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Photo- and chemically-produced phylloquinone biradicals: EPR and ENDOR study

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Abstract

Phylloquinone biradical triplet species were generated by 300 nm irradiation of frozen (77 K) solutions or by treatment with $AlCl_3$. The shape of the ($\Delta m_s = 1$) electron paramagnetic resonance (EPR) signal of the triplet is axially symmetric ($E = 0$) with $D = 19 \pm 0.5$ mT for photo-induced and $D = 11.2 \pm 0.5$ mT for chemically induced radicals. A half-field signal ($\Delta m_s = 2$) in the region of $g \approx 4$ was detected in both cases, confirming its assignment as a triplet. An additional line arising at the center of the ($\Delta m_s = 1$) signal with $g = 2.0048 \pm 0.0002$ was assigned to the phylloquinone radical anion ($PhQ^{\bullet -}$). Electron nuclear double resonance (ENDOR) measurements of the triplet revealed the sign of the D parameter. For photo-generated radicals it appeared to be negative, which is the characteristic of radical dimers with well-separated partners (biradicals). Spin–spin distances of 5.3 and 6.3 Å, respectively, were estimated from the D parameter of photo-generated and chemically prepared phylloquinone biradicals.

Keywords: Phylloquinone; Biradicals; Electron transfer; Electron paramagnetic resonance; Electron nuclear double resonance

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1. Introduction

Phylloquinone (Vitamin K_1) plays an important role in electron transport during photosynthesis. It servers as an intermediate electron acceptor in transmembrane electron transfer within Photosystem I (PS I). Upon excitation of the primary electron donor (P_{700}) of PS I to its lowest excited singlet state (P^*_{700}), an electron is transferred from P^*_{700} to a series of electron acceptors: A_0 (Chl *a*), A_1 (phylloquinone), and F_X, F_A, F_B (iron–sulfur clusters) [1–3]. The low-temperature photo-accumulated EPR signal of phylloquinone radical anion ($A_1{}^{\bullet -}$) typically has a g value of 2.004–2.005 and line width of about 10.5 G [4,5]. Other excited states of phylloquinone can be produced in photo-irradiated biological tissues. The excited triplet states of quinones, which are known to be good sensitizers of singlet oxygen production [6,7], have been studied in mixtures with Zn-porphyrins or other donors [8]. It has been found that metalloporphyrin/quinone mixtures produce large concentrations of triplet radical pairs with unusual stability in the solid-state matrix.

The presence of two phylloquinones, PhQ_A and PhQ_B, has been revealed in cyanobacterial PS I at 2.5 Å resolution [9]. It has been shown in purple bacterial photosynthetic reaction centers (RCs), where the photocycle involves electron transfer between two quinone molecules, Q_A and Q_B, separated by 17.2 Å, that the biradical $Q_A{}^{\bullet -}Q_B{}^{\bullet -}$ is an intermediate state in this process [10,11]. The $Q_A{}^{\bullet -}Q_B{}^{\bullet -}$ biradical state has been generated and stabilized in frozen solution of photosynthetic RCs containing Fe^{2+} or Zn^{2+} ions. Experiments at different frequencies (9–326 GHz) [10,11] have shown that the spectrum of the biradical is not simply a superposition of the individual radical spectra, indicating the existence of a magnetic interaction between the quinones. The observed small exchange interaction for the $Q_A{}^{\bullet -}Q_B{}^{\bullet -}$ biradical is typical for two spins connected by a diamagnetic bridge. Triplet states have also been detected when two or more anion radicals were stabilized by metal cations [12,13]. Butler et al. have predicted a symmetric arrangement of $Q_A{}^{\bullet -}$ and $Q_B{}^{\bullet -}$ with respect to the Fe^{2+} ion [14,15]. The formation of a reduced quinone–iron complex has been proposed. Iron exists as Fe^{2+} in this complex and does not change valence when the quinone is reduced. The electron is localized on the quinone, which is near the Fe^{2+} ion. A similar case, when two ketyl anions may be bound by two alkali ions, has been discussed [16]. The two alkali ions may be replaced by one divalent ion, such as Be^{2+} or Mg^{2+}. High-frequency EPR and ENDOR studies of metal-quinone high-spin complexes prepared by

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solid-state mechano-activation or chemical treatment in solution demonstrated the presence of ground-state triplet species in both cases [17,18].

Light-induced electron transfer reactions involving quinones are of great importance, especially with regard to the primary mechanisms of photosynthesis. In this work, we report the observation of triplet species of phylloquinone produced by photolysis of frozen solutions or by chemical treatment with $AlCl_3$. EPR and ENDOR studies of the phylloquinone biradicals in organic solvents may provide additional information on the role of quinones in photosynthetic RC electron transfer.

2. Experimental

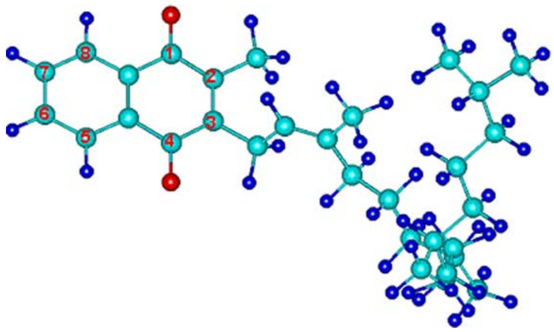
2.1. Materials and sample preparation

Vitamin K_1 (phylloquinone, see Scheme 1) was supplied by Roche Vitamins Inc. The solvents methylene chloride (Aldrich, anhydrous) and toluene (Fisher, HPLC grade) were used as received. Aluminum chloride ($AlCl_3$; anhydrous, 99%) was from Aldrich. $AlCl_3$ was purified by sublimation; only colorless samples of $AlCl_3$ were used.

The samples were prepared chemically by mixing phylloquinone and $AlCl_3$ at room temperature. One volume of 10^{-2} M $AlCl_3$ was mixed with three volumes of 10^{-2} phylloquinone solution in toluene or methylene chloride, and the resulting red mixture was transferred to a standard quartz EPR tube (WILMAD, 4 mm o.d.) and stored in liquid nitrogen before use. For the photochemically-generated triplet state, frozen phylloquinone solutions (77 K) were irradiated at 300 nm with light focused from a Xe/Hg lamp (1 kW) equipped with a Kratos monochromator.

2.2. EPR and ENDOR measurements

EPR spectra were measured at 77 K and 9.5 GHz with a Varian E-12 EPR spectrometer equipped with a rectangular cavity. The magnetic field was measured with a Bruker EPR 035M gaussmeter, and the microwave frequency was measured with a model HP 5245L frequency counter. ENDOR experiments were carried out with a Bruker ESR-300 EPR spectrometer, equipped with a DICE ENDOR facility.



Scheme 1. AM1-optimized structure of phylloquinone radical anion.

3. Results and discussion

3.1. Photolysis of phylloquinone frozen solutions

A typical EPR spectrum observed after 300 nm irradiation of a frozen toluene solution of phylloquinone at 77 K is shown in Fig. 1. The signal (a) in the central part of the $\Delta m_s = \pm 1$ spectrum ($g = 2.0048 \pm 0.0002$) originates from mono-radicals with $S = 1/2$ electron spin state, most probably from the phylloquinone radical anion ($PhQ^{\bullet -}$) [4,5]. The observed four-line signal (b) is indicative of axially symmetric systems with $S = 1$. It is, therefore, attributed to radical pairs with an axially symmetric zero-field splitting tensor. Distortion of the triplet (b) signal in Fig. 1 (low-field versus high-field bb, bb pair) is due to the mono-radical (a) that is located approximately 1 mT to the low-field side and causes broadening of the low-field features of the triplet. The observation of a "half-field" EPR signal near 160 mT ($g \sim 4$) confirms the formation of triplet species. The appearance of this semi-forbidden $\Delta m_s = \pm 2$ signal is characteristic of a triplet spin system in random orientation [19–21].

Triplet systems with $S = 1$ may be described by the spin Hamiltonian:

$$H = g\beta HS + DS_z^2 + E(S_x^2 - S_y^2) - g_n\beta H_n \sum I_k + \sum SA_k I_k \quad (1)$$

The symbols D and E are the fine structure splitting constants (so-called zero-field splitting parameters) and arise from the

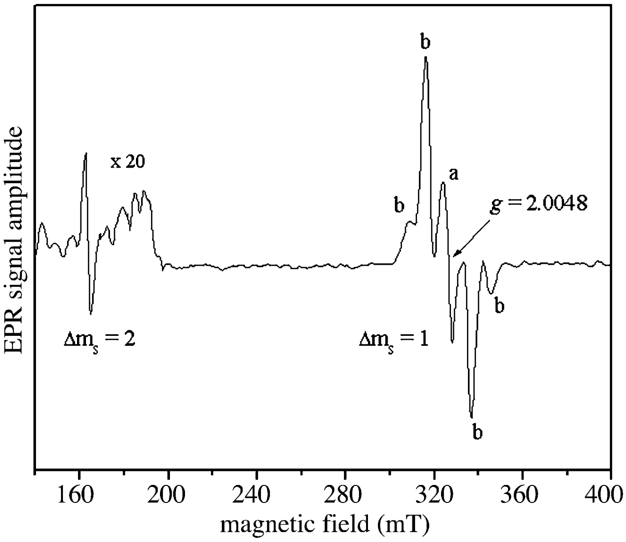


Fig. 1. The EPR spectrum of phylloquinone solution in toluene (10^{-2} M) irradiated (300 nm) and measured at 77 K. Microwave (MW) frequency, $\nu = 9.14$ GHz; MW power, 2.0 mW; modulation frequency, 100 kHz; modulation amplitude, 0.2 mT.

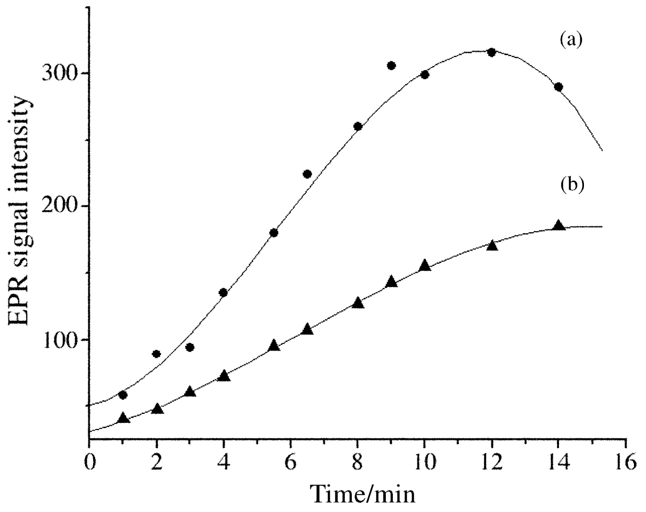


Fig. 2. Accumulation of the phylloquinone (a) biradicals, (b) mono-radicals under irradiation at 300 nm.

dipole–dipole interaction of the two unpaired electrons. A single EPR line for the triplet arising from the first electron Zeeman term in Eq. (1) and corresponding to degenerate transitions ($\Delta m_s = \pm 1$) is split by D in the case of axially symmetric ($E = 0$) dipolar or fine structure interactions [22].

The accumulation of the radicals upon irradiation at 300 nm is shown in Fig. 2. The line-widths of the triplet signal and the mono-radical signal are essentially the same. Therefore, we measured the EPR signal intensities to estimate the yield of the radicals. The yield of mono-radicals (Fig. 2b) is lower than that of the radical pairs and their production appears to be an independent process. The triplet signal is stable for more than 1 h at 77 K. After termination of illumination, the concentration of the mono-radicals and triplets decreased simultaneously (data not shown), inconsistent with triplet species decaying via dissociation into mono-radicals.

When irradiation of phylloquinone is performed in frozen CH_2Cl_2 solution, the EPR spectrum consists of a strong mono-radical signal in the middle ($g = 2.0047 \pm 0.0002$) with a triplet signal less intense than that seen in toluene (Fig. 3). This spectrum also has a “half-field” signal (data not shown). The zero-field axial parameter D estimated from triplet EPR spectra of phylloquinone in toluene or CH_2Cl_2 is 19.0 ± 0.5 mT ($0.0190\,\text{cm}^{-1}$). The parameters of the dipolar interaction D provide structural information about triplet species. It has been shown that when radicals exist in a monomer–dimer equilibrium, the dimeric forms show characteristic triplet EPR spectra with relatively small ZFS parameters ($D \sim 0.01\,\text{cm}^{-1}$ and $E \sim 0\,\text{cm}^{-1}$) [16,23]. It has been supposed that solvent molecules intervene between the two radicals; thus a small spin–spin dipole interaction is expected and D should be small [24,25].

If two unpaired electrons are well-separated, we may assume that D in Eq. (1) is only due to dipolar interactions with no contribution from exchange integrals. In this approximation, the value of D can be used to calculate the distance r between two unpaired electrons from Eq. (2):

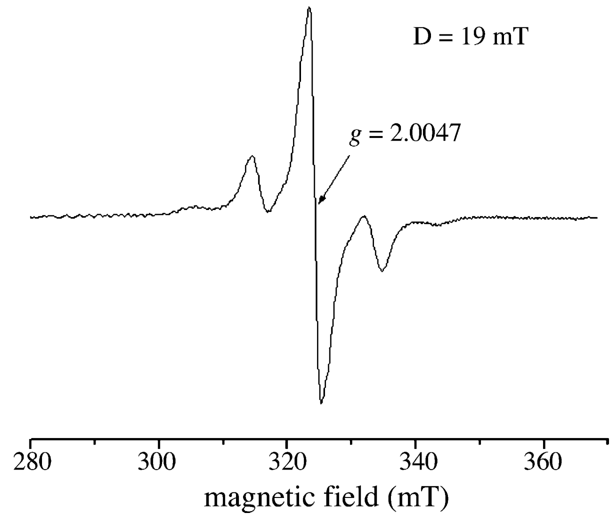


Fig. 3. EPR signal of phylloquinone solution in CH_2Cl_2 (10^{-2} M) irradiated (300 nm) and measured at 77 MW frequency, $\nu = 9.1$ GHz; MW power, 2.0 mW; modulation amplitude, 0.4 mT.

$$D = \frac{3g\beta}{2r^3} = 1.39 \times 10^3 \left(\frac{g}{r^3}\right) \tag{2}$$

where D is in mT and r is in Å [26].

The distance r estimated from Eq. (2) for photo-generated radical pairs is about 5.3 Å.

3.2. Chemically prepared triplet of phylloquinone

The triplet radical pair of phylloquinone was also produced chemically by treatment of quinone solutions with $AlCl_3$. Fig. 4 shows EPR spectra of triplets chemically generated with $AlCl_3$ (a) in toluene, or (b) in CH_2Cl_2. The spectra contain a strong signal of mono-radical. The EPR signal intensities of chemically-generated radical

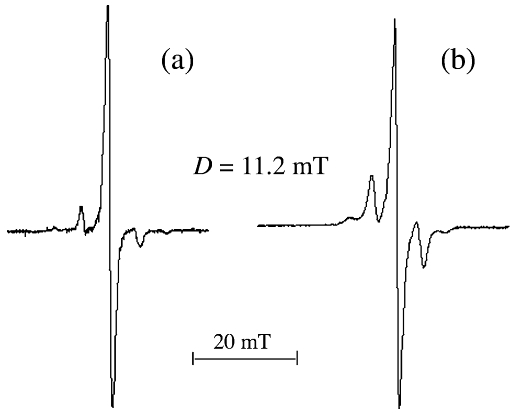


Fig. 4. EPR spectra of the phylloquinone/$AlCl_3$ mixture in (a) toluene or, (b) CH_2Cl_2 measured at 77 K. MW frequency, $\nu = 9.1$ GHz; MW power, 2.0 mW; modulation amplitude, 0.2 mT.

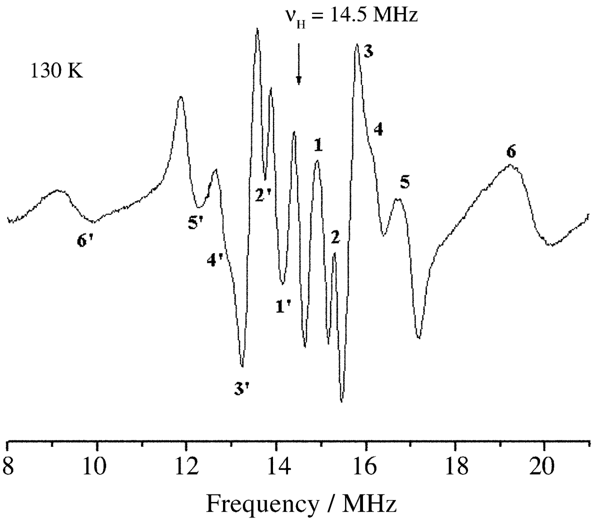


Fig. 5. ENDOR spectrum of phylloquinone radical anion measured at 130 K. MW frequency, $\nu = 9.53$ GHz; MW power, 6.4 mW; RF power, 150 W; modulation frequency, 12.5 kHz; time constant, 20.5 ms; gain, 1.6×10^4. ENDOR features 1,1′; 2,2′: ring protons; 3,3′; 4,4′: C3–CH_2 protons and 5,5′; 6,6′: C2–CH_3 protons.

pairs were smaller than those produced photochemically in toluene glasses. The dipolar–dipolar parameter D of chemically-generated triplets is 11.2±0.5 mT. The spin–spin distance r estimated from Eq. (2) is 6.3 Å. The magnitudes of D and r are found to be different for chemically- and photochemically-generated triplets, which may indicate that these triplet species have different structural arrangements.

3.3. ENDOR measurements

To understand the origin of the EPR signals observed, we performed ENDOR experiments, which allowed us to determine small hyperfine interactions. The ENDOR spectrum of phylloquinone/$AlCl_3$ in toluene obtained with the field set on the central mono-radical peak, is shown in Fig. 5. The ENDOR spectrum was measured at 130 K. The hyperfine coupling constants determined from the spectrum are similar to those reported for phylloquinone radical anion in proteino and in frozen solvents [27,28] (see Table 1). This is also in agreement with our UHF/INDO calculations of the $PhQ^{\bullet -}$ hyperfine couplings. Scheme 1 shows the AM1 geometry-optimized structure of the phylloquinone radical anion.

UHF/INDO MO calculations of AM1-optimized $PhQ^{\bullet -}$ structure showed that more than 90% of the electron spin density is localized on the naphthoquinone ring. Calculated spin densities on the protons of the phytyl tail were significantly less than those of the ring. The assignment of the phylloquinone ring protons was made on the basis of the ENDOR data reported for $PhQ^{\bullet -}$ in ethanol solution [27] and in an alkaline ethanol glasses [28].

We propose that the 6,6′ and 5,5′ features of the powder ENDOR spectrum of $PhQ^{\bullet -}$ arise from the $A_{||}$ (10.2 MHz) and $A_{\perp}$ (5.32 MHz) components of the axially symmetric hfc's due to the methyl protons at position C2. The estimated magnitude of isotropic hfc, $A_{\rm iso} = (A_{||} + 2A_{\perp})/3 = 7.0$ MHz is similar to $A_{\rm iso}$ reported for C2–CH_3 protons [27]. ENDOR features 4,4′ and 3,3′ can be assigned to the $A_{||}$ (3.78 MHz) and $A_{\perp}$ (2.6 MHz) components arising from the methylene protons at the position C3. Calculated $A_{\rm iso} =$ 3.0 MHz is in agreement with $A_{\rm iso}$ determined for C3–CH_2 protons [27]. Rigby et al. [28], and O'Malley and Babcock [29] have attributed the 5.32 and 2.6 MHz hfc to protons that are hydrogen-bonded to the quinone oxygens. However, hydrogen-binding is unlikely in the aprotic solvents used here. The high concentration of $PhQ^{\bullet -}$ allowed observation of protons with hfc <2.0 MHz, indicative of the ring protons.

The sign of the dipolar constant D allows the assignment of canonical peaks in the EPR spectrum. Radical dimers with separated partners (pairs and biradicals) are characterized by a negative D, while a positive sign of D is expected for molecular triplets with delocalized spins and radical trimers [30]. The sign of D may be determined from the ENDOR spectrum. For mono-radicals with $S = 1/2$ ($m_s = \pm 1/2$), the corresponding ENDOR frequencies, $\nu_{\rm ENDOR} = \nu_0 \pm A/2$, appear as mirror images around the free nuclear frequency ν_0. However, for systems with $S > 1/2$, EPR transitions can be selected and ENDOR lines appear separated from each other on one side of ν_0, at higher or lower frequency,

Table 1
Proton hyperfine coupling constants (MHz) of $PhQ^{\bullet -}$ in toluene glasses determined from ENDOR and calculated by INDO

Protons	Assigned features in ENDOR spectrum	ENDOR toluene glasses hfc	INDO calculated spin density	INDO calculated isotropic hfc	From [27,28]
C2–CH_3 $A_{\parallel}$	6,6′	10.2	0.0059	9.62	$A_{\parallel} = 10.0$
C2–CH_3 $A_{\perp}$	5,5′	5.32	0.0059	9.62	$A_{\perp} = 6.8$
					$A_{\rm iso} = 7.0, 7.9$
C3–CH_2 $A_{\parallel}$	4,4′	3.78	0.0022	3.59	$A_{\rm iso} = 3.8$
C3–CH_2 $A_{\perp}$	3,3′	2.6	0.0022	3.59	$A_{\perp} = 3.0$
C8	2,2′	1.55	−0.0008	−1.30	<2.0
C5	1,1′	0.73	−0.0005	−0.82	<2.0
C6			−0.0003	−0.50	<2.0
C7			−0.0006	−0.98	<2.0

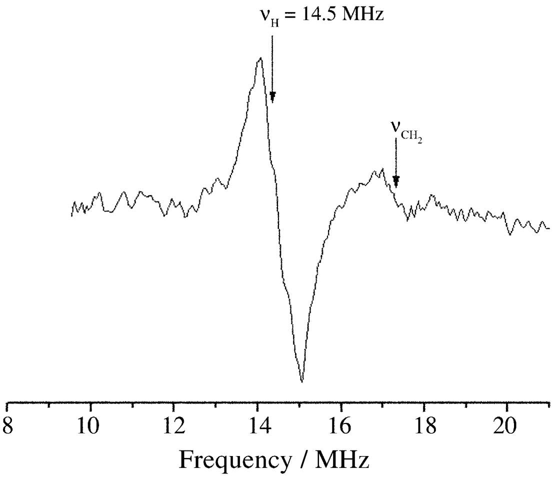


Fig. 6. ENDOR spectrum of the phylloquinone biradicals in toluene glasses with the low-field setting ($T = 120\,\mathrm{K}$; MW, $\nu = 9.55\,\mathrm{GHz}$; MW power, 6.4 mW; RF power, 150 W; modulation frequency, 12.5 kHz; time constant, 81.92 ms; gain, 8×10^4; sum of 20 scans).

depending on the sign of A (hfc constant) [18]. Typically an ENDOR spectrum of the triplet species consists of the line at ν_0 and, for each set of equivalent nuclei, an ENDOR line that is separated from the free nuclear frequency by hfc A [31,32].

When ENDOR measurements were carried out by setting the magnetic field on the low-field part of the triplet-type signal, the resultant spectrum consisted of an intense line at the free proton frequency ($\nu_H = 14.5\,\mathrm{MHz}$) and a line at 2.7 MHz higher than ν_H (Fig. 6). Apparent broadening of the ENDOR line centered at the free proton frequency originates from hfc's of the order of 1 MHz due to the phylloquinone ring protons.

The sign of D can be deduced from ENDOR spectra relative to the hfc's. For benzoquinones, the hfc's of the ring α-protons are known to be negative [29,30]. If α-protons are usually characterized by their negative isotropic hyperfine couplings, β-protons may be characterized by their positive isotropic hfc's [31]. This is consistent with our semi-empirical calculations, according to which the sign of the β-protons are positive. Positive hyperfine couplings correspond to a positive difference $\nu - \nu_0$ in ENDOR spectrum at the low-field canonical peak. According to considerations reported in [18,31], this observation corresponds to a negative sign of D, which is characteristic of biradicals.

For biradicals in which the unpaired electron is shared between two radicals, all hyperfine couplings are decreased by a factor of 2 compared to mono-radicals. Thus, the line at 17.2 MHz ($\nu - \nu_H = 2.7\,\mathrm{MHz}$) observed in the ENDOR spectrum in Fig. 6 is most likely due to the methyl protons at the C2 position (the $A_\perp$ of the mono-radical methyl protons is 5.32 MHz; see Table 1).

The obtained small D and relatively large r values are in agreement with those reported for solvent-shared ion pairs [33]. We propose that the origin of the photo-generated triplet species is the formation of the biradical $\mathrm{PhQ}^{\bullet -}\mathrm{PhQ}^{\bullet -}$, with the two anions separated by solvent molecule(s). Electron spins are localized on the naphthoquinone rings, which are in van der Waals interaction with solvent. The photo-generated biradical signal in frozen toluene solution is more intense than that in CH_2Cl_2, most likely because toluene gives good glassy matrices in which the biradicals are more stable [34]. Note that these distances are much closer than in PS I, where the $\mathrm{PhQ_A}$ and $\mathrm{PhQ_B}$ naphthoquinone rings are separated by ~15–25 Å [9].

In the case of the radical pairs generated by $AlCl_3$ treatment, we can assume a partial electron transfer of unpaired electrons from quinone radicals into orbitals of aluminum, which can modify the dipolar interaction between the electron spins. This is in contrast to the situation with photo-generated biradicals, where the spin density of the unpaired electrons is localized solely within the quinone radicals. Phylloquinone radical anions might form metal-quinone complexes similar to those reported for the reactions between quinones (Q) and metals (Me) such as Al, Zn, Cd, Ga, In, and Sn [18]:

$$\mathrm{Me}^{n+} + n\mathrm{Q}^{\bullet -} \rightarrow \mathrm{MeQ}_n^{\bullet -} \tag{3}$$

where n is the valence state of the metal.

Three $\mathrm{PhQ}^{\bullet -}$ can be symmetrically stabilized by an Al^{3+} ion, producing an $\mathrm{Al}^{3+}(\mathrm{PhQ}^{\bullet -})_3$ complex. A separation of about 5 Å has been found in contact ion pairs between ketyl radicals and alkali metal ions such as Li^+ [33]. The radius of the Al^{3+} ion (68 pm) is comparable with that of Li^+ (90 pm). Assuming that a phylloquinone radical is separated from an Al^{3+} ion by ~5 Å, the calculated distance of $r = 6.3$ Å between two $\mathrm{PhQ}^{\bullet -}$ in an $\mathrm{Al}^{3+}(\mathrm{PhQ}^{\bullet -})_3$ complex appears reasonable.

4. Conclusions

Triplet species of phylloquinone were produced by photo-excitation in frozen toluene or methylene chloride solutions or by chemical treatment with $AlCl_3$. The small D values (19.0 and 11.2 mT) and relatively large r values (5.3 and 6.3 Å) are typical for solvent-separated ion-radical pairs. ENDOR measurements of the photo-generated radical dimers revealed the sign of the D parameter. It appeared to be negative, which is characteristic of biradicals. It was proposed that photo-generated triplet signal is due to solvent-separated biradicals $\mathrm{PhQ}^{\bullet -}\cdots\mathrm{Sol}\cdots\mathrm{PhQ}^{\bullet -}$, where the spin density of the unpaired electrons is localized solely with the quinone radicals. Three-spin complexes of the type $\mathrm{Al}^{3+}(\mathrm{PhQ}^{\bullet -})_3$ are expected to be produced by chemical treatment of phylloquinone with $AlCl_3$ as a result of the overlap of the negative ion orbitals and the metal ion orbitals. EPR and ENDOR studies of the photo-produced biradicals of phylloquinone in organic solvents may provide additional information on the role of quinones in PSI electron transfer.

Acknowledgements

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