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CHLOROPHYLL ONE-ELECTRON PHOTOCHEMISTRY: FLASH PHOTOLYSIS
STUDIES OF THE CHLOROPHYLL-QUINONE REACTION IN ALCOHOL SOLVENTS[†]

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[†]Taken from a dissertation submitted by R. A. White in partial fulfillment of requirements for a Ph.D. degree, University of Arizona, 1971. Research supported in part by the U.S. Atomic Energy Commission (Contract No. AT(11-1)908) and the Air Force Cambridge Research Laboratory (Contract No. F-19628-68-C-0027).

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ABSTRACT

Flash photolysis of chlorophyll a alone in CBE (cyclohexanol-tert.-butanol-ethanol) yields a difference spectrum similar to those obtained upon steady illumination of chlorophyll a-quinone mixtures in this solvent. Decay kinetics in CBE and dimethylsulfoxide are faster at the Soret band than at 460-580 nm and red band regions. This difference is not obtained in other solvents (CHCl_3 , CCl_4 , tert.-butanol, ethanol), implying that two or more species are obtained in CBE and DMSO.

β -carotene in CBE increases the rate of decay of the flash-induced chlorophyll transients at 430 and 660 nm but only decreases the magnitude of the signal at 470 nm. This implies that the 470 nm absorbance is due to a product formed from the triplet state. This effect is not observed in ethanol.

Adding quinone to chlorophyll solutions results in slowly decaying species being generated by flash excitation in CBE. Three components can be distinguished: the first ($t_{1/2} \sim 0.2$ msec) corresponds to the triplet state; the second ($t_{1/2} = 5-10$ msec) is quinone concentration and species independent; the third ($t_{1/2} =$ several seconds) is dependent upon quinone concentration and species (rate is faster for higher concentrations and lower potential quinones). The ESR signal decay rate is approximately equal

to the third component flash decay rate when the chlorophyll and quinone concentrations are equal. With excess quinone, the flash decay rate becomes faster, and the ESR decay rate decreases slightly. These slowly-decaying species are not produced when quinone is added to chlorophyll a in ethanol or tert.-butanol, or to pheophytin in CBE. One observes merely a decrease in signal height with no accompanying increase in decay rate.

Mechanisms to account for all of these phenomena are presented which involve an initial chlorophyll triplet-solvent reaction with the subsequent formation of several species of chlorophyll-quinone radical complexes.

INTRODUCTION

In the preceding paper[1], we presented evidence for a chlorophyll-photosensitized one-electron transfer from solvent to quinone leading to the formation of a chlorophyll-semiquinone complex. This species is unstable in basic solvents and rapidly dissociates to liberate the quinone anion radical. Under some conditions of solvent and temperature the complex is stabilized and thus can be studied by steady-state methods. In the present paper we describe the results of a series of experiments using flash photolysis techniques which support and extend the steady-state measurements. This work also represents an extension of the flash studies of Raman and Tollin[2] of the chlorophyll-quinone reaction in pyridine, as well as of the investigations of Chibisov and co-workers[3] and of Seifert and Witt[4].

MATERIALS AND METHODS

The flash photolysis apparatus used in this work has been described earlier[2]. A Corning 3-66 orange filter was mounted between the flash lamp and the sample cell to eliminate exciting wavelengths below about 550 nm. The lamp was fired upon charging a 7.5 μ F capacitor to approximately 6kV. In the red region of the spectrum, the flash artifact lasted between 100 and 150 μ sec, so that most kinetic data were taken after this time. At shorter wavelengths, the flash artifact was small enough so that kinetics in the 50 to 100 μ sec range could be measured.

The sample compartment was surrounded on two sides by curved polished metal reflectors and cooled by forced air. A 5 cm quartz cylindrical cell contained the sample being studied. Degassing by the freeze-thaw method took place in a separate 20 ml round bottom flask equipped with a sidearm. The sample cell was attached to the sidearm through a high-vacuum Teflon stopcock. After the final cycle of degassing, the flask was tipped so that the sample solution flowed through the sidearm into the cell. The stopcock was then closed off and detached from the sidearm.

The photomultiplier was of the S-20 type (RCA 4463). Metal screens of known absorbance were used for calibration purposes. Oscilloscope traces were recorded on Tri-X Panchromatic 35 mm

film. For more slowly decaying absorbance changes, a Sanborn Type 151 recorder was used for readout. All other materials and methods were as described previously[1].

RESULTS

Flash Photolysis Experiments in the Absence of Quinones

In flash photolysis experiments, chlorophyll a by itself in CBE (cyclohexanol-tert.-butanol-ethanol) gives absorbance changes (Fig. 1) similar to those obtained in steady illumination experiments in this solvent in the presence of quinone[1], as well as to those observed upon flash excitation in other solvents[cf. 2]. Positive absorbance changes are found out to about 800 nm. Decay kinetics are very sensitive to the presence of traces of oxygen.* Fig. 2 shows a superposition of three oscilloscope traces obtained from the same sample on the first, sixth and thirteenth flashes. A slowing down of the decay and a small decrease in the maximum amplitude of the transient is observed. After about thirteen flashes, the decay rate and amplitude remain constant. A thirteen percent decrease in the maximum absorbance change occurs between the first and the thirteenth flash. Presumably, the oxygen and some of the chlorophyll are being consumed in a photochemical process. In the experiments reported below, decay curves were used only after an initial period of flashing was carried out.

Decay kinetics are shown for chlorophyll a in CBE, and solvents such as ethanol and dimethylsulfoxide (DMSO), for a

* These effects are probably due to the difficulty of degassing the viscous CBE solvent, inasmuch as we do not observe this in ethanol or other more fluid media.

few wavelengths in Figs. 3, 4 and 5. Decay rates do not follow any simple order, as has been observed earlier[2,5]. Semi-log plots of these decay curves show that in most cases the rates are approximately the same at all wavelengths. However, for two solvents,* CBE and DMSO, the decay of the bleaching of the Soret band (at 430 nm) appears to be considerably faster than at the other wavelengths. This may imply the presence of two or more species in these solvents which are either not obtained or not distinguishable in the other solvents.

If some of the absorbance changes we observe in these systems are due to the chlorophyll triplet state, we should be able to demonstrate quenching by β -carotene[1]. In Figs. 6 and 7 are shown the effects of the presence of this substance on the transients at 430, 470 and 660 nm in CBE. We observe marked increases in the rate of decay at 430 and 660 nm, whereas the transient at 470 nm is reduced slightly in magnitude with a much smaller increase in rate, even at considerably higher β -carotene concentrations. This shows that the absorbance changes at 430 and 660 nm reflect primarily the formation and decay of the chlorophyll triplet, while the changes at 470 nm result at least partly from another species which is a product

*Results in tert.-butanol, chloroform and carbon tetrachloride are similar to those obtained in ethanol and pyridine, except that the decay rates observed in chloroform and carbon tetrachloride are considerably faster than in the other solvents.

of a reaction of the triplet state, most probably with the solvent. The rather small decrease in signal size at 470 nm suggests that this product may also be formed from a chlorophyll singlet state reaction.

Different results are obtained in ethanol* (Fig. 8). In this solvent, on adding β -carotene, approximately the same amount of rate increase is observed both at 520 nm and at 665 nm (520 nm gives results which parallel those at 470 nm). Thus, it would appear that the second photoproduct is generated to a much lesser extent, if at all, in ethanol than in CBE. This is consistent with the results obtained at 430 nm in the absence of β -carotene (see above).

If one examines the flash decay curves of chlorophyll in CBE at slow sweep rates and higher amplification, one can observe a small, slowly-decaying component (half-life around 40 millisec). This is shown in Fig. 9. Similar results are obtained at all wavelengths. We will discuss a possible interpretation of this below.

Pheophytin also shows absorbance changes in CBE as measured by flash spectroscopy. Both positive and negative transients are obtained as with chlorophyll (Fig. 10). The decay rates at 470 and 660 nm for both chlorophyll and pheophytin are approximately the same, but the decay of the absorbance change in the Soret band

* β -carotene effects in pyridine parallel those observed in ethanol.

(410 nm) appears to be four times faster than the corresponding decay for the chlorophyll Soret.

Further similarities with chlorophyll are seen in the effects of β -carotene (Fig. 11). At 470 nm little or no difference is observed in the decay rates on addition of β -carotene, up to as high a concentration as 2×10^{-3} M. However, at the Soret band (410 nm), the decay rate is increased by the presence of 7.5×10^{-4} M β -carotene.

Flash Photolysis Experiments in the Presence of Quinones

The addition of quinones to solutions of chlorophyll in CDE results in slowly-decaying species being generated during the flash excitation period. This is shown for *p*-benzoquinone by the traces in Fig. 12. Three decay processes can be clearly distinguished in all wavelength regions in these flash experiments (Fig. 13). One is very fast, with a half-life of about 0.2 millisecc. This probably corresponds to triplet state decay (compare with trace of chlorophyll alone in Fig. 12). The second component (quantitatively the largest) has a half-life of 5 to 10 millisecc. This decay time is approximately independent of quinone concentration. The third (generally smaller) component plots as a first order decay and has a half-life of several seconds which is quite clearly a function of quinone concentration, becoming

faster at higher concentrations (Fig. 14). Note that the decay at 460 nm is more sensitive to quinone concentration than is the decay at 620 nm. This dependency is a consequence of a pseudo-first order process due to the presence of excess quinone. This can be seen by plotting the decay constants versus quinone concentration (Fig. 15).

Some results with other quinones are shown in Fig. 16. The fast initial decay is usually difficult to observe in these systems. The first slow decay process (half-life of 3 to 7 millisecc) is relatively independent of quinone species, with the exception of *p*-naphthoquinone. In the case of this derivative, its redox potential may be too low to elicit a sizeable amount of the species responsible for the slow decays. That naphthoquinone has little effect on the decay of chlorophyll absorbance changes is seen in Fig. 16.

The decay rates of the slowest processes are clearly dependent upon quinone species. The rates decrease as the quinone potential increases (Fig. 17). This is similar to what was observed in the steady-state experiments[1].

Quinone potential is also a factor in the divergence of decay rates between the 460 nm band and the red band. In flash experiments using different quinones at equal concentrations (quinone to chlorophyll ratio of 5.8), it was found that, for quinones whose potential is less than that of benzoquinone, the decay rates were about equal (Fig. 18).

If one compares the slowest decay observed in the flash photolysis experiments (red band bleaching), the steady-state ESR experiments and the steady-state optical experiments, one finds that the steady-state optical decay is much slower than the other two decay processes (Fig. 19). The difference in decay rate observed between the steady-state ESR signal and the slowest flash decay depends on the molar ratio of chlorophyll to quinone. If the concentrations are about equal, the two decay rates approach each other. With increasing quinone, the ESR decay rate decreases slightly but the slowest flash decay becomes faster (Fig. 20).

Quite different flash behavior is obtained in ethanol or tert-butanol upon addition of quinone. In these solvents, one observes a decrease in the signal size at all wavelengths (Figs. 21 and 22). Decay kinetics (in ethanol) are not affected at 460 and 665 nm, although they seem to be slightly accelerated at 430 nm. This behavior parallels that observed in pyridine[2] and so we may assume that the nature of the reaction is the same (see below). It is worth recalling here that, in these solvents, one obtains no chlorophyll bleaching in steady-state experiments and the paramagnetic species generated is always the semiquinone anion radical.

Addition of quinone to pheophytin solutions in CBE produces similar quenching of the signal size as with chlorophyll in ethanol

or tert.-butanol. No slowly decaying species are generated.

This is consistent with our observations of no steady-state photobleaching of pheophytin and formation of semiquinone radical species[1].

DISCUSSION

In the preceeding paper[1], we have presented arguments for solvent involvement in chlorophyll-quinone photochemistry. The simplest reaction of chlorophyll triplet with an alcoholic solvent would be hydrogen abstraction (inasmuch as quinone radical formation also occurs in tert.-butanol, the hydroxyl hydrogen could be involved^{*}):

k_1 Represents all physical modes of decay of the triplet state.

Triplet quenching by β -carotene would lead to an increase in triplet decay rate and a decrease in the amount of $\text{ChlH}\cdot$ formed.

* It is not possible to preclude the occurrence of α -hydrogen abstraction. However, if the reaction occurs within a chlorophyll-solvent complex in which the hydroxyl oxygen is coordinated to magnesium[6], hydroxyl hydrogen transfer could be favored. Garcia-Morin, et al.[7] have postulated that a similar hydrogen transfer occurs in chlorophyll-water micelles formed in hydrocarbon solvents. Also, irradiation of quinones with visible light in aqueous propanol, N-ethyl acetamide or dimethyl formamide has been shown to lead to semiquinone radical formation and proton ejection[8] which suggests that hydrogen atom transfer from solvent to excited quinone can occur.

This can account for the difference in behavior of the transients at 430 nm, 460 nm and 660 nm in the presence of this quencher* (Figs. 6 & 7).

Because of the overall reversibility of the photoreaction, we propose that the reverse hydrogen transfer process is fast:

However, an alkoxy radical should also be capable of dimerization⁺, which would compete with reaction (3):

Inasmuch as the peroxide so generated is an oxidizing agent, it could also function to oxidize ChlH·:

* It is interesting that β -carotene in hydrocarbon solvents, in ethanol or in pyridine quenches chlorophyll transients at all wavelengths with approximately equal effectiveness (Fig. 8 and Ref. 2). This suggests that in these solvents, little or no hydrogen abstraction from solvent by excited chlorophyll occurs in the absence of quinone. In the case of the hydrocarbon solvents, no quinone radical signals are generated upon illumination.

⁺ Weiner & Hammond[9] have observed that the tert.-butoxy radical decays via a second order process (dimerization?) with a rate constant of $10^4 \text{ M}^{-1} \text{ sec}^{-1}$.

Equations (3), (4) and (5) account for the lack of simple kinetics at 460 nm, and can also explain the very slow decay process observed in flash experiments in CBE (Fig. 9).

Reactions (1)-(5) are consistent with all of the flash photolysis results obtained in the absence of quinones. When quinones are present in ethanol or tert.-butanol, one observes a decrease in the amount of flash-induced transient with little or no change in decay rates (at least at 460 and 660 nm) and the rapid formation of semiquinone anion radical (within the flash lifetime). This is the same type of behavior observed by Raman and Tollin[2] in pyridine, and thus the mechanism proposed for this solvent probably also applies to these alcohols. This involves a photochemical reaction* proceeding within a ternary complex of solvent, chlorophyll and quinone:

The increase in decay rate at 430 nm (Figs. 21 & 22) may reflect the disappearance of some of the quinone radical via reaction with oxidized solvent.

*

It is also known that quinone quenches Chl_s and Chl_t via collisional processes [2]. However, since the chlorophyll bleaching decreases in the presence of quinone, these are probably not photochemical processes.

Reaction (6) can easily explain all of our steady-state ESR and optical results at room temperature (no chlorophyll bleaching or radical signals, semiquinone anion radical formation)[1].

The quinone radical may also disproportionate (this was demonstrated by our earlier ESR experiments[10]):

Regeneration of Q can proceed via the following reactions, both of which would be expected to be rapid:

This accounts for the complete reversibility of the quinone-chlorophyll photosystems.

In attempting to formulate the reactions which are occurring in CBE, we must keep in mind the following additional facts:

1. Two decay processes are observed in the flash experiments. One is relatively fast and quinone concentration and species independent. The other is slow (although faster than the ESR and steady-state optical decays at higher quinone concentrations) and increases in rate with increasing quinone concentration and

decreasing potential (with a difference of magnitude between 460 and 660 nm for high potential quinones).

2. As shown in the preceeding paper[1], the rise kinetics for the steady-state optical signals are complex and are faster in the initial portion at 460 nm and 430 nm than at 660 nm. Only the rapidly-produced species is paramagnetic. The decay kinetics of the optical signals are the same at all wavelengths and are the slowest of all.

The initial, rapid formation of a slowly-decaying species (occurring within the flash lifetime) is undoubtedly the following (by analogy with the pyridine and ethanol results):

Such a complex of chlorophyll and quinone radical would be metastable and its decay should occur by dissociation (the electron leaving with the quinone):

With metal-free porphyrin derivatives, the complex is either not formed or is quite unstable, and thus slow decays are not observed. Reaction (11) could correspond to the intermediate, quinone concentration independent*, decay process observed in the

* It is difficult to rationalize why such a dissociation should be relatively independent of quinone potential unless the unpaired electron is largely localized on the quinone[1].

flash experiments (the quinone radical decaying by disproportionation, as before). We propose that the electron remains with the quinone so as to be consistent with reaction (6). This is also to be expected on chemical grounds.

At the high levels of quinone used in these experiments, it is possible that the 1:1 complex reacts with another quinone molecule to form a 1:2 complex:

This would again decay by a similar dissociation:

However, the additional quinone molecule would further delocalize the unpaired electron and, in terms of our previous arguments[1], would probably make reaction (13) intrinsically slower than reaction (11). We suggest that the 1:2 complex is what is being observed in the ESR experiments, which are generally carried out at high quinone concentrations. Reaction (13) is also consistent with the first order nature of the ESR decay. The additional quinone molecule, by further decreasing the electron density on the chlorophyll, and perhaps also by partially removing the proton through hydrogen bonding, might tend to make the absorption spectrum of this species more similar to that of ordinary chlorophyll than would be the case for the 1:1 complex.

Thus, the quinone concentration and quinone species-dependent absorbance decrease observed in the flash experiment could be reaction (12). This can also account for the differing decay rates seen at 460 nm and 660 nm in the flash results with benzoquinone (Fig. 15). Thus, if the 1:2 complex has lost much of the 460 nm absorbance, while still retaining some bleaching in the red,^{*} the slower rate of reaction (13) would tend to compensate at 660 nm but not at 460 nm for the increase in decay rate caused by reaction (12). Lower potential quinones would form less stable 1:2 complexes, thus causing reaction (13) to become rapid (Fig. 17). This would tend to make the differences at 460 nm and 660 nm less apparent, as is observed (Fig. 18).

One would not expect much of the 1:2 complex to be formed in a flash experiment due to the bimolecular nature of its formation and the instability of the 1:1 complex. Thus, a separate decay process reflecting reaction (13) might be difficult to observe. On the other hand, this species would tend to build up in a steady-state experiment such as ESR.

*This is perhaps not unreasonable, inasmuch as the unpaired spin density on the chlorophyll should be quite low but there should still be an appreciable perturbation of the chlorophyll absorption spectrum by the complexed quinone molecules.

The biphasic rise kinetics observed in the optical experiments can be explained in terms of a photoproduct interacting with the 1:2 complex. This is most likely $\text{ChlH}\cdot$ (although it could also be another molecule of the 1:1 complex):

or electron dipole-dipole interaction

If appreciable electron pairing occurs, this species would be diamagnetic. This is in agreement with our results[1]. Also, if the ChlH_2 form makes an important contribution, one might expect the absorbance in the normal chlorophyll bands to be smaller than for the 1:2 complex. This would explain the very slow bleaching process observed in the steady-state optical experiments. The rate of formation of this species should depend on the nature of the quinone used, again in agreement with our results. The biphasic nature of the rise curve can be accounted for by the intermediacy of the 1:2 complex. The fact that reaction (14) produces a diamagnetic species explains the monophasic nature of the ESR rise curve.

* It is also possible to formulate this as follows:

Such a 2:2 complex can again decay by a first order dissociation:

The extensive electron delocalization and lack of radical character in such a species should result in appreciable stability, thus accounting for the very slow decay and the build-up of this product upon prolonged illumination. This also explains the differences observed between the ESR and optical decays.

Inasmuch as all of the species which we are observing (the 1:1 complex which is the predominant form seen in the flash experiments, the 1:2 complex which is the predominant ESR-active product and the 2:2 complex which produces the largest steady-state optical signals) contain both porphyrin and quinone moieties, we would expect their decay rates and activation energies to respond to changes in porphyrin and quinone structure in similar ways. This is in agreement with our observations[1].

The above scheme, while perhaps not unique, is probably the simplest which can successfully account for all of our results. Even if all of the details of interpretation are not entirely correct, a clear and very interesting feature of these experiments is the apparent ability of relatively small changes in solvent environment (e.g., ethanol vs. CBE) to cause interactions which

stabilize otherwise very highly reactive species ($\text{ChlH}\cdot$ or ChlH^+ , Q^- , ChlH_2). Such effects could have important implications for the photosynthetic system, in which such reactive entities must have long enough lifetimes to effectively participate in energy-conserving redox reactions. Furthermore, the formation of alkoxy radicals from alcohols (and peroxides by dimerization), if hydroxyl hydrogen abstraction occurs, provides a simple chemical model for photosynthetic oxygen production (which can easily proceed via peroxide decomposition).

Another interesting feature of the present results is the clear difference observed between the photochemical behaviors of chlorophyll and pheophytin in CBE. This may have important implications for the role of the chelated magnesium in photosynthetic energy conversion.

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LEGENDS FOR FIGURES

Figure 1. Flash photolysis difference spectrum for chlorophyll a ($4.7 \times 10^{-6} \text{M}$) in CBE (refluxed) at room temperature. Absorbance change measurements were made 0.15 millisecc. after the flash in a 5 cm cuvette.

Figure 2. Oscilloscope traces showing a comparison of decay kinetics for the first, sixth and thirteenth flashes at 460 nm for chlorophyll a ($1.3 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature. Each division on the ordinate corresponds to 5 mv.

Figure 3. Top-oscilloscope traces showing decay kinetics after flash photolysis of chlorophyll a ($1.8 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature. Bottom-semi-log plots.

Figure 4. Top-oscilloscope traces showing decay kinetics after flash photolysis of chlorophyll a ($4.0 \times 10^{-6} \text{M}$) in ethanol at room temperature. Bottom-semi-log plots.

Figure 5. Top-oscilloscope traces showing decay kinetics after flash photolysis of chlorophyll a ($4.4 \times 10^{-6} \text{M}$) in dimethylsulfoxide at room temperature. Bottom-semi-log plots.

Figure 6. a) Oscilloscope traces showing decay kinetics at 430 nm after flash photolysis of chlorophyll a ($2.5 \times 10^{-5} \text{M}$) and chlorophyll a plus β -carotene ($1.0 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature.

b) Semi-log plots of data in (a).

c) Oscilloscope traces showing decay kinetics at 660 nm after

flash photolysis of chlorophyll a (2.4×10^{-5} M) and chlorophyll a plus β -carotene (1.0×10^{-5} M) in CBE (refluxed) at room temperature.

d) Semi-log plots of data in (c).

Figure 7. Oscilloscope traces and semi-log plots showing decay kinetics at 470 nm after flash photolysis. a) Chlorophyll a (4.7×10^{-6} M) and chlorophyll a plus β -carotene (2.3×10^{-3} M) in CBE (refluxed) at room temperature.

b) Chlorophyll a (1.8×10^{-5} M) and chlorophyll a plus β -carotene (7.7×10^{-5} M) in CBE (refluxed) at room temperature.

Figure 8. Oscilloscope traces and semi-log plots showing decay kinetics after flash photolysis of chlorophyll a (4.5×10^{-6} M) and chlorophyll a plus β -carotene (1.8×10^{-5} M) in degassed ethanol at room temperature.

a) 520 nm.

b) 665 nm.

Figure 9. Oscilloscope traces at 5 and 100 millisec. full scale sweep showing decay kinetics at 460 nm for chlorophyll a (1.1×10^{-5} M) in CBE (refluxed) at room temperature.

Figure 10. Flash photolysis difference spectrum for pheophytin a (5×10^{-6} M) in CBE (refluxed) at room temperature. These absorbance changes are for a 5 cm cuvette and were measured 0.2 millisec. after the flash.

Figure 11. Oscilloscope traces and semi-log plots showing decay kinetics

after flash photolysis for pheophytin a ($5 \times 10^{-6} \text{M}$) and pheophytin a plus β -carotene ($7.5 \times 10^{-4} \text{M}$) in CBE (refluxed) at room temperature.

a) 470 nm

b) 410 nm

Figure 12. Oscilloscope traces at 470 nm (top) and 650 nm (bottom) showing decay kinetics after flash photolysis for chlorophyll a ($1.2 \times 10^{-5} \text{M}$) and chlorophyll a plus p-benzoquinone ($2.0 \times 10^{-4} \text{M}$) in CBE (refluxed) at room temperature. Each division on the ordinate corresponds to 2 mv.

Figure 13. Oscilloscope traces at 5 and 50 millise. and 50 sec. full scale sweep showing decay kinetics at 460 nm for chlorophyll a ($1.2 \times 10^{-5} \text{M}$) plus p-benzoquinone ($2.0 \times 10^{-4} \text{M}$) in CBE (refluxed) at room temperature.

Figure 14. Recorder traces showing a comparison of decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis for various concentrations of p-benzoquinone and chlorophyll a ($1.6 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature.

Figure 15. Plots of first order decay constants for the slowest flash decay versus benzoquinone concentration. System: chlorophyll a ($1.6 \times 10^{-5} \text{M}$), p-benzoquinone (various concentrations) in CBE (refluxed) at room temperature.

Figure 16. Semi-log plots of decay curves obtained after flash excitation of various chlorophyll-quinone systems at 460 nm in CBE.

$[\text{Chl}] = 1.6 \times 10^{-5} \text{M}$. $[\text{Quinone}] = 9.3 \times 10^{-5} \text{M}$.

Figure 17. Recorder traces showing a comparison of decay kinetics at 460 nm (top) and 620 nm (bottom) after flash photolysis of chlorophyll a ($1.6 \times 10^{-5} \text{M}$) and various quinones ($9.3 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature.

Figure 18. Plot of the ratio of the 460 nm first order decay constant to the 620 nm first order decay constant (k_{460}/k_{620}) versus quinone redox potential for slowest flash decay (measured by strip-chart recorder). System: chlorophyll a ($1.6 \times 10^{-5} \text{M}$), quinone ($9.3 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature.

Figure 19. Kinetic curves for ESR and optical experiments with chlorophyll a ($0.9 \times 10^{-5} \text{M}$) and p-benzoquinone ($1.3 \times 10^{-5} \text{M}$) in CBE (refluxed) at room temperature. (a) Steady-state ESR signal rise and decay. (b) Steady-state optical bleaching rise and decay at 430 nm. (c) Flash optical decay at 650 nm.

Figure 20. Plots of the log of the first order flash decay constant for bleaching at 620 nm (slowest decay from strip-chart recorder) and the log of the first order steady-state ESR decay constant versus the molar ratio of p-benzoquinone to chlorophyll a (BQ/Chl); CBE (refluxed) at room temperature.

Figure 21. Oscilloscope traces showing decay kinetics at 430 nm (top), 460 nm (center) and 665 nm (bottom) after flash photolysis of chlorophyll a ($4 \times 10^{-6} \text{M}$) and chlorophyll a plus p-benzoquinone ($7.6 \times 10^{-4} \text{M}$) in degassed ethanol at room temperature.

Figure 22. Semi-log plots of oscilloscope traces for decay kinetics

after flash photolysis of chlorophyll $\bar{a}$ (4×10^{-6} M) and chlorophyll $\bar{a}$ plus p-benzoquinone (7.6×10^{-4} M) in degassed ethanol at room temperature.

- a) $\odot$ chlorophyll alone, 460 nm
 Δ plus benzoquinone, 460 nm
 $\odot$ chlorophyll alone, 665 nm
 Δ plus benzoquinone, 665 nm
 b) $\odot$ chlorophyll alone, 430 nm
 Δ plus benzoquinone, 430 nm

FIG. 1

FIG. 2

OSCILLOSCOPE SIGNAL

OSCILLOSCOPE SIGNAL

ABSORBANCE CHANGE

OSCILLOSCOPE SIGNAL

ABSORBANCE CHANGE

OSCILLOSCOPE SIGNAL

OSCILLOSCOPE SIGNAL

ABSORBANCE CHANGE

ABSORBANCE CHANGE

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OSCILLOSCOPE
SIGNAL

ABSORBANCE CHANGE

ABSORBANCE CHANGE

FIG. 10

FIG. 12

OSCILLOSCOPE
SIGNAL

OSCILLOSCOPE SIGNAL

OSCILLOSCOPE
SIGNAL

FIG. 14

FIG. 15

FIG. 10

FIG. 17

FIG. 12

→ | | ← 5 SEC (TIME SCALE PERTAINS TO ALL ABOVE KINETIC CURVES.)

FIG. 20