

An EPR study of Signal II in oriented photosystem II membranes

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Signal II of plant photosynthesis, which is thought to be due to a plastosemiquinone cation radical, has been studied by EPR at 9 and 35 GHz in non-oriented and partly oriented PS II particles. The spectra measured of the oriented particles at 35 GHz show that the molecular Z-axis, which is the axis perpendicular to the plane of the radical, makes an angle of 60° with the membrane normal. All spectra could be computer-simulated with one set of parameters. This set is essentially the same as that given earlier on the basis of EPR spectroscopy on non-oriented membranes [(1985) *Biochim. Biophys. Acta* 809, 421–428], except that the bond bending of the hydroxyl group on ring position 1 is found to be 60°, resulting in a somewhat smaller isotropic hyperfine splitting of the hydroxyl proton.

Signal II EPR Orientation Hyperfine coupling

1. INTRODUCTION

The structure of the secondary donor of PS II, labeled Z, has been under debate for many years. By heptane extraction Kohl and Wood [1] showed that a plastoquinone molecule was likely to be the precursor of an EPR signal, called Signal II [2], that subsequently was assigned to Z^+ [3].

Recently, O'Malley and Babcock [4] found EPR support for the idea that Z is a semiquinone, the cationic form of which gives rise to Signal II. From a comparison of the shape of Signal II with EPR signals of in vitro semiquinone cations, they concluded that the spin density due to the unpaired electron of Z^+ is mainly in an antisymmetrical orbital.

Dekker et al. [5,6] and Diner and De Vitry [7] found by optical absorbance difference spectroscopy that the optical absorbance difference spectrum of Z^+ -Z is in good agreement with the absorbance difference spectrum of a cationic durosemiquinone minus durosemiquinol.

In [8] we have shown by EPR spectroscopy at 9 and 35 GHz of Z^+ in deuterated and protonated

PS II particles and computer simulation of the resulting EPR signals that a consistent set of spectroscopic parameters could be found that accounted for all the available spectra. Our EPR parameters, which also accounted quite well for the EPR lineshape of Signal II at 9 GHz in oriented PS II particles [4,9], agreed well with the assignment of Z^+ to a plastosemiquinone cation, which has the unpaired spin density in the antisymmetrical π -orbital because of bond bending of the hydroxyl proton on ring position 1.

A severe test of our spectral assignments in [8] is Q-band (35 GHz) EPR spectroscopy on oriented PS II particles. Because of the 4-fold higher g resolution compared to EPR at 9 GHz, the hyperfine anisotropies are expected to show up more clearly, and accurate determination of the orientation of the g tensor (and therefore of the molecule) in the membrane should then be feasible.

This paper reports on such experiments. We find that our earlier interpretation was basically correct, the only important change in our refined data compared to that of [8] being a more pronounced bond bending of the hydroxyl group on ring posi-

tion 1. In addition we find that the molecular Z-axis (which is perpendicular to the quinone plane) makes an angle of 60° with the membrane normal.

2. MATERIALS AND METHODS

Oxygen-evolving PS II particles were prepared according to Berthold et al. [10]. The final chlorophyll concentration was 12 mg/ml. This suspension was painted on mylar strips, which were cut to fit exactly in a 2 mm inner diameter quartz EPR tube. The painted strips were dried at 4°C for 48 h in a closed dark box containing argon gas and a saturated ZnSO_4 solution which kept the relative humidity at 90%. A stack of 5 dried, painted strips was carefully transferred under ambient light into the EPR tube. The tubes were frozen and stored in liquid nitrogen until use.

The orientation measurements were carried out on a home-built superheterodyne Q-band (35 GHz) EPR spectrometer which was equipped with a metal immersion type liquid He cryostat. The sample was cooled down to 1.8 K by pumping on the liquid He. Some X-band (9 GHz) EPR spectra were recorded with a Varian E9 spectrometer equipped with an Oxford 900 He flow cryostat or a home-built nitrogen flow cryostat using the same sample as for the Q-band measurements.

For the Q-band measurements the angle between the magnetic field and the plane of the mylar sheets was varied by rotating the magnet in steps of 10° . For symmetry reasons we have chosen a cylindrical TE_{011} cavity in which the mylar sheets were centered along the axis. For the X-band measurements two orientations, the planes of the mylar sheets parallel and perpendicular to the magnetic field, were set by rotating the sample over 90° .

The simulations were carried out using the program of [8], which was extended to include the nuclear Zeeman interaction [11]. Calculations were done on a VAX/780 computer.

3. RESULTS AND DISCUSSION

Fig.1 presents Q-band EPR data of Signal II in PS II particles that are oriented by drying on mylar sheets. The changes in g value and hyperfine structure in these spectra are much more pronounced than in the corresponding X-band spectra (fig.2,

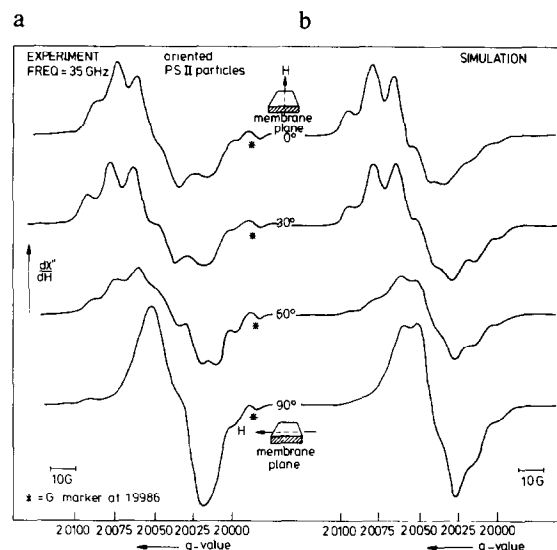


Fig.1. Q-band (35.0 GHz) EPR spectra of Signal II in partially oriented PS II particles. Experimental conditions as in fig.5B. The peak at the position of the asterisk indicates the P-doped Si g marker.

which agree with previously published results [4]).

The g value of the zero crossing of the oriented Q-band spectra is plotted in fig.3 vs the angle between the magnetic field and the normal to the surface of the mylar sheet (which is assumed to coincide with the 'averaged' normal to the membrane surface). The principal values of the anisotropic g tensor of Signal II are $g_x = 2.0074$, $g_y = 2.0044$ and $g_z = 2.0023$ [8]; its principal axes coincide with the molecular axes as defined in fig.4. If one of the molecular axes is parallel to the membrane normal, then the EPR signal measured with the magnetic field along this direction is expected to be almost symmetrical. Since the Q-band EPR spectrum in fig.1 with its magnetic field along the membrane normal ($\theta = 0^\circ$) is not symmetrical and the g value of the zero crossing is not one of the principal values we conclude that the radical is not oriented with one of its molecular axes along the membrane normal.

By course grid variation of the orientation angles of the radical with respect to the membrane normal we found a best fit to the orientation-dependent spectra of Signal II (of which fig.1 gives a few examples) for an angle of 60° between the molecular Z-axis and the membrane normal (fig.4). The g value of the zero crossing of the

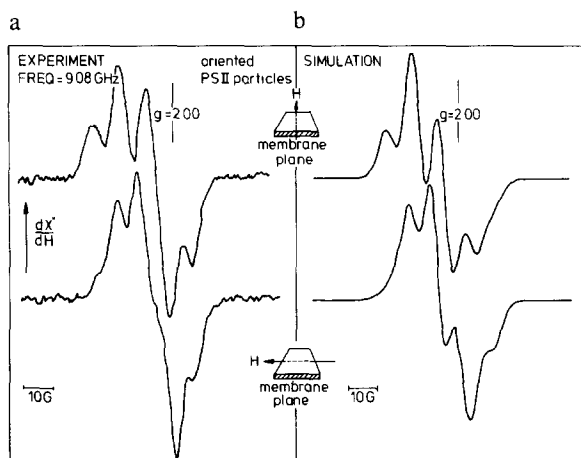


Fig.2. X-band (9.08 GHz) EPR spectra of Signal II in partially oriented PS II particles. Microwave power, 200 μ W; modulation amplitude, 2 G; modulation frequency, 100 kHz; temperature, -100°C . Computer simulations were carried out using the parameters listed in table 1.

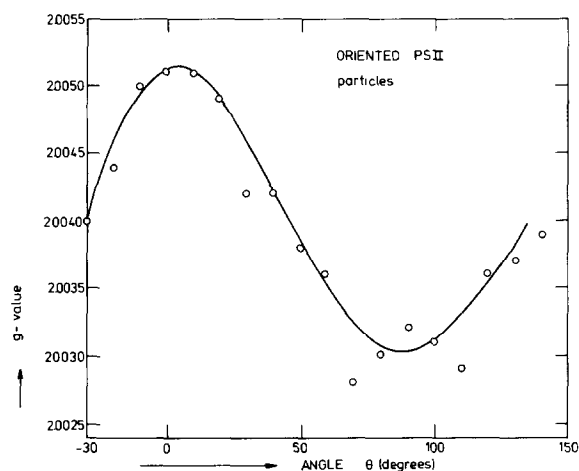


Fig.3. Plot of the g value of the zero crossing of the oriented Q-band EPR signals of Signal II against the angle between the magnetic field and the membrane normal.

simulated spectra followed the angle dependence depicted in fig.3.

The orientation of the plastosemiquinone cation in the membrane is an essential parameter for the simulations of the oriented spectra of figs 1a and 2a. With the above-determined orientation of PQH_2^+ we have simulated the oriented spectra using as starting values for the hyperfine tensors the

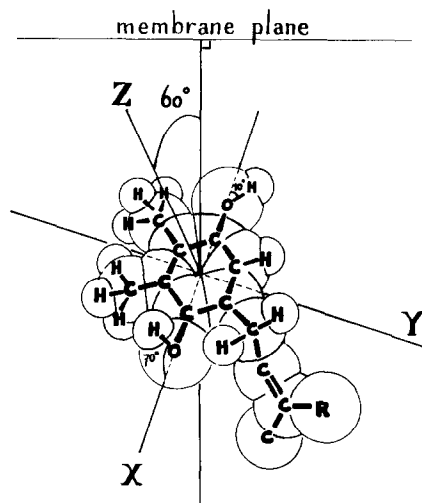


Fig.4. Proposed orientation of the Z species with respect to the membrane.

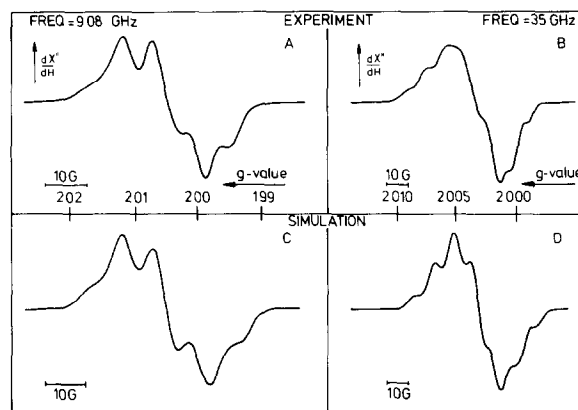


Fig.5. EPR powder spectra of Signal II. (A) Microwave frequency, 9.08 GHz; microwave power, 2 μ W; modulation amplitude, 2 G; modulation frequency, 100 kHz; temperature, 4.2 K. (B) Microwave frequency, 35.0 GHz; microwave power, 5 nW; modulation frequency, 73 Hz; temperature, 1.8 K. (C,D) Computer simulations with parameters listed in table 1.

parameters given in [8]. The refined values, corresponding to the simulations of figs 1b and 2b, are tabulated in table 1. It is seen that the new values of the principal components and orientations of the hyperfine tensors deviate little from those given earlier, the only exception being the angle that the hydroxyl group on C1 makes with the X-axis in the molecular plane, whose new value

Table 1
Principal components of the proton hyperfine tensors of PQH_2^+ ,
and its principal g values

Signal II	X	Y	Z	iso	θ	ϕ	ψ
A(C5-H)	9.7	8.2	6.0	8.0	45	10	45
A(C5-H)	9.7	8.2	6.0	8.0	45	50	45
A(C2-CH ₃)	1.7	1.5	1.5	1.6	0	60	0
A(C1-OH)	11.2	2.5	2.0	5.2	0	10	0
A(C4-OH)	10.5	1.8	1.3	4.5	-70	0	70
g	2.0074	2.0044	2.0023	2.0048			

θ , ϕ and ψ are the Euler angles ($^\circ$) relating the principal axes of the hf tensors to the coordinate frame of the g tensor (see [8])

is close to 10° (was 35°). We note that with the interpretation of the X-band spectra given in [12] it is impossible to fit the spectral shape of Signal II of figs 1a and 2a.

With the values of the various parameters listed in table 1 we have simulated the X- and Q-band spectra of Signal II in randomly oriented material (fig.5). It is seen that although the overall fit is somewhat better than that with our previous parameters (cf. fig.5 with fig.5 of [8]), the difference is not very large. As the older set of parameters did not yield acceptable fits of the oriented Q-band spectra, this points to the importance of performing X- and especially Q-band spectroscopy on oriented membranes. Note that the Q-band EPR spectrum of fig.5A was recorded at 1.8 K. There are no significant changes in lineshape between this spectrum and that obtained previously at 90 K [8]. Since methyl groups are supposed to stop rotating at temperatures well above 4.2 K [13], this observation is a compelling argument against the notion advanced in [12] that a rotating methyl group is responsible for the dominant hyperfine structure of Signal II.

The angle between the C1-OH proton and the X-axis, 10° (table 1), is somewhat less than that found previously from a fit of randomly oriented spectra of Signal II (35° [8]), and deviates considerably from the value found from a space filling model (70° [8]). Our new value of the angle is similar to that inferred from ENDOR studies on the OH group of the radical anion of ubisemiquinone in bacterial reaction centers [14], sup-

porting our earlier suggestion that the bond bending is caused by hydrogen bonding to the protein matrix. It is likely this bond bending that stabilizes the symmetrical orbital of PQH_2^+ , leaving the antisymmetrical orbital for the unpaired electron.

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