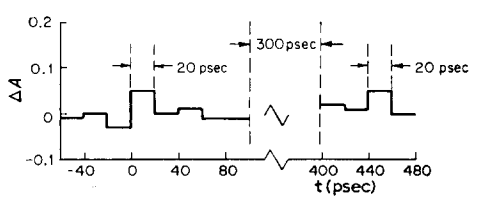

Fig. 6. Time-resolved ΔA at 900 nm of chlorophyll in ethanol solution. Note that no detectable absorbance changes are evident in the range of 0–500 ps.

reliability and weak light intensity of the continuum beam at 820 nm. The kinetics of the 820 nm absorption (Fig. 2, bottom) resemble the observed 500 nm rates of < 10 ps formation and > 0.5 ns decay. Fig. 6 shows that no absorbance change occurred at 900 nm for the time interval of 0–500 ps.

The ΔA of 0.12 at 820 nm was not enhanced when the experiment was repeated with the binary mixture of Q and chlorophyll. Similarly, we were unable to observe any changes in kinetics with this mixture. The small absorbance of this band did not allow us to make quantitative statements with regard to ΔA vs. t after excitation. However, in an identical solution where Q was added, we observed a lower ΔA than that observed in chlorophyll/ethanol immediately after excitation, which continued to decrease in time.

(C) *Bleaching at 660 nm*

Simultaneous with the formation of the two absorption bands in the 820 and 500 nm regions, we observed bleaching at 660 nm. Furthermore, this bleaching per-

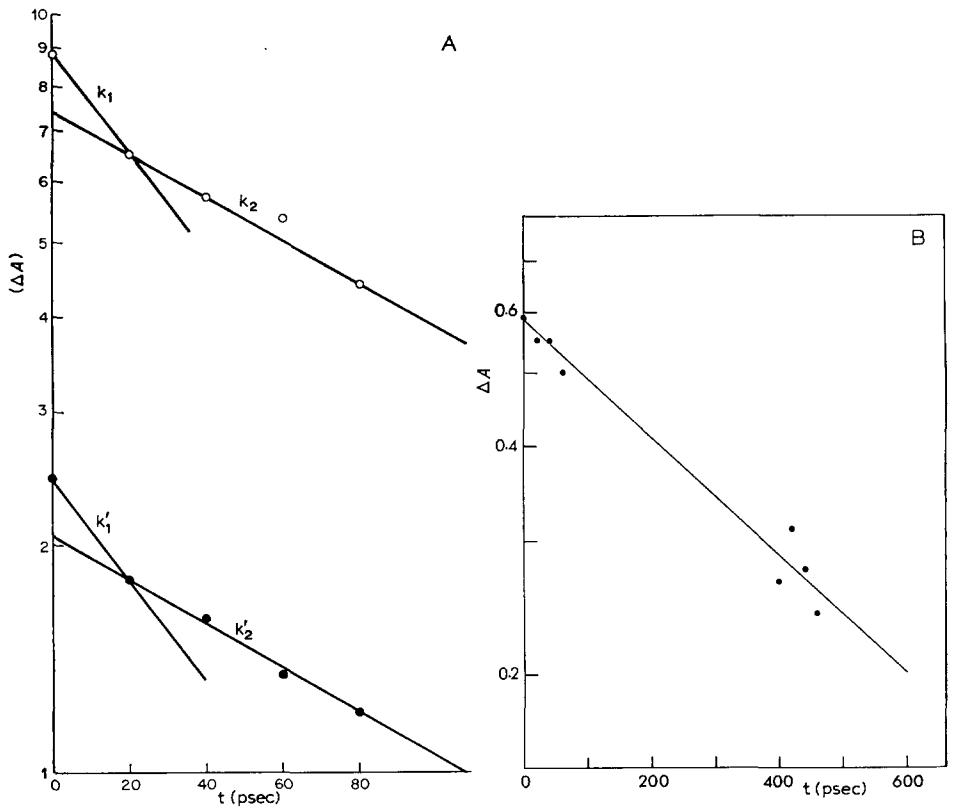

Fig. 7. Plot of the absorbance changes (ΔA) of the binary systems chlorophyll *a* plus 2,6-dimethylbenzoquinone in ethanol solution. (A) 2,6-Dimethylbenzoquinone concentration is 0.15 M; ○, 660 nm; ●, 480 nm. (B) 2,6-Dimethylbenzoquinone concentration is 0.05 M and the wavelength is 660 nm.

sisted for an interval of time longer than 0.5 ns (Fig. 2, middle), similar to the kinetic behavior of the two absorption bands.

Repetition of the experiment after addition of 0.15 M Q to the $2 \cdot 10^{-4}$ M chlorophyll/ethanol solution indicated that the band recovered from bleaching within 400 ps (Fig. 3) lower. Thus, little or no long-lived chlorophyll species were produced. Examination of the recovery kinetics as a function of ΔA revealed that the rate could not be described by a simple exponential, but could be fitted by the two completely different rates which appear in Fig. 7a. The observed dependence of the rate on [Q] suggests that at lower concentrations of Q the faster recovery times of Fig. 7a should disappear. Indeed this is the case at $\simeq$ 0.05 M of Q (see Fig. 4) where the fast recovery slope of Fig. 7a was not observed (Fig. 7b) and the only decay lifetime found was about 400 ps, a value which is in close agreement with the lower rate constant, approx. $5 \cdot 10^{10}\ s^{-1} \cdot M^{-1}$, (Fig. 7b).

DISCUSSION

The 630-nm laser pulse excites chlorophyll into the lowest excited singlet state which is known in vivo to result in energy transfer and an oxidation-reduction process with nearly unit quantum efficiency. As will be shown below, the present experiments seem to indicate that the interaction of this excited state with an acceptor such as quinone in vitro results in energy degradation without the formation of stable electron transfer products.

The absorbance changes observed after excitation, both positive and negative (bleaching) achieved their maximum value in less than 10 ps and persisted for more than 0.5 ns in pure ethanol. The absence of any decrease of the maximum intensity after 0.5 ns would suggest that it is reasonable to assume that the decay is several nanoseconds long. Therefore we can assign our data to an excited state of chlorophyll *a* with a character similar if not identical to the previously observed lowest singlet state, which has a measured fluorescence lifetime of 6.3 ns [10].

The assumption does not exclude the presence of $Chl^{\cdot +}$, since this radical also shows absorption at these wavelength regions at least in dichloromethane solutions [11]. Therefore it is possible that the observed absorbance variations reflect, at least in part, the formation of this species.

The observed increase in the rate of recovery of the 660 nm bleaching with added quinone can be assigned to a quenching process resulting from the interaction between the excited state chlorophyll and quinone. This causes the return of the population to the ground state, i.e. recovery of bleaching and the transfer of energy to Q. The fact that instead of a single exponential decay we obtain (Fig. 7a) a ΔA vs. t curve composed of two different decay slopes, suggests that at high Q concentration we observe the formation of a supermolecule or aggregate composed of a chlorophyll *a* molecule surrounded by several quinone molecules within the interaction radius. In this case the ultrafast rate constants are attributed to near-neighbor static quenching, rather than to a collisional mechanism involving diffusion. This mechanism is further substantiated by the disappearance of the faster of the two decay components of Fig. 7a at lower quinone concentrations, i.e. 0.05 M as displayed in Fig. 7b.

Similar kinetics were observed for the 460–550 nm photoinduced absorption band; however, the relatively small absorption cross-section of the 820-nm state pre-

vented an accurate measurement of its decay. These experimental observations, and the fact that no long lived absorbance in the 460–560 nm spectral region was observed, suggest that the probability for the formation of a $Chl\cdot^{+}$ species is very small, unless its lifetime is less than 10 ps. The absence of a $Chl\cdot^{+}$ species which lives longer than 10 ps is also supported by the fact that: (1) the addition of quinone clearly failed to produce any additional absorption in the 800–830 nm region at any time up to 0.5 ns. (2) The 480-nm absorption intensity did not increase after addition of Q. One would expect enhancement at these wavelengths [11] if a mechanism which generated $Chl\cdot^{+}$ during the quenching process was operative to any appreciable extent. (3) The absence of any absorption changes in the 850–950 nm region immediately after excitation rules out the possibility of electron photoejection from chlorophyll *a* into the ethanol. The absorption spectrum of the solvated electron [12] is well known and its solvation kinetics have been the subject of several recent studies [13, 14]; therefore, we believe that if present, its absorption would be displayed as increased ΔA in Fig. 6.

In view of the results presented in this paper, we believe that the singlet state quenching mechanism involves predominantly energy transfer and degradation. Therefore, either electron transfer is not involved at all, or, if such transfer occurs, the resulting ion pair ($Chl\cdot^{+}-Q\cdot^{-}$) disappears via recombination with a rate faster than $10^{11}\ s^{-1}$. Inasmuch as electron transfer readily occurs from the lowest chlorophyll *a* triplet to quinones to form stable radical products which are easily observed (refs. 3–5 and Rizzuto, F., Castelli, F. R. and Tollin, G., unpublished), we are compelled to conclude, in view of the present experimental data, that the operative mechanism for the interaction of the chlorophyll *a* singlet state with quinone in solution involves quenching without such stable radical formation.

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