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An ESR study of the light-induced interaction between porphyrins and quinones in amide solvents

The light-induced ESR signals* observed with photosynthetic organisms have been widely investigated and may be tentatively identified as originating from the oxidized form of chlorophyll[1-3]. The electron acceptor and its form produced in the primary photoreaction is unknown. Speculation that the light-induced form of the electron acceptor is a semiquinone of either a ubiquinone or a plastoquinone has received little support from ESR studies since no hyperfine sturcture corresponding ro a semiquinone has ever been observed.

The photoreactions between porphyrins and quinones *in vitro* has received considerable study[4-7]. TOLLIN AND GREEN[5] demonstrated the presence of the semiquinone in the light-sensitive chlorophyll-*p*-benzoquinone system with the aid of the ESR method. These authors observed only a broad signal when the chlorophyll–ubiquinone Q_6 system in ether–isopentane–ethanol (8:3:5, v/v/v) was irradiated. The lack of hyperfine structure was attributed to complex formation between the positive chlorophyll ion radical and the ubisemiquinone Q_6. Broad signals have also been observed for porphyrin–benzoquinone systems in aerated media[8,9,**]. These are attributed to a porphyrin-oxygen complex.

The present study was undertaken to investigate the effect of high polar solvents on the light-induced interactions between porphyrins and quinones. The solvents studied were the monosubstituted amides (*N*-methylformanide and *N*-ethylacetamide and the disubstituted amides *N*, *N*-dimethylformamide and *N*, *N*-diethylacetamide). The structures of these molecules are the same as the planar configuration of the peptide linkages[10]. These solvents provided an opportunity to study the influence of hydrogen bonding of the N–H group on the light-induced electron transfer of the porphyrin–*p*-benzoquinone systems.

The present investigation shows that when either pheophytin or a chlorophyll a is excited in the presence of ubiquinone Q_1f in either aerated or deaerated *N*-ethylacetamide, the 9-line ESR spectrum characteristic of the ubisemiquinone is observed. The porphyrin–benzoquinone systems in the aerated and deaerated amide solvents demonstrate the necessity of the N–H group to observe the hyperfine structure in the presence of oxygen.

Chlorophylls *a* and *b* were prepared by the method of ZCSHEILE AND COMAR[11]. The pheophytin a was prepared from chlorophyll *a* by treatment of the ether solution with HCl followed by chromatography on sucrose. Bacteriochlorophyll and the chromatophores of *Rhodopseudomonas spheroides* were kindly supplied by Dr. E. Fujimori of this laboratory. Ubiqiunone Q_{10} and Q_6 were used as supplied by the Mann Research Laboratories, New York, N.Y. *p*-Bensoquinone was purified by sublimation. The amide solvents, *N*-methylformamide (Eastman), *N*-ethylacetamide (Eastman), *N*, *N*-dimethylformamide ("Baker Analyzed" Reagent), and *N*, *N*-diethylacetamide (Eastman) were purified by vacuum distillation. Chloroplasts were prepared from spinach by the method of PARK AND PON[12].

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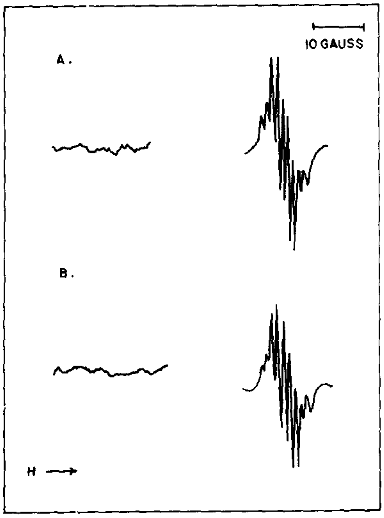
[*] This is signal II in ref. 1 or signal I in ref. 2.

[**] K. P. QUINLAN, unpublished results. The chlorophyll *b* or pheophytin *a*–benzoquinone systems exhibit light-induced broad ESR signals with modulation amplitude values of 1.75 gauss or less.

The method of measuring the light-induced ESR signals has recently been described[8]. The lamp setup described in ref. 8 was used with a Corning CS2-61 red filter (opaque to wavelengths below 600 nm). Air-free solutions were obtained by flushing with nitrogen which was previously cleaned with either alkaline pyrogallol or chromous chloride solution.

Fig. 1 shows the reversible light-induced ESR signals when pheophytin *a* is excited with red light in the presence of ubiquinone Q_{10} in either aerated or deaerated *N*-ethylacetamide. The same signal is observed with the irradiated chlorophyll *a*–ubiquinone $Q_1$1 system in aerated *N*-ethylacetamide. No signal is observed when either pigment is irradiated alone in these solvents. The 9-line spectrum characteristic of the ubisemiquinone is the result of a light-induced electron transfer from the porphyrin to the ubiquinone. The ESR spectrum has been characterized by BLOIS AND MALING[13]. The observation of the hyperfine structure is probably the result of the ability of the *N*-ethylacetamide to inhibit the complex formation between the pigment positive ion radical with the ubisemiquinone. The failure to detect an ESR signal corresponding to the porphyrin positive ion has been discussed by TOLLIN[5]. These observations suggest that in photosynthesis either the chlorophyll undergoes a light-induced complexing reaction with the native quinones or that the native quinones are not involved in the primary photoreaction.



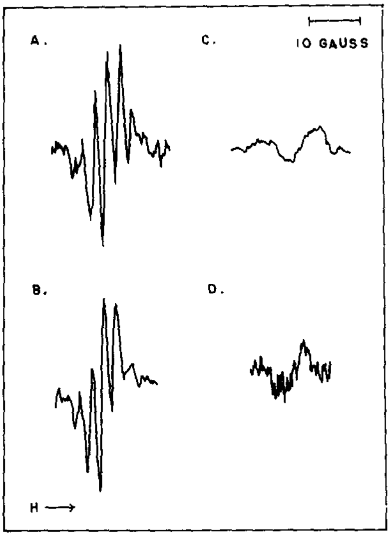


Fig. 1. Light-induced ESR signals of the pheophytin *a*–ubiquinone Q_{10} systems in deaerated and aerated *N*-ethylacetamide. Left-hand figures, light off; right-hand figures, light on. A. Deaerated *N*-ethylacetamide. Pheophytin *a* concn., $6.6 \cdot 10^{-5}$ M; ubiquinone Q_{10} concn., $2.0 \cdot 10^{-3}$ M; modulation amplitude, 0.45 gauss. B. Aerated *N*-ethylacetamide. Pheophytin *a* concn., $6.6 \cdot 10^{-5}$ M, ubiquinone Q_{10} concn., $2.9 \cdot 10^{-3}$ M; modulation amplitude, 0.45 gauss.

Fig. 2. Light-induced ESR signals of the pheophytin *a*–benzoquinone system in aerated amide solvents. A. *N*-Ethylacetamide. Pheophytin *a* concn., $9.5 \cdot 10^{-5}$ M; benzoquinone concn., $3.3 \cdot 10^{-2}$ M; modulation amplitude, 0.75 gauss. B. *N*-Methylformamide. Pheophytin *a* concn., $9.5 \cdot 10^{-5}$ M; benzoquinone concn., $3.6 \cdot 10^{-2}$ M; modulation amplitude, 0.75 gauss. C. *N*,*N*-diethylacetamide. Pheophytin *a* concn., $6.6 \cdot 10^{-5}$ M; benzoquinone concn., $3.0 \cdot 10^{-2}$ M; modulation amplitude, 4.0 gauss. D. *N*,*N*-Dimethylformamide. Pheophytin *a* concn., $6.8 \cdot 10^{-5}$ M; benzoquinone concn. $3.5 \cdot 10^{-2}$ M; modulation amplitude, 0.75 gauss.

The lack of influence of oxygen on the light-induced ESR observed for the porphyrin–ubiquinone Q_1I system in *N*-ethylacetamide was unexpected. A study of the light-induced ESR spectra of the porphyrin–*p*-benzoquinone in the monosubstituted amides (*N*-methylformamido and *N*-ethylacetamide) and the disubstituted (*N*,*N*-dimethylformamide and *N*,*N*-diethylacetamide) shows that the presence of the N–H group lessens the influence of oxygen on these electron transfer reactions.

The mono- and di-N-substituted amides all possess large dipole moments in the range of 3.8 D[14,15]. The dielectric constants of the monosubstituted amides are much larger than the disubstituted amides and have been attributed to the association of the monosubstituted amides into chain polymers[16,17]. This association is achieved through the formation of hydrogen bonds between the N-H group of one molecule and the O^-–C of the other. In the disubstituted amides, association through hydrogen bonding is no longer possible and hence a large decrease in dielectric constant occurs. The ability of these solvents to form hydrogen bonds through the N–H group is manifested in their dielectric constants. It is not certain whether the aldehydic C–H group is capable of forming hydrogen bonds[18].

Fig. 2 shows the light-induced ESR signals observed with the pheophytin *a*–bensoquinone system in the four aerated solvents. The ESR spectrum characteristic of the benzosemiquinone ion radical is observed in the monosubstituted amides (*N*-methyl formamide and *N*-ethylacetamide). Only small broad ESR signals are observed in the disubstituted amides. The chlorophyll a systems give similar results in the amide solvents. The results for the two pigment systems are tabulated in Table I. The N–H group of the monosubstituted amides possibly bonds to those positions of the porphyrin necessary for oxygen complex formation .The typical light-induced 5-line spectrum is observed for these porphyrin–quinone systems in deaerated *N*,*N*-dimethylformamide and *N*,*N*-diethylacetamide.

TABLE I

THE PRESENCE (+) AND ABSENCE (—) OF THE LIGHT-INDUCED ESR HYPERFINE STRUCTURE OF THE SEMIQUINONE IN THE VARIOUS AMIDE SOLVENTS

Pigment	*Solvent*			
	N-Methylformamide	*N-Ethylacetamide*	*N,N-Dimethylformamide*	*N,N-Diethylacetamide*
Pheophytin *a*	+	+	—	—
Chlorophyll *a*	+	+	—	—

Attempts to determine whether *N*-ethylacetamide treatment of chloroplasts of spinach or chromatophores of *R. spheroides* could induce light-induced ESR hyperfine structure were unsuccessful.

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