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THE CATIONIC PLASTOQUINONE RADICAL OF THE CHLOROPLAST WATER SPLITTING COMPLEX

HYPERFINE SPLITTING FROM A SINGLE METHYL GROUP DETERMINES THE EPR SPECTRAL SHAPE OF SIGNAL II

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The proposal that EPR Signal II in spinach chloroplasts is due to a plastoquinone cation radical (O'Malley, P.J. and Babcock, G.T. (1983) Biophys. J. 41, 315a) has been investigated in further detail. The similarity in spectral shape between Signal II and the 2-methyl-5-isopropylhydroquinone cation radical is shown to arise from hyperfine coupling to one methyl group for both radicals. A well-resolved four line EPR spectrum of approximate relative intensity 1:3:3:1 for membrane orientation parallel and perpendicular to the applied magnetic field direction also indicates that the partially resolved structure of Signal II is due to hyperfine interaction with one methyl group, i.e., the 2-CH_3 group of the plastoquinone cation radical. The ENDOR band observed for this coupling is similar to that observed for methyl group bands of model quinone radicals. The principal hyperfine tensor values obtained for the methyl group interactions are $A_\perp = 27.2$ MHz and $A_\parallel = 31.4$ MHz. The large isotropic coupling value (28.6 MHz) of the plastoquinone cation radical's 2-methyl group in vivo indicates that the antisymmetric orbital is the sole contributor to the spin-density distribution of Signal II. The orientation data also suggest that the plastoquinone cation radical is oriented such that the C–CH_3 bond direction, and hence the aromatic ring plane, lies perpendicular to the membrane plane.

Introduction

The radical species responsible for the EPR Signal II of green plant phososynthesis has been known for some time to be associated with the oxygen-evolving complex of Photosystem II [1–4].

In recent years, it has been shown that a fast transient component of Signal II functions as an electron donor to $P680^+$ in both oxygen-evolving and nonoxygen-evolving chloroplasts [5,6]. The role of this species, generally termed Z, is to transfer oxidizing equivalents which are generated photochemically at P680 to the site of water oxidation. Recent work with isolated PS II reaction center complexes indicates that Z is bound to the same polypeptide to which the PS II reaction center chlorophyll binds [7].

We have recently proposed [8–10] that the molecular origin of Signal II is a plastoquinone

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Abbreviations: ENDOR, electron nuclear double resonance; P680, primary donor reaction center chlorophyll of Photosystem II; Q, McConnell proportionality factor between carbon spin density and proton-electron hyperfine coupling values; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

cation radical * in which substantial stabilization of the benzene antisymmetric orbital has occurred. In this report, we have used EPR orientation studies and ENDOR spectroscopy to explore this proposal in more detail. In particular, we have focused on the origin of the unusual, partially resolved hyperfine structure in the Signal II EPR spectrum and in that of a quinone cation radical model compound, 2-methyl-5-isopropyl quinone. Our results indicate that this structure is primarily the result of the 2-methyl group in the model; we conclude that a similar splitting arises from the methyl group para to the polyprenyl substituent of the plastiquinone cation radical in vivo.

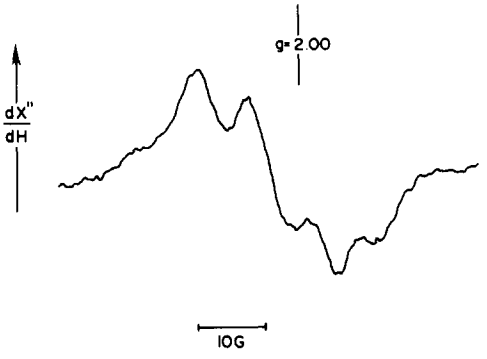

Fig. 1. Spectral shape of EPR Signal II in chloroplast membranes. Temperature, 298 K; power, 10 mW; modulation, 3.2 G; time constant, 200 ms; sweep time, 100 s.

Experimental

Spinach chloroplasts and Photosystem II particles were prepared as previously described [11,12]. The final buffer in which the chloroplast samples were resuspended contained 0.4 M sucrose/0.05 M Hepes/0.01 M NaCl (pH 7.6); the final buffer for the PS II particles was 0.4 M sucrose/0.5 M Mes/0.01 M NaCl (pH 6.0). Orientation of the membranes was achieved by slow drying on mylar strips over a saturated solution of K_2HPO_4 [13]. Preparation of the 2-methyl-5-isopropylhydroquinone cation radical was carried out as described in [9,10]. The X-band EPR and ENDOR spectra were recorded on a Bruker ER200D spectrometer equipped with a Bruker ENDOR accessory by using procedures similar to those already described [14]. The temperatures and instrument settings used are given in the figure legends. Spectral simulations were performed on a Nicolet 1180 computer using an EPR simulation program supplied by Bruker.

Results and Discussion

Recently, we have shown that the characteristic spectral shape of Signal II (Fig. 1) closely resembles the spectral shape observed for the immobilized 2-methyl-5-isopropylhydroquinone cation radical (Fig. 2) [8–10]. A 26% contribution from the antisymmetric benzene molecular orbital to the spin distribution of the 2-methyl-5-isopropylhydroquinone cation radical [10] leads to relatively large spin density values at the 1, 2, 4 and 5 ring carbon positions (Fig. 3a). Immobilization of the bulky isopropyl group can be expected in the frozen state, hence leading to a reduced Q value for the –CH β proton in comparison with the $C–CH_3$ fragment [15]. The McConnell Q factor for a C–OH fragment has a complex dependence on the β carbon spin density, the oxygen spin density and the carbon-oxygen bond angle [16]. In general, however, the Q value of the C–OH frag-

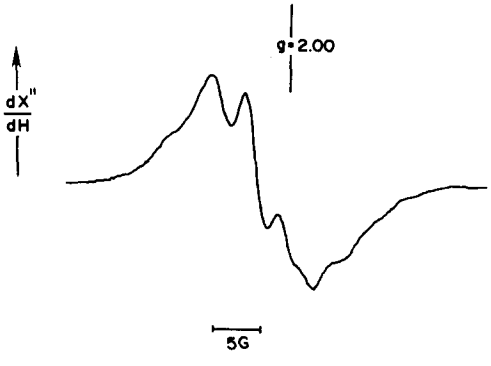

Fig. 2. Spectral shape of immobilized 2-methyl-5-isopropylhydroquinone cation radical. Temperature, 123 K; power, 0.063 mW; modulation, 0.16 G; time constant, 500 ms; sweep time, 200 s.

* Plastoquinone: 2,3-dimethyl-5-nonaprenyl benzoquinone. Note that with this numbering it is the 2-methyl group which is para to the polyprenyl substituent. Chloroplasts also contain plastoquinones with allylic hydroxyls and their esters. Such substituents are sufficiently remote from the ring that they have no significant effect on the magnetic resonance and redox properties of the semiquinones, and would not affect the interpretations presented here.

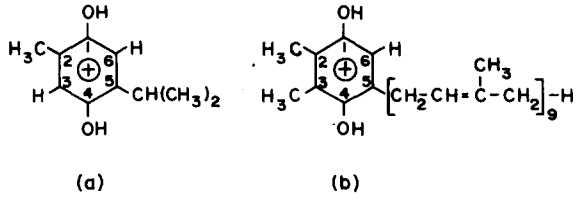

Fig. 3. (a) 2-Methyl-5-isopropylhydroquinone cation radical. (b) Plastoquinone cation radical.

ment is much less than for a $C-CH_3$ fragment [16,17]. Therefore, although similar spin densities are found at the 1, 2, 4 and 5 positions of Fig. 3a, the large Q_{C-CH_3} value results in a much larger hyperfine coupling for the 2-methyl group compared with the other substituents. Owing to the relatively large $-CH_3$ hyperfine coupling value and the low anisotropy of β ($-CH_3$) proton couplings, the partial structure due to the three equivalent methyl protons is retained in the immobilized radical EPR spectrum and is responsible for the partially resolved structure observed in Fig. 2 (see Ref. 10).

By analogy to the model quinone cation radicals, we proposed that Signal II was due to a plastoquinone cation radical in which substantial stabilization of the antisymmetric orbital occurred [10]. Such a condition leads to large spin-density values at the 1, 2, 4, and 5 ring carbon positions (Fig. 3b). In this case as well, immobilization of the isoprenoid chain is expected to occur leading to a substantially reduced Q value for the $-CH_2$ β proton of the isoprenoid chain and the hydroxyl group coupling constant is predicted to be substantially smaller than that observed for the $-CH_3$ group *. Therefore, similar to the 2-methyl-5-isopropylhydroquinone radical, the *para*-methyl group hyperfine coupling is greater than the other hyperfine couplings, which leads to the similar spectral shape observed for Signal II and the immobilized 2-methyl-5-isopropylhydroquinone cation radical. That the hyperfine splittings in Signal II are substantially greater than those in the 2-methyl-5-isopropylquinone cation radical indicates a greater contribution of the antisymmetric orbital in this case [10].

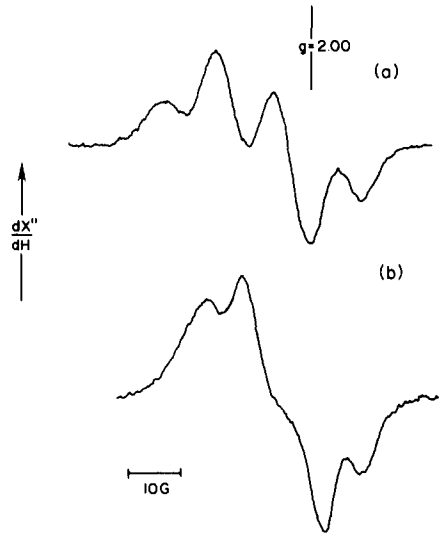

Fig. 4. Effect of membrane orientation on Signal II spectral shape. (a) Magnetic field direction perpendicular to membrane plane; temperature, 17 K; power, 20 mW; modulation, 1.6 G. (b) Magnetic field direction parallel to membrane plane. Temperature, 17 K; power, 20 mW; modulation, 1.6 G.

Hyperfine coupling to a freely rotating $-CH_3$ group would be expected to lead to a four line spectrum of intensity ratio 1:3:3:1 [18]. However, this is only true for liquid solution spectra or for single-crystal spectra, and not for powder spectra of the type in Figs. 1 and 2 where all possible orientations contribute to the spectral shape observed. As a result, the anisotropy in hyperfine interaction at the 2-CH_3 position and the smaller contributions at the other ring positions are likely to obscure the predicted 1:3:3:1 pattern in powder spectra. Because the in vivo radical is membrane-bound [10], however, orienting the membrane also orients the plastoquinone cation radical in vivo, and eliminates the distribution of orientations which complicates the powder spectrum observed in Fig. 1. For example, Fig. 4 illustrates that for membrane-plane orientation parallel and perpendicular to the applied magnetic field the spectral shape of Signal II has simplified considerably and consists in both cases of four broad lines. In Fig. 5, computer-simulated spectra

* An illustration of the lower Q_{C-OH} value compared with the Q_{C-CH_3} value for similar spin densities on the β carbon atom can be obtained by comparing the proton coupling constant values of the $-CH_3$ groups in the durene cation radical (30 MHz) and the $-OH$ groups in the tetrahydroxybenzene cation radical (4.8 MHz). In both cases, the spin density at the β carbon atom was approx. 0.27 [17,22], thereby giving rise to $Q_{C-CH_3}/Q_{C-OH} \approx 6$.

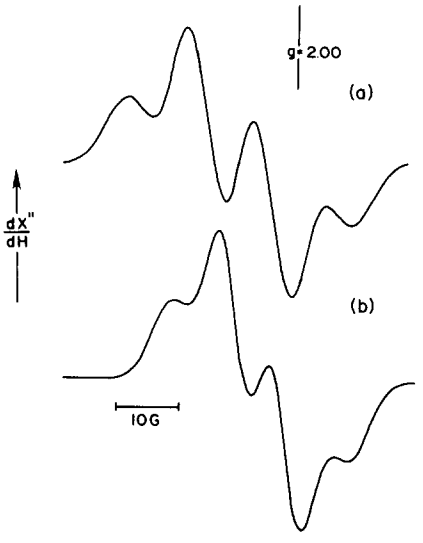

Fig. 5. Computer-simulated spectra for isotropic coupling by three equivalent protons. (a) Hyperfine coupling = 10 G; g = 2.0076; linewidth = 8.0 G. (b) Hyperfine coupling = 8 G, g = 2.0030, linewidth = 7.5 G.

for three equivalent protons are illustrated. The hyperfine coupling values * chosen for the simulations, 10 G ($\perp$) and 8 G ($\parallel$), were based on the best spectral fit achieved with the experimental spectra. These values also correspond closely to the expected anisotropy of a methyl group hyperfine coupling (10–20%) as is discussed in more detail below. The linewidth broadening apparent in the experimental spectra was simulated by choosing a linewidth value which gave the closest correspondence between calculated and experimental spectra. The close similarity in spectral shape between experimental and simulated spectra agrees with our prediction that the spectral shape of Signal II is principally determined by the 2-methyl group of the plastoquinone cation radical. The slight deviation from the exact 1 : 3 : 3 : 1 ratio for both oriented spectra can be attributed to underlying small hyperfine interactions from the other ring substituents and also to imperfections in the orientation procedure.

The ENDOR spectrum of Signal II in an unoriented (i.e., powder) sample in the spectral region 27–33 MHz is illustrated in Fig. 6b. Two bands are observed, a relatively sharp band at 28.1 MHz and a broad band at 30.3 $\pm$ 0.2 MHz. Recently, we have shown that it is possible to obtain the principal values of the hyperfine tensor for α and β proton-electron interactions from the turning points in the powder ENDOR spectrum [19,20]. For example, the ENDOR band observed for the 2-methyl group of the 2-methyl-5-isopropylhydroquinone cation radical is illustrated in Fig. 6a. The shape of the band is remarkably similar to that of Signal II and consists of a sharp band at 20.0 MHz with a broad band at 21.2 $\pm$ 0.1 MHz. Axial symmetry is expected for a rotating methyl group [21] so, therefore, we can assign the band at 20.0 MHz ($\frac{1}{2}A$ = 5.3 MHz, ν_n = 14.7 MHz) to $A_\perp$, whereas the broad band at 21.2 MHz ($\frac{1}{2}A$ = 6.5 MHz) corresponds to $A_\parallel$. These values give the trace of the hyperfine tensor $(2A_\perp + A_\parallel)/3$ at 11.4 MHz, which is similar to the isotropic value of 11.0 MHz obtained from the room temperature EPR spectrum (unpublished data).

From Fig. 5a, the $A_\perp$ and $A_\parallel$ components of Signal II are (ν_n = 14.5 MHz) 27.2 and 31.4 MHz, respectively. This corresponds to an isotropic value of 28.6 MHz which is similar to the value observed for both the durene and pentamethylbenzene cation radicals (30.0 and 28.2 MHz), respectively [18,22]. For both of these catio radicals, the spin-density distribution is determined solely by the

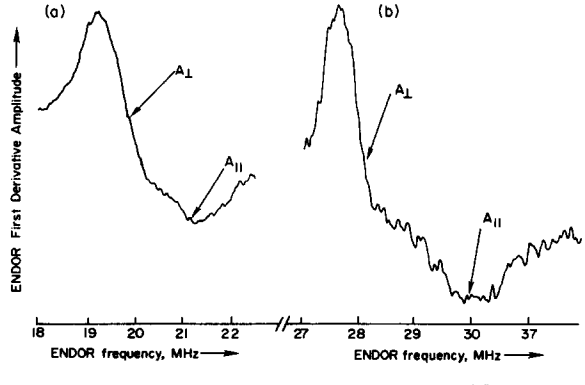

Fig. 6. Portion of the high frequency proton ENDOR spectrum for (a) the 2-methyl-5-isopropylhydroquinone cation radical and (b) Signal II. (a) Temperature, 123 K; microwave power, 6.3 mW; rf power at 10 MHz, 150 W; fm deviation 150 kHz.

* Due to the predominance of one orientation (see text), isotropic g and A values can be used to simulate the spectral shape. The g values used were those obtained from the corresponding experimental spectra.

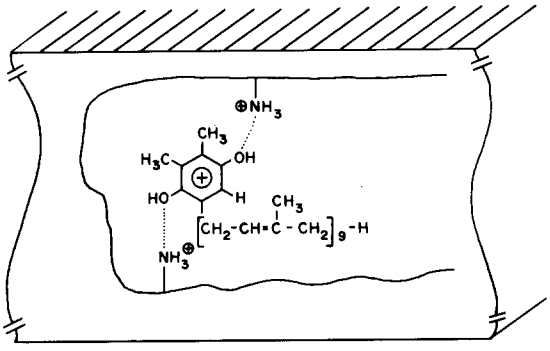

Fig. 7. Proposed orientation of the plastoquinone cation radical (Signal II) in the membrane, illustrating the quinone ring plane orthogonal to the membrane plane. The –OH groups of the plastoquinone cation radical are shown to be hydrogen bonded to protonated amino groups on the surface of the binding site as discussed in Ref. 10 to explain the decreased electron donating ability of the –OH groups which allows stabilization of the antisymmetric orbital of the semiquinone ring.

antisymmetric orbital [10,22]. The similar $-CH_3$ coupling value observed for Signal II suggests that the antisymmetric orbital is the sole contributor to the spin-density distribution of this radical species as well. This contrasts with the situation for the 2-methyl-5-isopropylhydroquinone cation radical where the antisymmetric orbital makes only a 26% contribution to the spin distribution [10]. This results in the lower 2-CH_3 coupling value for this radical observed in its ENDOR spectrum and gives rise to the narrower spectral extent observed for this radical in comparison with Signal II (Figs. 1 and 2).

The well-defined spectral characteristics in Fig. 4 indicate that in the oriented spectra the magnetic field lies close to a principal axis of the methyl group hyperfine interaction tensor. For a rotating methyl group, the unique axis ($A_{||}$) is expected to be along the C–CH_3 bond direction and corresponds to the largest value of the electron dipole-proton dipole interaction [21]. The well-resolved spectral features of the spectrum in Fig. 4a plus the large hyperfine coupling value observed (approx. 28 MHz) for orientation of the membrane plane perpendicular to the applied magnetic field indicates that the magnetic field is closely aligned with the C–CH_3 bond direction. The decreased coupling value, compared with the $A_{||}$ component obtained from the ENDOR data (31.4 MHz) is probably due to the fact that a Gaussian distribution of orientations is likely to exist in the oriented sample [23] with some of the C–CH_3 axes deviating slightly from the magnetic field axis. The above data indicate, however, that the plastoquinone cation radical is positioned in the membrane such that the C–CH_3 bond, and hence the aromatic ring plane lie closely perpendicular to the membrane plane. This orientation is schematically outlined in Fig. 7.

In conclusion, therefore, the orientation properties of Signal II provide further support for its assignment as a plastoquinone cation radical in which the spin-density distribution approximates to the antisymmetric benzene molecular orbital. The ENDOR data obtained are also in accord with this assignment and allow us to estimate the principal values of the 2-methyl group hyperfine interaction tensor. Finally, the orientation data also indicate that the plastoquinone cation radical is oriented on the membrane such that the C–CH_3 bond direction and the aromatic ring plane are closely perpendicular to the membrane plane. Previous studies [11] have indicated that the various kinetic components of Signal II have similar orientation properties. Hence, the membrane orientation given above for the slow component can also be applied to the faster components.

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