CHLOROPHYLL-QUINONE PHOTOCHEMISTRY IN LIPOSOMES: MECHANISMS
OF RADICAL FORMATION AND DECAY[†]

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Running Title: Chlorophyll-Quinone Photochemistry in Liposomes

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ABSTRACT

Laser flash photolysis has been used to investigate the mechanism of formation and decay of the radical species generated by light induced electron transfer from chlorophyll a triplet to quinone in egg phosphatidyl choline bilayer vesicles. Chlorophyll triplet quenching by quinone is controlled by diffusion occurring within the bilayer membrane ($k_q \sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$, as compared to $\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ in ethanol) and reflects bilayer viscosity. Radical formation via separation of the intermediate ion pair is also inhibited by increased bilayer viscosity. Cooperativity is observed in this process due to an enhancement of radical separation by electron transfer from semiquinone anion radical to a neighboring quinone molecule. Two modes of radical decay are observed, a rapid ($t_{1/2} = 150 \mu\text{s}$) recombination occurring within the bilayer and a much slower ($t_{1/2} = 1\text{-}100 \text{ ms}$) recombination occurring across the bilayer. The latter is also cooperative, which accounts for a $t_{1/2}$ which is dependent upon quinone concentration. The slow decay is only observed with quinones which are not tightly anchored into the bilayer, and is probably the result of electron transfer from semiquinone anion radical formed within the bilayer to a quinone molecule residing at the bilayer-water interface. With benzoquinone, approximately 60% of the radical decay occurs via the slow mode. Triplet to radical conversion efficiencies in the bilayer systems are comparable to those obtained in fluid solution ($\sim 60\%$). However, radical recombination, at least for the slow decay mechanism, is considerably retarded.

INTRODUCTION

The one-electron reduction of quinones by photoexcited chlorophyll a (Chl) has been extensively studied (Chibisov, 1969; Kelley and Porter, 1970; Tollin et al., 1979) as a means of exploring the mechanism of the primary event of photosynthesis, i.e. Chl photo-oxidation. It is now generally accepted that bacteriopheophytin serves to mediate electron transfer to quinone in bacterial photosynthetic systems (Fajer et al., 1975). This finding has led to a search for a similar role for pheophytin in green plant photosynthesis and some evidence for this in Photosystem I has been reported (Klimov et al., 1977). The situation in Photosystem II, which utilizes quinone as an acceptor, is less clear. Continued study of Chl-quinone photochemistry in vitro should help to further elucidate the molecular-electronic basis of the one-electron oxidation of Chl excited states, including perhaps the rationale for the in vivo employment of pheophytin as mediator. It may also provide insight into possible ways of employing Chl in practical biomimetic photochemical energy conversion systems.

Inasmuch as the solution photochemistry of quinone reduction is now quite well understood (Tollin et al., 1979 and references therein) we have begun to study this reaction in heterogeneous systems so as to develop an understanding of environmental effects on Chl photoelectron transfer processes. In one such system, Chl has been incorporated into a spherical phospholipid bilayer (hereafter referred to as Chl-liposomes). Such bilayers are of biological relevance in that they provide Chl with an environment that more closely mimics its natural state. A previous report from this laboratory (Hurley et al., 1980) has examined Chl triplet state (Chl_t) quenching in liposomes and electron transfer from Chl_t to quinone, although mechanistic analysis of this latter process was

not considered. The present communication describes a more detailed study of this electron transfer reaction, the results of which allow a plausible mechanism to be postulated.

MATERIALS AND METHODS

Chl was purified according to Strain and Svec (1966). Chromatographically pure egg phosphatidyl choline (egg PC) was obtained and purified according to the method of Hanahan and Wells (1969), with the additional step of deionization of the PC using Bio Rex 70 and Ag3-X4A ion exchange resins (Bio-Rad Laboratories). Dioleoyl-L- α -phosphatidylcholine (DOPC) and dipalmitoyl-L- α -phosphatidylcholine (DPPC) were purchased from Sigma Chemical Co. and didodecyldimethylammonium bromide from Eastman Organic Chemicals. These were used without further purification. Benzoquinone (Eastman Organic Chemicals) was purified by vacuum sublimation (5x). Ubiquinone-30 (Sigma Chemical Co.) and 2,6-di-t-butyl-p-benzoquinone (Aldrich) were used without further purification. Duroquinone (Aldrich) and 2,5-dichloro-p-benzoquinone (Eastman Organic Chemicals) were recrystallized from ethanol. All solvents and buffers were AR grade.

Liposomes were prepared by the injection method described previously (Batzri and Korn, 1973; Hurley et al., 1980). For preparation of DPPC and didodecyldimethylammonium bromide liposomes, the buffer was heated to 90°C before injection of the lipid solution. This resulted in no noticeable degradation of the Chl as judged by its absorption spectrum. For experiments with benzoquinone (BQ), this compound was added following liposome preparation. As previously reported (Hurley et al., 1980) equilibration throughout the entire system was rapid. In the case of ubiquinone (UQ), duroquinone (DQ) and 2,6-di-t-butyl-p-benzoquinone (DTBQ), the quinone was present in the

injected ethanol solution along with the Chl and phospholipid. With 2,5-dichloro-p-benzoquinone (DCQ), due to its insolubility in either water or ethanol, solid quinone was added to preformed liposomes and then sonicated in a bath sonicator (Heat Systems Ultrasonics) for several minutes until solubilization occurred. As will be discussed more fully below, this method of quinone incorporation may have resulted in a somewhat different final distribution.

Each sample contained enough Chl to give a final optical density (at 667 nm) of 0.5. Chl, DQ, DTBQ, DCQ and UQ concentrations within the liposome were estimated by calculation as described previously (Hurley et al., 1980), assuming that all of the material was bound to the liposome. All concentrations specified as apparent refer to those which would result from uniform dispersion of the material throughout the total volume of the solution. The appreciable water solubility of BQ required an estimation of the extent of its partitioning between the vesicle and aqueous phases. This was attempted in two different ways. First the binding was measured by spinning the liposomes upward through a solution of BQ in D₂O. The BQ removed from solution was estimated by measuring the UV absorbance (240 nm) of the cleared solution which was obtained by puncturing the bottom of the centrifuge tube. Binding was also measured by ultrafiltration using an Amicon Model 10-PA ultrafiltration device and a UM10 (10,000 MW retention) Diaflo ultrafiltration membrane. Although the data obtained with both methods showed appreciable scatter (this is due in part to the instability of BQ solutions (Poupe, 1947; Lindsey, 1974)), both were in fairly good agreement with an association constant of approximately 10^3 M^{-1} and approximately 500 binding sites when subjected to Scatchard analysis. Binding of other small molecules to PC liposomes has been analysed using the latter method (Huang and Charlton, 1972).

All samples were prepared in minimal light and were bubbled and sealed under nitrogen prior to being subjected to laser flash photolysis. The

determination of triplet and radical concentrations and the laser flash photolysis apparatus have been described previously (Tollin et al., 1979), except that the Biomation Model 805 Waveform Recorder was replaced with the Biomation Model 8100 which has a better time resolution (10 ns per point). Extinction coefficients used at 465 nm were $3.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for Chl_t (Linschitz and Sarkanen, 1958) and $1.26 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for the chlorophyll cation radical (C^+) (Borg et al., 1970). An extinction coefficient of $1.7 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ was used for C^+ at 395 nm. This was calculated from the data after having determined the concentration of C^+ at 465 nm. Excitation was at 655-660 nm utilizing a mixture of Rhodamine 6G and cresyl violet (1mM in ethanol) as the dye laser.

RESULTS AND DISCUSSION

The presence of BQ or UQ in a preparation of Chl-liposomes results in the flash-induced production of the electron transfer products (Hurley et al., 1980), chlorophyll cation radical (C^+) and benzoquinone anion radical (BQ^-) or ubisemiquinone anion radical (UQ^-). The flash-induced difference spectra produced in the presence of BQ and of UQ are shown in Fig. 1. In the case of BQ two spectra are shown, one representing a rapidly decaying transient and the other a more slowly decaying species. The origin of these two decay processes is discussed below. The spectrum of the fast component is not shown in the red region due to the fact that the light scattering artifact interferes with measurements at early time points in this region of the spectrum. All three difference spectra are similar to those previously observed for C^+ and Q^- in ethanol solution (Chibisov, 1969) and in cellulose acetate films (Cheddar et al., 1980). The electron transfer resulting in C^+ production occurs from Chl_t (Hurley et al., 1980) and the concentration of C^+ increases steadily with increasing quinone concentration until the Chl_t concentration begins

to drop due to Chl singlet state quenching. At this point the concentration of both Chl_t and C^+ decrease in parallel fashion as shown in Fig. 2 for an experiment in which UQ was used. The quenching of Chl_t by both BQ and UQ follows Stern-Volmer kinetics over the concentration range of interest (Fig. 3). The quenching constants so determined are given in Table 1. We cannot be certain as to the significance of the larger k_q for BQ in egg PC as compared to UQ, due to the uncertainty in the calculation of the BQ concentration within the vesicle. However, we feel confident that they are at least of the same order of magnitude. These values should be compared to those obtained previously ($3.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) for BQ in ethanol (Tollin et al., 1979). Thus, quinone diffusion is approximately 10^3 times slower within the vesicles.

The decay of the radical products obtained with BQ is biphasic (Fig. 4) with approximately 60% of the radicals decaying via the slower process (the half-time for this can be as long as 40 ms). Both decays exhibit first order kinetics as shown by the invariance of the decay rate upon decreasing the laser intensity (Fig. 4 inset). However, exponential behavior is not observed, probably because of the inherent heterogeneity of the distribution of both Chl and BQ among the liposomes. It is evident that the rate of this decay ($t_{1/2}$ approx. 150 μs) is similar to that of the rapidly decaying radical observed with BQ as acceptor. The biphasic decay observed with BQ is most probably due to the fact that this compound can partition into the membrane (Shah et al., 1980) (i.e., it has a finite solubility in both hydrophilic and lipophilic solvents). On the other hand, UQ, a highly lipophilic quinone, is constrained to be situated within the bilayer. We thus attribute the faster decay process observed with both quinones to recombination of C^+ and Q^- occurring within the

membrane. The more slowly decaying C^+ is due to recombination across the interface, i.e. with external BQ^- located at the H_2O -bilayer interface. The first order nature of these decays indicates that the radical products do not become independent of one another, i.e., the transferred electron recombines with the C^+ from which it originated. This can be likened to geminate recombination and similar first order processes in other heterogeneous systems have been reported. For example, Frank et al., (1976) have observed that intramolecular disproportionation of Br_2^- in cetyltrimethylammoniumbromide micelles follows first order kinetics. They envisioned this reaction as a two-dimensional surface diffusion process. Undoubtedly, a similar situation exists in the present system for Q^- at the aqueous-lipid interface.

Concerning the biphasic nature of the C^+ decay, it should be pointed out that a similar biphasic decay has been observed in a system where an amphipathic ruthenium complex solubilized in a PC liposome photo-oxidized N,N-dimethylaniline (DMA) (Nagamura et al., 1980). These authors reported that both decays followed second order kinetics, although apparently no experiments were done at varied flash intensities. The fast decay was attributed to direct recombination of radicals while no extensive attempt was made to explain the slower decay except that the possibility of distal interliposome migration of DMA^+ was mentioned.

The decay rate of the slow C^+ in liposomes is independent of chlorophyll concentration but increases with increasing quinone concentration (Fig. 5). The fast decay, as evidenced by the $C^+ - UQ^-$ recombination, is independent of both of these parameters (Fig. 6). The surprising result here is the dependence of the slow decay on quinone concentration. This can be understood if one assumes the mechanism shown in Fig. 7 for the production and decay of this species. We suggest that Q^- to Q electron transfer can occur in both the

forward (radical formation) and reverse (radical recombination) reactions (more direct evidence for this is given below). Increasing the BQ concentration will then serve to increase the number of quinone molecules which can function as intermediates in the reverse electron transfer reaction. This allows a greater number of possible return routes by which the external BQ^- can transfer its electron back to C^+ in the membrane and results in an increase in rate for the slowly decaying C^+ upon increasing the BQ concentration. The concentration independence of the rate of the fast decay component implies that the rate-limiting step is the Q^- to C^+ electron transfer, as opposed to any secondary electron transfer steps involving C or Q. Similarly, the fact that the slow decay is independent of Chl concentration indicates that any C to C^+ electron transfer that may be occurring is not rate-limiting. As an extension of these arguments, we can conclude that the rate limitation for the slow decay process must be the transfer which occurs across the bilayer-water interface.

Fig. 8 shows the C^+ yield as a function of apparent concentration of UQ, DQ and BQ. The relative positions of these curves is consistent with the partitioning of each quinone between the aqueous and liposome phases. Thus, UQ, which is water insoluble, achieves its maximum C^+ yield at a much lower apparent concentration than does BQ which has an appreciable water solubility. DQ, only slightly water soluble, is intermediate but closer to UQ in this effect. More revealing in terms of mechanistic arguments is that the midpoint of the yield in the case of BQ occurs at a much lower concentration than does the midpoint of the C^+ halftime dependence (0.2×10^{-4} vs 6×10^{-4} M. Fig. 3). That the yield reaches half its maximum value at lower concentrations indicates that additional quinone in this region is still penetrating the membrane and

contributing to the internal membrane electron transfer reaction. As this saturates, further addition of quinone will act to increase the interfacial quinone concentration, thereby increasing the effect on the slow return pathway, which shows up as an increase in the slow C^+ decay rate. Additional evidence that the slow C^+ recombination is associated with the interfacial region and its properties will be given below, as will further evidence of Q^- to Q electron transfer.

Studies of these electron transfer processes in bilayers of different fluidities clearly show the effects of viscosity. Table I presents triplet quenching constants and conversion efficiencies for radical production in bilayers of different composition. Egg PC and DOPC exist in a fluid state at room temperature (-15 to -7°C and -22° respectively) (Ladbrooke and Chapman, 1969). DPPC, on the other hand, is much less fluid, its transition being above room temperature (41°C) (Ladbrooke and Chapman, 1969). These fluidities (viscosities) are reflected in Chl_t decay half-times. In egg PC and DOPC, the half-time is about the same (165 vs. $145\mu\text{s}$) while in DPPC, Chl_t is much more long-lived ($t_{1/2}=650\mu\text{s}$). The increased lifetime is due to decreased collisional quenching in the more viscous bilayer (see Hurley et al., 1980, for evidence showing the collisional nature of Chl_t self-quenching in liposomes). A viscosity effect also appears in the quenching of Chl_t by UQ (as would be expected for a diffusional process) as well as in the radical conversion efficiencies. Thus, the quenching constants (Table I) are similar in egg PC and DOPC but substantially smaller in DPPC. The triplet to radical conversion efficiencies reflect this same effect- 15% conversion in egg PC and 13% in DOPC, while no measurable radical at all is produced in DPPC. The fact that no C^+ is produced from UQ in DPPC is presumably due to an internal viscosity high enough to prevent UQ^- from diffusing away from the radical-pair complex before reverse electron

transfer occurs. The conversion efficiencies obtained when BQ was employed as acceptor are similar in all three lipids (approximately 60-70%). That we observe no appreciable reduction in radical yield in DPPC with BQ implies that this quinone, being a much smaller molecule than UQ (108 vs. 591 MW), and having no long hydrocarbon tail, is still able to diffuse away from the electron transfer site with high efficiency. There is however, an appreciable decrease (approximately two times) in the fast C^+ decay rate in DPPC (Fig. 9). Thus, radical pair separation is much more dependent on viscosity than is recombination.

Figure 10a shows the concentration of radical produced as a function of the fraction of Chl_t quenched ($1-\tau/\tau_0$). For a simple one-step mechanism, i.e., electron transfer followed by radical-pair separation, a linear relationship would be expected. The non-linear dependence which we obtain (this was also found with DQ and DTBQ) implies a cooperativity among quinone molecules in producing C^+ . In Fig. 10b, the data are analyzed in terms of the Hill equation (van Holde, 1971). The maximum slope obtained yields a Hill coefficient of between 2 and 3, which implies that at least this many quinone molecules are involved in the cooperativity. The mechanism presented in Fig. 7 includes Q^- to Q electron transfer in the forward reaction and we believe that this is responsible for the cooperativity that we observe. In other words, efficient radical pair separation requires Q^- to Q electron transfer (presumably via a diffusional interaction) in order to effectively compete with recombination. A similar exchange mechanism has been described previously (Moroi et al., 1979; Gratzel, 1979) in micellar systems where a photochemically reduced transition metal ion exchanges with an unreduced ion present in relatively high concentration at the micelle surface. This physical exchange mechanism is ultimately equivalent to the electron exchange mechanism we propose, with both

leading to a cooperative and efficient means of charge separation. Such an electron exchange mechanism as we propose has been well characterized for many organic free radical systems including several quinone systems (Layloff, et al., 1965; Miller and Adam, 1966; deBoer and vanWilligen; 1967). It might also be pointed out that Hauska (Hauska, 1977) has postulated domain formation of UQ in PC liposomes, an effect which might readily lead to the cooperativity which we observe.

Still further evidence for interquinone electron transfer is given by the results in Table 2. In this experiment, BQ was added to an egg PC liposome sample which contained enough UQ to highly quench (98%) Chl_t . Even at 10^{-6} M added BQ, an appreciable amount of slowly decaying radical was produced. At the lowest (micromolar) BQ concentrations, the results suggest that the slow C^+ was formed at the expense of the fast C^+ i.e., upon going from 1×10^{-6} to 9×10^{-6} M BQ, the total C^+ remained essentially constant but the proportion of fast to slow C^+ decreased. It should be pointed out that in the experiment with no UQ present, we were not able to accurately estimate the concentrations of total and fast C^+ at the lowest BQ concentrations because of the overlap of the Chl_t (which is still present in significant concentration) and C^+ spectra. When one considers that the Chl_t decay rate at these concentrations of UQ is approximately fifty times faster than it is with no UQ present, the amount of slow C^+ produced is far greater than one would expect if BQ were reacting directly with Chl_t (for example, in the absence of UQ, 10^{-6} M BQ has virtually no effect on the Chl_t lifetime; thus, it could not compete with UQ as a triplet quencher). The above results provide strong support for the inclusion of Q^- to Q electron transfer in the mechanism of radical formation depicted in Fig. 7. It is interesting that a similar type of electron transfer

is operative in photosynthetic systems (Vermeglio and Clayton, 1977).

Several other quinones have also been looked at in terms of quenching and conversion efficiencies and proportion of fast and slow radical components and these results are shown in Table 1. For duroquinone (DQ), which has a redox potential equal to that of UQ but no long hydrocarbon tail, we observe a much higher conversion efficiency than is seen with UQ as well as the production of substantial slow radical. This further demonstrates the effect of diffusion on radical pair separation. DTBQ which also has a relatively low redox potential, shows a reduced conversion efficiency, although not as low as with UQ. Since this smaller molecule would be expected to diffuse more readily than UQ, we attribute the lower yield to steric hindrance or to some combination of steric and diffusional properties which inhibit a productive encounter complex leading to subsequent electron transfer. Again, however, a substantial slow radical signal was observed. When the higher potential acceptor DCQ was used, somewhat anomalous results were obtained. Thus, while radical production was as efficient as with BQ and DQ, 80% of the radical species produced decayed by the fast route. Furthermore, Stern-Volmer kinetics were not obeyed. These results may be due to some more complex partitioning of DCQ due to its method of incorporation into the liposomes. Whatever the explanation, it suggests that quinone redox potential is not very important in determining radical yields. The fact that slowly-decaying radical and the quinone concentration dependence of the decay rate was observed with all of the quinones used, with the exception of UQ, suggests that the important factor in allowing this pathway of radical decay is the mobility of the quinone within the vesicle, rather than the degree of water solubility. Presumably, the anchoring of UQ by its hydrocarbon tail prevents it from assuming the required orientation within the interface region.

Chl was also incorporated into liposomes having a positively charged surface (didodecyldimethylammonium bromide) (Kunitake et al., 1977; McNeil and Thomas (1980)) in order to determine if surface charge might affect the reaction mechanism. When BQ was added, Chl_t^+ was quenched and C^+ was produced, but with only 10% conversion efficiency. The decay of C^+ was biphasic, with approximately 60% decaying by the slow route, similar to what was seen with PC. However, over the concentration range examined (0.01 - 6.00 mM), the slow component was independent of BQ concentration, decaying with a half-time of about 22 ms. This result and the 10% conversion efficiency are dramatically different from what is observed with PC. Although it is not entirely clear at this point precisely what the basis for these effects are, it seems apparent that the nature of the interface can markedly influence the electron transfer reactions occurring in these systems. In particular, the lack of cooperativity in the slow decay process is consistent with our interpretation that the latter reflects a reaction which occurs across the bilayer-water interface.

CONCLUSION

The results presented above have shown that quinone quenching of Chl_t^+ within a lipid bilayer is a diffusional process which results in the formation of ion-radical species. Effective separation of these radicals is also diffusion-dependent and is greatly enhanced by electron transfer from the primary Q^- to another Q molecule either within the bilayer or at the bilayer- H_2O interface. In the latter case, a more highly stabilized radical pair is produced than is formed within the bilayer, i.e., the rate of reverse electron transfer is considerably slower, by as much as a factor of several hundred at low quinone concentrations. Conversely, the fact that approximately equal numbers of Q^- are produced in the two environments indicate that the electron transfer which occurs in the forward direction across the bilayer is at least as fast as the transfer occurring within the bilayer. This

combination of fast forward and slow reverse rates, of course, results in a very favorable situation with regard to energy storage and subsequent utilization of Q^- . It remains to be determined by what mechanisms membrane structural parameters influence these rates. Studies along these lines are presently underway.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Francesco Castelli for his valuable comments and discussions and Mr. Malcolm S. Capel for his help with the binding experiments.

Table 1: Electron Transfer to Quinones in Various Lipid Bilayers

Quinone	Redox Potential(V)	Lipid	$k_q \times 10^6 (M^{-1}s^{-1})$	%Conversion $C_t \rightarrow C^+$	%Fast	%Slow
BQ	-0.510 ^a	egg PC	7.2	60	40	60
		DOPC	nd	70	50	50
		DPPC	nd	70	40	60
UQ	-0.840 ^b	egg PC	2.2	15	100	0
		DOPC	1.7	13	100	0
		DPPC	0.3	0	0	0
DQ	-0.840 ^a	egg PC	1.2	60	50	50
DTBQ	-0.730 ^a	egg PC	2.0	30	50	50
DCQ	-0.180 ^a	egg PC	nd	60	80	20

^aPeover, M.D., (1962) J. Chem.Soc. 4540-4549

^bMarcus, M.F. and M.D. Hawley (1971) Biochim. Biophys. Acta 226, 234-238.

Table 2: Radical Production and Triplet Quenching Parameters Indicating
Quinone-Quinone Electron Transfer

(UQ) $\times 10^5 \underline{M}$	(BQ) $\times 10^6 \underline{M}$	$1-\tau/\tau_0$	τ_0/τ	(C ⁺) (arb. units)		
				Total	Fast	Slow
0	0	0	1.0	0	0	0
0	1	0.01	1.0	nd	nd	8
0	3	0.28	1.3	nd	nd	14
0	9	0.65	2.8	nd	nd	25
0	50	0.90	10.4	64	20	44
0	100	0.96	25.0	96	47	49
0	300	0.97	30.5	134	70	58
2×10^{-5}	0	0.98	46.3	18	18	0
2×10^{-5}	1	0.98	52.1	23	20	3
2×10^{-5}	3	0.98	52.1	22	16	6
2×10^{-5}	9	0.98	52.1	22	14	8
2×10^{-5}	50	0.98	65.8	46	25	21
2×10^{-5}	100	0.99	83.3	60	28	32
2×10^{-5}	300	0.99	89.3	119	77	42
2×10^{-5}	900	0.99	178.6	145	82	63

FIGURE LEGENDS

- Fig. 1. Flash-induced difference spectra of Chl-liposomes in the presence of quinones. With BQ present ($7.0 \times 10^{-4} \text{ M}$ app), points were taken 1 ms after the flash for the slow component (X) and $50 \mu\text{s}$ after the flash for fast component (●). The absorbance at $500 \mu\text{s}$ was subtracted from this latter measurement in order to correct for absorbance due to the slow component. With UQ present ($1.0 \times 10^{-4} \text{ M}$ app) (○), points were taken at $50 \mu\text{s}$ after the flash. Chl:PC mole ratio was 1:500 (approximately 5 Chl per liposome).
- Fig. 2. C^+ and Chl_t yields as a function of UQ concentration. Yields were obtained by extrapolating semilog plots of the decay curve at 395 nm back to zero time in the case of C^+ . In the case of Chl_t , the decay curve at 465 nm was extrapolated back to zero time after the baseline was adjusted to correct for the more slowly decaying radical species. Chl:PC mole ratio was 1:500 (approximately 5 Chl per liposome).
- Fig. 3. Stern-Volmer plots showing the quenching of Chl_t by BQ(●) and UQ(○). The values of τ_0 were $160 \mu\text{s}$ and $55 \mu\text{s}$ respectively. The τ values were estimated by dividing the areas of the Chl_t decay curves by the intensity at zero time as previously described (Hurley et al., 1980).
- Fig. 4. C^+ decay curves measured at 395 nm with either BQ (5 mM app) or UQ ($1 \times 10^{-5} \text{ M}$ app) present. The inset shows a semilog plot of these data for BQ(●) and UQ (Δ) as well as the data obtained after cutting the laser intensity by a factor of 4 and 3 for BQ (▲) and UQ (○) respectively. Chl:PC mole ratio was 1:500 (~ 5 Chl/liposome).

- Fig. 5. Dependence of the slow C^+ decay halftime on the apparent BQ concentration. These data are from semilog plots of 395 nm traces with the slow decay being extrapolated to zero time. Shown are data for Chl:PC ratios of 1: 2500, 1:500 and 1:90 representing approximately 1, 5 and 30 Chl per liposome respectively.
- Fig. 6. Invariance of fast C^+ decay rate as a function of Chl and UQ concentrations. Data are from 395 nm traces. Apparent UQ concentrations are shown. 2,5,10,20 and 120 Chl/per liposome represent Chl:PC mole ratios of 1:1250, 1:500, 1:250, 1:125 and 1:21 respectively. Intensities are not comparable.
- Fig. 7. Hypothetical scheme for electron transfer between Chl and quinones in liposomes.
- Fig. 8. Concentration of C^+ produced as a function of quinone concentration. Data obtained with UQ ($\blacksquare$), DQ (X) and BQ(O) are shown. In the cases of DQ and BQ it is the slow C^+ component that is shown. The data were obtained by extrapolating semilog plots of 395 nm traces to zero time. In each case the Chl:PC mole ratio was 1:500 (approximately 5 Chl/per liposome).
- Fig. 9. Decay of C^+ at 395 nm in DPPC and DOPC liposomes at apparent BQ concentrations of 1.1 and 0.8 μ respectively. Chl:PC mole ratio was 1: 500 (approximately 5 Chl per liposome) in both cases.
- Fig. 10a. Concentration of C^+ produced as a function of the fraction of Chl_t quenched ($1-\tau/\tau_0$). Data for BQ, UQ and DTBQ are shown. The values of τ were calculated as described in Fig. 3. In the cases of BQ and DTBQ it is the concentration of slow C^+ that is shown. The

data are from semilog plots of decay curves obtained at
395 nm extropolated to zero time

b. Hill Plot of the data shown in Fig. 10a. The term $\frac{C^+}{C_{\text{max}}^+ - C^+}$
is analogous to $\frac{\bar{v}}{n - \bar{v}}$ employed in the usual usage of the
Hill equation which represents the ratio of filled to vacant
sites. Also shown are lines whose slopes represent Hill
coefficients of 2 and 3.

R E F E R E N C E S

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