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# Reversible Photooxidation of Chlorophyll. A Study of the Chlorophyll–Benzoquinone System Utilizing Flash Photolysis and Electron Spin Resonance Spectroscopy[1]

**Brian J. Hales[2] and James R. Bolton*[3]**

*Contribution from Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455.* *Received July 20, 1971*

**Abstract:** The photochemical reaction of chlorophyll a with *p*-benzoquinone in alcoholic solvents is studied using the new esr flash photolysis technique. The semiquinone anion which is produced is shown to decay by both recombination with the chlorophyll a cation and by disproportionation with itself. The chlorophyll a cation similarly decays by recombination and is quenched by an unknown species in a pseudo-first-order reaction. A new fast-decaying esr signal is detected under conditions of high modulation amplitude and microwave power. This signal is assigned to a chlorophyll–quinone biradical complex. A new mechanism is developed incorporating the new findings and discussed in terms of previous work on this system.

The photochemistry of chlorophyll has been a popular subject of study, probably because of the central role that chlorophyll plays in the photochemical reactions of photosynthesis. There it acts as a photosensitizer or photocatalyst by promoting essential redox reactions of various substrates. Thus it is natural that *in vitro* studies have concentrated on the oxidations and reductions of substrates mediated by photoexcited chlorophyll molecules.

The reversible photoreduction of chlorophyll has been shown to occur in pyridine solutions containing ascorbic acid as the reducing agent;[4] the kinetics of this reaction have been thoroughly investigated using optical flash photolysis.[5,6] The photooxidation reactions of chlorophyll with various electron acceptors have also been studied,[6-15] but here the mechanism is less well understood. This is probaly due to the fact that the kinetics of these reactions are much faster than those of the corresponding photoreductions; an additional difficulty is that experimental findings have not been consistent with one another.

The quenching of chlorophyll fluorescence by quinones in solution was shown by Livingston and Ke.[7] In addition to quenching the excited singlet state of chlorophyll, it has been demonstrated through the technique of flash photolysis and esr that various quinones will also quench the lowest triplet state of chlorophyll. Linschitz and Rennert[8] were the first to show a direct reaction of the excited chlorophyll molecule with quinone to yield a bleaching of the chlorophyll absorption. Later, Evstigneev[9] showed that this reaction leads to the formation of the chlorophyll cation.

Tollin, *et al.*,[10,11] were the first to carry out an extensive study on the photooxidation of chlorophyll using esr spectroscopy. They were able to produce steady-state signals of semiquinone anion radicals ($Q\cdot^-$) by irradiating alcoholic solutions of chlorophyll (Chl) containing various quinones (Q). Since the action spectrum corresponded to the absorption spectrum of Chl, they proposed the following mechanism

$$\mathrm{Chl} + h\nu \longrightarrow \mathrm{Chl}^* \quad (1)$$

$$\mathrm{Chl}^* + \mathrm{Q} \xrightarrow{k_2} \mathrm{Chl}\cdot^+ + \mathrm{Q}\cdot^- \quad (2)$$

$$\mathrm{Chl}\cdot^+ + \mathrm{Q}\cdot^- \xrightarrow{k_3} \mathrm{Chl} + \mathrm{Q} \quad (3)$$

This mechanism is consistent with the complete reversibility of the system and the fact that the decay of the $Q\cdot^-$ signal is second order. No esr spectrum from $Chl\cdot^+$ was detected; this was explained in terms of the signal being too broad to detect.

Chibisov, *et al.*,[6,12] have obtained optical spectra for $Chl\cdot^+$ and $Q\cdot^-$ in ethanol and ethanol–gylcerol mixtures using flash photolysis techniques. Both intermediates appeared to decay by second-order kinetics according to reaction 3, with $k_3^{(C)} = (2.0 \pm 0.5) \times 10^9$ l. mol$^{-1}$ sec$^{-1}$ in ethanol at room temperature.

Seifert and Witt[13] have also observed a second-order decay of $Chl\cdot^+$ and $Q\cdot^-$. They found $k_3^{(SW)} = 4.0 \times 10^9$ l. mol$^{-1}$ sec$^{-1}$ in 2-butanol at 22°. They also observed a short-lived intermediate with a half-life of 20 μsec, which they ascribed to chlorophyll in its lowest triplet state.

While Chibisov, *et al.*, and Seifert and Witt have used only benzoquinone in these reactions, Kelly and Porter have studied the photooxidation of chlorophyll by duroquinone, α-tocopherylquinone, and vitamin $K_1$. These quinones were studied in both neutral and acidic ethanolic solutions. In addition to finding many new side reactions in acidic media, their results show that the dominant reaction for the decay of the chlorophyll and quinone radical species is the recombination reaction 3. They obtained a rate constant of $k_3^{(KP)} =$

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(2) This paper was written while the author was at The Royal Institution of Great Britain, London, England.

(3) Address correspondence to the Department of Chemistry, University of Western Ontario, London 72, Ontario, Canada.

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(5) R. Livingston and A. C. P. Pugh, *Nature (London)*, **186**, 969 (1960).
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(15) D. C. Mukherjee, D. H. Cho, and G. Tollin, *Photochem. Photobiol.*, **9**, 273 (1969).

$(3.2 \pm 0.5) \times 10^9$ l. mol$^{-1}$ sec$^{-1}$ for the reaction of duroquinone with chlorophyll in ethanol at room temperature.

Thus, the optical flash photolysis results were consistent with Tollin's original mechanism. However, recently Mukherjee, Cho, and Tollin[15] measured the second-order decay of the $Q\cdot^-$ esr signal in ethanol at room temperature and found $k_8^{(T)} = 2 \times 10^7$ l. mol$^{-1}$ sec$^{-1}$. They used a shutter technique to terminate the photochemical reaction. From these results they suggested that the simple recombination reaction 3 was not sufficient to explain the results, and thus proposed an alternative mechanism

$$\text{Chl} + h\nu \longrightarrow \text{Chl}^* \quad (4)$$

$$\text{Chl}^* \longrightarrow \text{Chl}^T \quad (5)$$

$$\text{Chl}^T + Q \xrightarrow{k_6} \text{Chl}\cdot + Q\cdot^- + H^+ \quad (6)$$

$$2\text{Chl}\cdot \xrightarrow{k_7} \text{Chl}^{ox} + \text{Chl} \quad (7)$$

$$2Q\cdot^- + 2H^+ \xrightarrow{k_8} Q + H_2Q \quad (8)$$

$$\text{Chl}^{ox} + H_2Q \xrightarrow{k_9} \text{Chl} + Q \quad (9)$$

where Chl· represents the chlorophyll neutral radical, and $\text{Chl}^{ox}$ stands for chlorophyll which has undergone a two-electron oxidation.

Tollin's mechanism is not consistent with the optical results,[6,13,14] where it was found that the $Q\cdot^-$ optical absorption decays with an average rate constant of $(3.0 \pm 1.0) \times 10^9$ l. mol$^{-1}$ sec$^{-1}$, as opposed to Tollin's results of $2 \times 10^7$ l. mol$^{-1}$ sec$^{-1}$.

The present situation is one of confusion. The group of workers using flash photolysis has suggested a recombination reaction (3) for the decay of $\text{Chl}\cdot^+$ and $Q\cdot^-$; their results, however, are confounded by the complicated and overlapping absorption spectra of the intermediates, which lead to difficulties in interpretation of their data. The well-known absorption of the Soret band of Chl[16] in ethanol strongly overlaps the semiquinone anion spectrum at 435 nm.[14] In addition to this, the broad absorption of the chlorophyll triplet[8] masks most of the chlorophyll cation spectrum[14] in the visible region. On the other hand, the group of researchers using esr spectroscopy suggest a more complicated mechanism[15] involving the disproportionation of both $Q\cdot^-$ and $\text{Chl}\cdot^+$. This esr work is aided by the fact that the $Q\cdot^-$ spectrum is distinct and easily recognized. However, until now esr research has been limited to radicals which can be generated by continuous lumination.

In order to provide further experimental results to test the various mechanisms proposed, we undertook a study of this system using a flash photolysis esr technique which we have developed.[17] We feel that the flash method is superior to the shutter technique in studying the kinetics of the intermediates, as much higher light intensities may be utilized and the time resolution is much improved.

## Experimental Section

A schematic diagram of the flash photolysis esr apparatus is shown in Figure 1. The esr spectrometer, triggering unit, and flash

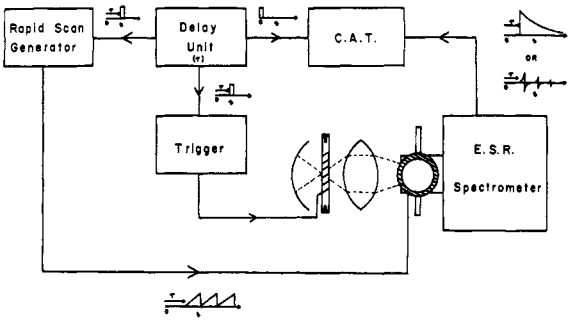


Figure 1. Block diagram of the flash photolysis esr apparatus used. In one mode of operation, pulse from the delay unit triggers the CAT at $t = 0$. At $t = \tau$, a pulse from the delay unit initiates the firing of the flash lamp. The signal from the esr spectrometer is stored in the CAT. In the rapid-scan mode, a pulse from the delay unit starts the rapid-scan generator at $t = \tau$ so that the magnetic field is swept during the time following the flash.

housing have been described elsewhere.[17] A red filter (Corning CS2-64) was used. Instead of an oscilloscope to monitor kinetic traces, as is usually used in optical flash photolysis, the output of the esr spectrometer is fed into a computer of average transients (CAT). The CAT used (Varian, Model C-1024) consists of 1024 channels and has the ability to sweep through these channels in times from 25 msec to 1000 sec.

Since it is not usually convenient to record a radical's decay back to the base line, a delay unit was built to trigger the CAT a set amount of time ($\tau$) prior to the triggering of each flash. In this way a base line could be recorded to be used later in the determination of signal amplitude. Enough time was always allowed between flashes to permit the signal intensity to decay virtually to zero.

It is often desirable to record the spectrum of the intermediates produced immediately after the photolyzing flash. A rapid-scan generator was designed for this purpose. This unit allows the magnetic field to be scanned quickly so that spectra can be recorded.

In the rapid-scan generator, a sawtooth voltage of variable frequency (1–400 Hz) is generated by a unijunction transistor which drives an operational amplifier (OP-21, Kepco). This amplifier is attached to a set of Helmholtz coils positioned about the sample in the resonance cavity.[18]

The pulse used to trigger the CAT from the delay unit was also used to initiate the sawtooth wave form. Since multiple scans were taken to improve the signal-to-noise ratio, the triggering unit was synchronized with the rapid-scan unit to assure the firing of the flash tube at a given place on the sawtooth wave form. The sawtooth generator was made variable not only to coincide with the various scan rates of the CAT but also to allow the intermediate's spectrum to be recorded several times during each scan of the CAT.

Figure 2 shows how the rapid scan records a signal. The comparison is made between the recording of the central line of the benzosemiquinone anion with the rapid scan during steady illumination and after a kinetic flash. Thus, these two spectra can easily be compared to detect any change in $g$ factor or the presence of another possible intermediate.

The amplitude of the sawtooth wave form was also made available to sweep a wide range of magnetic fields (0–40 G in as little as 2 msec). The sweep range was calibrated by recording the spectrum of various samples of known hyperfine splittings while noting the number of lines recorded at different sweep amplitudes.

The rapid-scan generator was also used in the determination of second-order rate constants. The procedure used to calculate rate constants has been described previously.[17]

Ethanol (absolute, Worum Chemical) was used as received. All other alcohols (reagent, Aldrich), diethyl ether (analytic reagent, Mallinckrodt), and 2-methylbutane (reagent, Matheson Coleman and Bell) were also used without further purification. *p*-Benzoquinone-$h_4$ (K and K Chemicals) was purified by sublimation *in vacuo*. *p*-Benzoquinone-$d_4$ was synthesized according to the method of Charney and Becker.[19]

All solution samples were thoroughly degassed prior to illumination as previously described. All degassing was performed under

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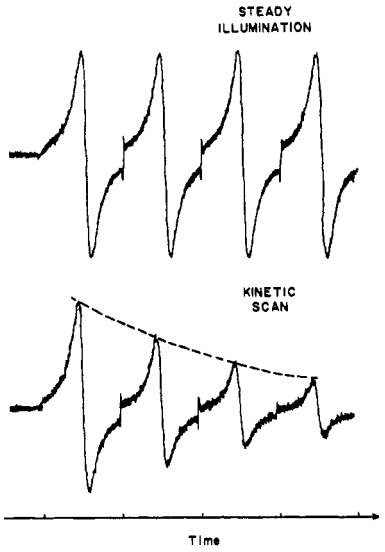


Figure 2. An example of how the flash photolysis esr system works in the rapid-scan mode. The recording is the center line of the *p*-benzosemiquinone spectrum. In the upper series of spectra, steady illumination was used; in the bottom series, a flash occurred immediately prior to the first trace. Each unit on the time base is 20 msec.

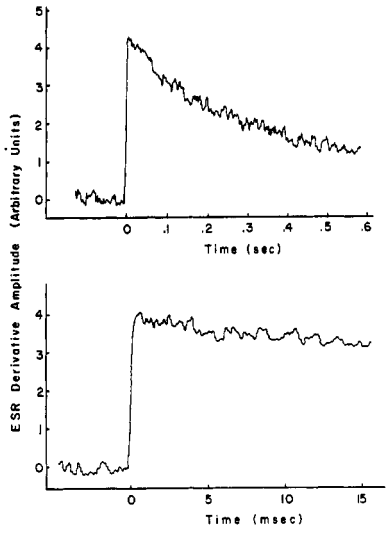


Figure 3. (Top) Time response of the center line of the esr spectrum of *p*-benzosemiquinone following a flash at $t = 0$. The result shown is the average of 50 scans. (Bottom) Same but on a shorter time scale. [Chl] $\sim 10^{-4} M$; [Q] $\sim 10^{-2} M$.

dim illumination from an incandescent light. For low-temperature photolysis, a standard Varian low-temperature flat cell was employed. Unless otherwise specified, all the chlorophyll and quinone concentrations used were [Chl] $\sim 10^{-4} M$ and [Q] $\sim 10^{-2} M$.

Chlorophyll a and b were extracted from corn leaves and purified by chromatography according to the method of Strain.[20] Purity of the material was checked by visible absorption spectroscopy.[16] Perdeuteriochlorophyll was a gift from Dr. H. H. Strain.

## Results and Discussion

**(A) Determination of the Rate Constant for the Decay of the $Q \cdot^-$ Esr Signal.** Solutions of chlorophyll and *p*-benzoquinone (Q) in absolute ethyl alcohol exhibited no esr signal in the dark. However, when the solution was irradiated with a steady red light ($\lambda \geq 650$ nm), a strong esr signal assignable to $Q \cdot^-$ was observed, in agreement with Tollin's results.[10,11] This signal decayed completely within a second after cessation of illumination. The $Q \cdot^-$ esr signal resolved into five lines of intensity ratios 1:4:6:4:1. The spectrum is described by $g = 2.0046$[21] and $a = 2.36$ G.[22] At room temperature the line width is ~0.2 G.

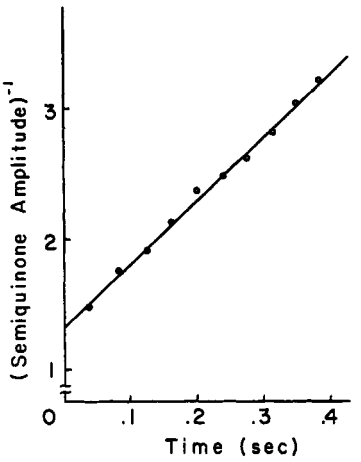


Figure 4. Second-order plot of the kinetic data from Figure 3.

The decay rate constant of the $Q \cdot^-$ esr signal was determined at various temperatures using the flash photolysis esr apparatus. All kinetic measurements were performed on the central line of the quintet. Figure 3 (top) illustrates a typical kinetic trace. The data fit best to second-order kinetics (see Figure 4) and were monophasic over the entire decay. In particular, when this decay is recorded in a 25-msec scan (Figure 3, bottom), there is no evidence for any short-lived (within the 0.2-msec time resolution) $Q \cdot^-$ component. This finding will be important in later discussions.

From an Arrhenius plot of the rate constants determined between −40 and 25°, we obtain (in absolute alcohol)

$$k_{20°} = (1.7 \pm 0.5) \times 10^7 \text{ l. mol}^{-1} \text{ sec}^{-1}$$

$$E_a = (5.1 \pm 0.2) \text{ kcal mol}^{-1}$$

These compare with

$$k_{20°} = 2.0 \times 10^7 \text{ l. mol}^{-1} \text{ sec}^{-1}$$

$$E_a = 5.4 \text{ kcal mol}^{-1}$$

measured by Tollin, *et al.*,[15] using a shutter technique. This rate constant is assigned accordingly to the disproportionation reaction 8 of the semiquinone anion, in agreement with analogous rate constants determined for duroquinone[23] and chloranil.[24]

We have also investigated the semiquinone decay in a number of other solvents. In isopropyl alcohol and *sec*-butyl alcohol, the decay rate is very similar to that in ethyl alcohol. For instance, in *sec*-butyl alcohol, $k_{20°} = (2.2 \pm 1.0) \times 10^7$ l. mol$^{-1}$ sec$^{-1}$. However, no $Q \cdot^-$ signal was detected in *tert*-butyl alcohol either with flash or steady-state illumination. These results

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(23) N. K. Bridge and G. Porter, *Proc. Roy. Soc., Ser. A*, **244**, 276 (1958).

(24) D. R. Kemp and G. Porter, *J. Chem. Soc. D*, 1029 (1969).

suggest two possible explanations. Either primary and secondary alcohols react rapidly with Chl·+, thus impairing its recombination with Q·−, or the higher viscosity of the *tert*-butyl alcohol aids in the deactivation (either radiative or nonradiative) of the photoexcited chlorophyll before it can react with Q.

Of special interest is the rate constant for Q·− decay in *sec*-butyl alcohol at room temperature; this is the solvent system in which Seifert and Witt[13] observed only the recombination reaction 3. From the return of the chlorophyll absorption, they determined a second-order rate constant of $2.0 \times 10^9$ l. mol$^{-1}$ sec$^{-1}$. However, from the decay of Q·−, we find a second-order rate constant of $2.2 \times 10^7$ l. mol$^{-1}$ sec$^{-1}$, in accord with the disproportionation reaction 8. Thus, the disparity still exists. Considering all of the above results, it seems probable that both recombination and disproportionation occur as decay processes for Q·−. Since recombination is the faster of the two reactions, its decay process is probably unobservable to the flash photolysis esr apparatus, where the time constant is of the order of 0.2 msec. Calculations show that if the concentration of Q·− decaying by recombination is *ca.* ten times that decaying by disproportionation, the recombination decay will not be observed in our apparatus. This would be consistent with the observation that recombination, which has a half-life of around 100 μsec,[13] is the predominant decay measured by optical flash photolysis. Similarly, even though the initial quinone and chlorophyll concentrations are $10^{-2}$ and $10^{-4}$ *M*, respectively, the concentration of Q·− calculated to be observed by our esr is only $10^{-7}$ *M*. This implies that the disproportionation reaction, although observable by esr, represents only a small portion of the total semiquinone decaying in the system.

**(B) The Chlorophyll Cation.** If we accept that Q·− must be decaying by both recombination and disproportionation, the question arises as to what happens to the chlorophyll cation (Chl·+) that does not decay by recombination. In view of the fact that some of the Q·− is left to undergo disproportionation after all of the Chl·+ has decayed, it seems logical that there is a side reaction competing with recombination for the decay of Chl·+. (Instead of recombination, two groups[15,25] have independently proposed disproportionations as the main mode of decay of the Chl·+. Although differing in fine points, both groups consider Chl·+ as decaying *via* a second-order mechanism.) This competing process must have a rate comparable to that of recombination in order to leave a significant amount of Q·− to undergo disproportionation. If this competing process obeys second-order kinetics, the maximum rate constant would be that for a diffusion-controlled process, *i.e.*, $k \sim 5 \times 10^9$ l. mol$^{-1}$ sec$^{-1}$ in ethanol at room temperature. Since the initial concentration of Q·− decaying by disproportionation must be the same as that of Chl·+ decaying by this competing process, we can compute an exact expected half-life for Chl·+ from

$$\tau_{1/2}(\text{Chl}\cdot^+) = \tau_{1/2}(\text{Q}\cdot^-)\frac{k_{\text{Q}\cdot^-}}{k_{\text{Chl}\cdot^+}}$$

In our experiment, $\tau_{1/2}(\text{Q}\cdot^-) \sim 0.2$–$0.4$ sec, $k_{\text{Q}\cdot^-} = 2 \times 10^7$ l. mol$^{-1}$ sec$^{-1}$. Hence $\tau_{1/2}(\text{Chl}\cdot^+) \sim 1$–$2$ msec. Because this half-life is within the time resolving power of our instrument, the flash transient of Chl·+ should be detectable if present.

The esr characteristics of Chl·+ are well known;[25] $g = 2.0025$, $\Delta H_{pp} \sim 9$ G, and its esr signal does not saturate until fairly high levels of microwave power (~30 mW) are reached. A systematic search for the Chl·+ signal was conducted, accumulating as many as 1000 kinetic scans for one magnetic field value. Calculations based on the signal-to-noise ratio, signal amplitude, and line width of the Q·− signal indicated that we should have been able to detect the broader Chl·+ signal if it were there. Therefore, we must conclude from this, that the competing reaction in the decay of Chl·+ must be a first-order or a pseudo-first-order process with a half-life of less than the time constant of the spectrometer; that is

$$\text{Chl}\cdot^+ + \text{X} \xrightarrow{k_{10}} \text{Chl} + \text{X}\cdot^+ \qquad (10)$$

with X in excess and $k_{10}[\text{X}] \geq 10^3$ sec$^{-1}$.

Chibisov[6] has detected a first-order component in the decay of Chl·+ which he suggests is the reaction of Chl+ with the alcoholic solvent. He quotes a rate constant of ~$10^3$ sec$^{-1}$. Thus, X could be the solvent. However, we were not able to detect the presence of X·+ by its esr spectrum ($CH_3$–$CH_2$–OH·+ would deprotonate rapidly and the esr characteristics of $CH_3$–$\dot{C}$H–OH are well known[26]). Similarly, the amounts of semiquinone anion produced did not significantly depend on the alcohol used.

Tollin[27] has recently suggested a ternary complex among quinone, chlorophyll, and solvent molecules which, upon photochemical excitation, breaks into the oxidized solvent radical and the reduced quinone radical

$$(\text{solvent}\cdots\text{Chl}\cdots\text{Q}) + h\nu \longrightarrow \text{solvent}_{\text{ox}} + \text{Chl} + \text{Q}\cdot^- \qquad (11)$$

Although it has been shown that alcoholic solvents will coordinate with Chl molecules,[28] these complexes do not appear to break up upon photoexcitation,[29] as is suggested in eq 11. Likewise, this mechanism is suspect due to the lack of detection of a solvent radical in the esr results mentioned above.

Kelly and Porter[14] have reported that durohydroquinone readily quenches Chl·+ in solution. It is well known that all quinones, especially *p*-benzoquinone, contain small amounts of hydroquinone ($QH_2$) impurity which is extremely difficult to remove below about the 1% level. This is an attractive hypothesis as the product radical, the semiquinone anion, would join the pool of Q·− already present. Kelly and Porter have represented this reaction as

$$\text{Chl}\cdot^+ + \text{QH}_2 \xrightarrow{k_{12}} \text{QH}\cdot + \text{Chl} + \text{H}^+ \qquad (12)$$

with

$$\text{QH}\cdot \longrightarrow \text{Q}\cdot^- + \text{H}^+ \qquad (13)$$

occurring in pure ethanolic solvents, where $k_{12} = 5.5 \times 10^5$ l. mol$^{-1}$ sec$^{-1}$. In agreement with reactions 12 and

(25) D. C. Borg, J. Fajer, R. H. Felton, and D. Dolphin, *Proc. Nat. Acad. Sci. U. S.*, **67**, 813 (1970).

(26) R. Livingston and H. Zeldes, *J. Chem. Phys.*, **44**, 1245 (1966).
(27) R. Raman and G. Tollin, *Photochem. Photobiol.*, **13**, 135 (1971).
(28) J. J. Katz, H. H. Strain, D. L. Leussing, and R. C. Dougherty, *J. Amer. Chem. Soc.*, **90**, 784 (1968).
(29) J. R. Norris, R. A. Uphaus, H. L. Crespi, and J. J. Katz, *Proc. Nat. Acad. Sci. U. S.*, **68**, 625 (1971).

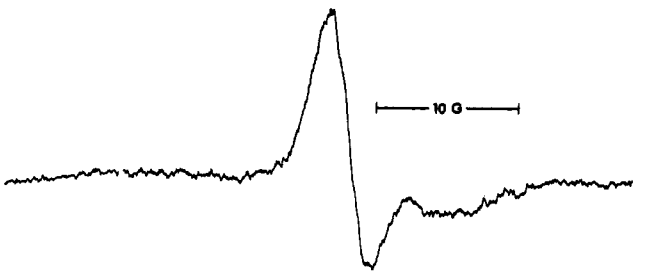


Figure 5. Esr spectrum of a solution of chlorophyll a and *p*-benzoquinone-$d_4$ in ethanol containing 0.1 *M* acetic acid. Modulation amplitude 2 G, microwave power 10 mW.

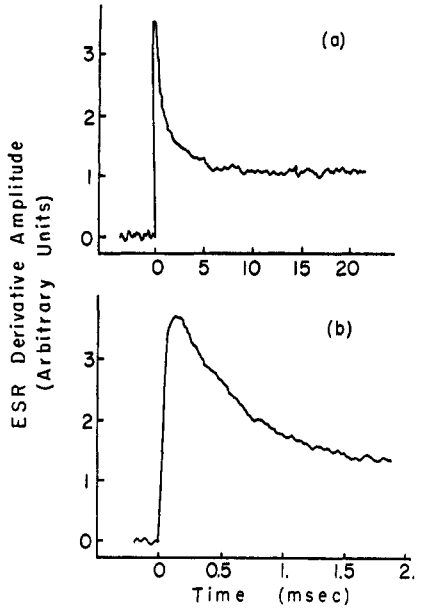


Figure 6. (a) Time response of the esr spectrometer at one of the extremes of the fast-decaying signal following a flash at $t = 0$. (b) Same as (a) except on a shorter time scale.

13, negative pH changes have been detected[30,31] upon irradiation of these systems.

Considering that the benzoquinone concentration is at least $10^{-2}$ *M*, a hydroquinone impurity of a few per cent will yield a $QH_2$ concentration comparable with that of Chl and about $10^3$ times greater than the $Q\cdot^-$ observed. Using the rate constant for reaction 12 calculated by Kelly and Porter, along with this amount of $QH_2$ impurity, a half-time of $\tau_{1/2} \sim 10^{-2}$ sec can be calculated for reaction 10; this is, however, more than an order of magnitude greater than the predicted half-life that has been discussed above. Therefore, the quenching rate constant calculated by Kelly and Porter suggests that $QH_2$ is not the dominant quencher (X) of $Chl\cdot^+$.

A possible insight into the identity of X can be gained through the investigation of the system at low pH. It has been observed[14] with optical techniques that under the conditions of low pH the chlorophyll cation is stabilized. If this is true, then the esr spectrum of $Chl\cdot^+$ should be observable under similar conditions. Figure 5 shows the spectrum obtained from the irradiation of Chl a with perdeuterioquinone in ethanol containing 0.1 *M* acetic acid. It can be seen that in the

(30) K. P. Quinlan and E. Fujimori, *J. Phys. Chem.*, 71, 4154 (1967).
(31) K. P. Quinlan, *ibid.*, 73, 2058 (1969).

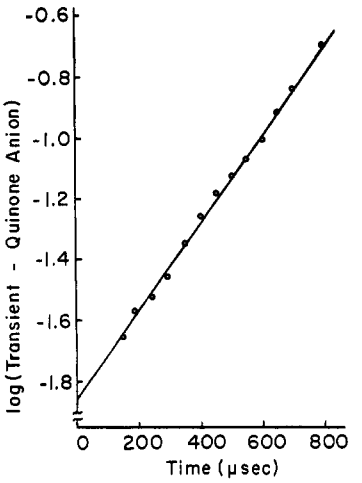


Figure 7. First-order plot of the residual signal in Figure 5 after the $Q\cdot^-$ signal is subtracted out.

presence of acid a second radical becomes detectable. This radical is centered at a higher field relative to $Q\cdot^-$ and is much broader. Furthermore, this second radical does not significantly saturate at high powers (40 mW) as does $Q\cdot^-$ (see discussion below). The spectrum in Figure 5 is described by $g \simeq 2.0026 \pm 0.0002$ and $\Delta H_{pp} \sim 7$ G. It is felt that this second signal is due to $Chl\cdot^+$, since the line width and *g* factor are very close to those for $Chl\cdot^+$.[25]

The effect of acid on the system can be explained with the use of reaction 10. Acid may protonate X to produce $XH\cdot^+$. If $XH^+$ does not quench $Chl\cdot^+$, then the steady-state concentration of $Chl\cdot^+$ will increase with decreasing pH, as has been observed.[14] With regard to this effect, a possible assignment of X is that of a solvent molecule as has been suggested by both Chibisov[6] and Tollin.[27] Further experiments are being undertaken to elucidate this hypothesis. It should finally be noted that the decay of $Chl\cdot^+$ under acid conditions is pseudo first order in kinetics[14] as has been proposed from the esr results above.

**(C) Fast-Decaying Component.** During the search for the $Chl\cdot^+$ esr signal, a short-lived esr signal was detected under conditions of high modulation amplitude (~5 G) and high microwave power (~50 mW) at room temperature. Figure 6a illustrates the decay trace after 900 kinetic scans, with Figure 6b showing an enlargement of the fast decay. The longer decaying portion is residual $Q\cdot^-$ signal. When this signal is subtracted, the data fit first-order kinetics (see Figure 7) with a rate constant of $(1.5 \pm 0.4) \times 10^3$ $sec^{-1}$. By changing the magnetic field in steps and recording the resultant amplitude of the fast decay, a profile of the esr signal component was constructed (see Figure 8). The signal has a single line with $\Delta H_{pp} \sim 7$–8 G and $g = 2.0046 \pm 0.0004$. The *g* factor of this signal rules out the possibility of it being due to $Chl\cdot^+$, which has $g = 2.0025$.[25] Detection of any changes in line width or rate constant with temperature was impaired by decreased signal intensity at low temperatures. This effect of temperature was more noticeable for the fast signal than the $Q\cdot^-$ signal.

If this new signal is due to a solvent radical, its esr characteristics should be affected by a change of solvent. With *sec*-butyl alcohol as a solvent, no change in the esr

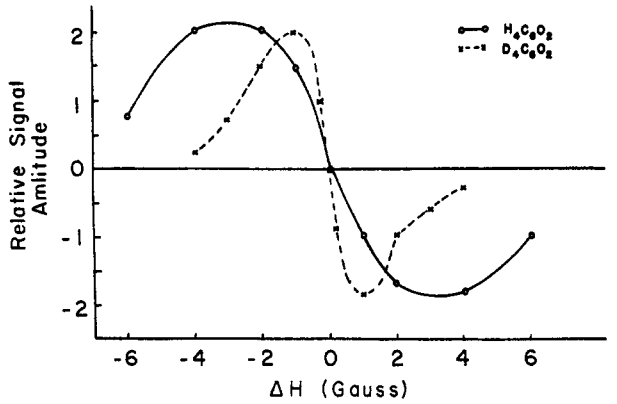


Figure 8. Profile of the esr signal of the fast transient. Solid line corresponds to the use of *p*-benzoquinone-$h_4$ and the dotted line to the use of *p*-benzoquinone-$d_4$.

signal could be detected. Thus, the transient is probably not a solvent radical.

The possible involvement of quinone in the transient was tested by use of *p*-benzoquinone-$d_4$. Owing to the fact that the total width of *p*-benzoquinone-$h_4$ is about 3.3 times that of *p*-benzoquinone-$d_4$, one would expect a reduction in line width if the quinone is involved in the species giving rise to the transient. The dotted line in Figure 8 shows that indeed there is a reduction in line width when *p*-benzoquinone-$d_4$ is used. Now $\Delta H_{pp} \sim 2.5$ G. The first-order rate constant is $(1.0 \pm 0.4) \times 10^3$ sec$^{-1}$. It was also found that this fast transient occurs in solutions containing quinone and chlorophyll b.

The above information implies that the fast transient species contains quinone, yet it is not the free $Q\cdot^-$, as $Q\cdot^-$ is seen separately under the same conditions. Thus, this new signal must be due to a paramagnetic quinone complex with an unknown molecule(s).

**(D) Nature of the Quinone Complex.** To determine the nature of the quinone complex and its involvement in the reaction mechanism, several facts have to be considered: (1) The esr line is quite broad (~7 G) for a free radical in solution at room temperature. (2) The esr signal does not saturate until moderately high microwave power levels (~50 mW). This is also unusual, as the esr spectra of most free radicals saturate at microwave power levels of ~1–2 mW. (3) The decay of the transient follows first-order kinetics.

The last point suggests that the decay is due to the breaking up of the complex. However, a complex comprised of a free radical and a diamagnetic molecule *must* break up to yield a free radical by spin conservation. It is logical to assume that the free radical would be $Q\cdot^-$. The concentration of the complex was estimated by comparison with the concentration of the $Q\cdot^-$ already present. From this analysis, it is concluded that the complex and $Q\cdot^-$ are initially present in about the same concentration. *Thus, the formation of $Q\cdot^-$ from the decay of the complex should have been observable.* Examination of Figure 3 (bottom) shows that no such formation is detected. This presents a problem, as no free radical is detected from the breakup of the radical complex. One possible answer is that the complex is a *biradical, i.e.*, comprised of two free radicals perhaps separated by a solvent molecule. This hypothesis would explain the unusual line width and saturation behavior, as another free radical close by would provide a strong relaxation mechanism.

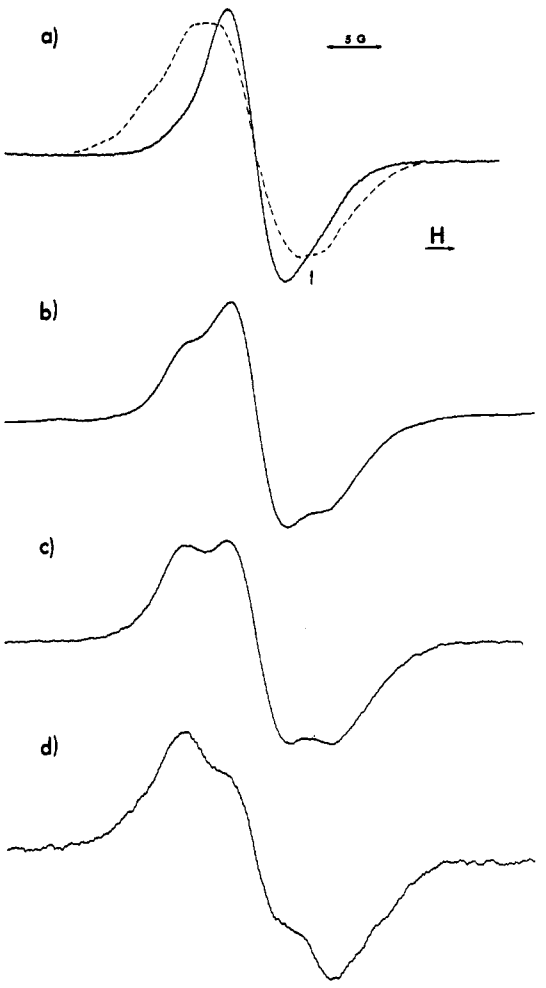


Figure 9. Esr spectrum of a solution of Chl and Q in ether–isopentane–ethanol (8:3:5) at −140° under steady red-light illumination. (a) Spectrum at low microwave power (~1 mW). Dotted line corresponds to *p*-benzoquinone-$h_4$ and the solid line to *p*-benzoquinone-$d_4$. (b–d) Spectra as the microwave power is increased to ~100 mW in (d).

A chlorophyll–quinone complex was first suggested by Livingston and Ke.[7] They reported that the fluorescence quenching of chlorophyll by quinone did not follow the Stern–Volmer equation. From this they suggested that the residual fluorescence was due to a complex. The presence of an "unquenchable" complex species has subsequently been detected by others.

Of particular interest is the proposal put forth by Tollin and Green.[10] They observed that at low temperatures the typical esr spectrum of the semiquinone anion was displaced by a much broader single-line signal. This signal decayed when the temperature was raised, and the investigators proposed it to be a chlorophyll–quinone complex stabilized at low temperature.

We have repeated this work at −140° in an ether–isopentane–ethanol (8:3:5) solvent. The spectrum obtained is shown by the dotted-line trace in Figure 9a and is identical with that obtained by Tollin.[10] The use of *p*-benzoquinone-$d_4$ produced a narrowing (see solid trace in Figure 9a) similar to that noted above for the flash transient at room temperature. Furthermore, now with the steady-state illumination, much better

resolution is possible at low temperatures. With this improved resolution, a shoulder is seen on the high-field side of the deuterated signal (the arrow in Figure 9a). On increasing the microwave power (Figures 9b–d), it is seen that a broad signal underlies the sharp signal.

It is tempting to propose that the broad and narrow signals are due to the $Chl\cdot^+$ and $Q\cdot^-$ partners, respectively, of a complex. The $g$ factor of the broad signal, however, is $2.0042 \pm 0.0004$, which is too high[25] for $Chl\cdot^+$. In addition, an experiment with perdeuteriochlorophyll did not produce a further narrowing of either signal.

Therefore, it must be said that the low-temperature esr signals are not those of a chlorophyll–quinone complex such as that proposed for the fast-decaying signal at room temperature. It is in fact difficult to assign them to any light-induced quinone complex. At temperatures where both the broad and $Q\cdot^-$ signals are clearly distinguishable ($\sim -110°$), the decay kinetics of each signal was observed. In agreement with previous results,[10] extinguishing the irradiating source caused complete decay of the $Q\cdot^-$ signal, but only a slow partial decay of the broad signal. If the broad signal alone were due to a complex, it would be assumed that the rate of the back-reaction to a neutral complex, *i.e.*

$$(Chl\cdot^+\cdots Q\cdot^-) \longrightarrow (Chl\cdots Q)$$

would be fast and mainly independent of temperature. Such a fast decay is observed only with the room-temperature broad signal.

The double signal observed at low temperature is not unique. Similar "anomalous" saturations have been detected with both flavoproteins[32] and flavin[33] radicals at low temperatures. One possible explanation of the double signal in our system evolves from the high concentrations ($10^{-1}$–$10^{-2}$ $M$) of the quinones used in these systems. At low temperatures, where the quinone solubility is only a fraction of that at room temperature, some of the quinone crystallizes out of solution. These quinine crystals would encompass some of the $Q\cdot^-$ in solution. This produces two forms of the semiquinone anion, the free anion ($Q\cdot^-$) and the aggregated form ($(Q)_h\cdot^-$). These two forms would explain the two signals of different saturation behaviors yet similar $g$ factors. This hypothesis is strengthened by the fact that at low temperatures where the two signals were detected, quinone crystals were also observed in the solutions. Furthermore, the broad signal is most easily produced by continuous irradiation of the system while the temperature is being lowered. Sudden irradiation at low temperatures caused only a very slow formation of the signal. This implies that the broad signal depends on the $Q\cdot^-$ in solution and not on a complex whose rate of formation is diffusion controlled. Further studies of this problem are now in progress.

**(E) The Proposed Mechanism.** Considering all of the above facts, the following reaction mechanism seems the most reasonable.

$$Chl + h\nu \longrightarrow Chl^*$$

$$Chl^* + Q \longrightarrow Chl\cdot^+ + Q\cdot^-$$
$$k = (7.0 \pm 1.0) \times 10^9 \text{ l. mol}^{-1} \text{ sec}^{-1} \text{ (ref 13, 14)}$$

$$Chl^* + Q \longrightarrow (Chl\cdot^+\cdots Q\cdot^-)$$
$$k = (5.0 \pm 2.0) \times 10^9 \text{ l. mol}^{-1} \text{ sec}^{-1} \text{ (ref 14)}$$

$$(Chl\cdot^+\cdots Q\cdot^-) \longrightarrow Chl + Q$$
$$k = (1.2 \pm 0.4) \times 10^3 \text{ sec}^{-1} \text{ (this work)}$$

$$Chl\cdot^+ + Q\cdot^- \longrightarrow Chl + Q$$
$$k = (3.0 \pm 1.0) \times 10^9 \text{ l. mol}^{-1} \text{ sec}^{-1} \text{ (ref 6, 13, 14)}$$

$$Chl\cdot^+ + X \longrightarrow Chl + X\cdot^+$$
$$k \geq 10^3 \text{ sec}^{-1} \text{ (this work; ref 6, 14)}$$

$$2Q\cdot^- \longrightarrow Q + Q^{2-}$$
$$k = (2.0 \pm 1.0) \times 10^7 \text{ l. mol}^{-1} \text{ sec}^{-1} \text{ (this work; ref 15)}$$

Here Chl* represents either the photoexcited singlet or triplet state of chlorophyll, $(Chl\cdot^+\cdots Q\cdot^-)$ symbolizes the fast-decaying chlorophyll–quinone complex, and $Chl\cdot^+$ and $Q\cdot^-$ represent the separated solvated radicals.

Identification of the quencher X of the chlorophyll cation and the nature of the fast decay complex are being undertaken.

**Acknowledgment.** We are grateful to Dr. H. H. Strain for his gift of deuterated chlorophyll a.

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