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Very high frequency (135 GHz) EPR of the oxidized primary donor of the photosynthetic bacteria *Rb. sphaeroides* R-26 and *Rps. viridis* and of $Y_D^{\bullet}$ (signal II) of plant photosystem II

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Abstract - 2 mm EPR spectroscopy reveals significant differences between the *g*-tensors of the oxidized primary donor in reaction centers of the photosynthetic bacteria *Rb. sphaeroides* R-26 and *Rps. viridis*: the principal values are g_{xx} = 2.00159, g_{yy} = 2.00224 and g_{zz} = 2.00402, and g_{xx} = 2.00180, g_{yy} = 2.00235 and g_{zz} = 2.00380, respectively. Similarly, the *g*-tensors of the $Y_D^{\bullet}$ tyrosyl radical in the D_2 subunit of the reaction center of plant photosystem II and of oxidized tyrosine in vitro are found to be quite different, with principal values of g_{xx} = 2.00212, g_{yy} = 2.00426 and g_{zz} = 2.00752, and g_{xx} = 2.00181, g_{yy} = 2.00381 and g_{zz} = 2.00613, respectively. The observed differences probably reflect differences in the molecular structure of the radicals investigated.

INTRODUCTION

EPR spectroscopy has been a powerfool tool for characterizing the primary reactants in photosynthesis. Linewidth and *g*-factor report on the structure of the oxidized and reduced co-factors in photosynthetic reaction centers, and have been instrumental in the assignment of the primary donor in bacterial photosynthesis as a bacteriochlorophyll (BChl) dimer (see for a recent review[1]). More detailed information on the hyperfine couplings, and thus on the electronic structure, of the co-factors has become available from electron-nuclear double resonance (ENDOR) spectroscopy by virtue of its much higher resolving power (reviewed in[2]). In principle, also the *g*-tensor would yield detailed information on the structure of the oxidized or reduced co-factors, but this information is much more difficult to extract because of the generally small anisotropies of the *g*-tensor of organic radicals. Most work has been carried out with randomly-oriented samples at X- or Q-band frequencies (9 and 35 GHz, respectively). Even at the latter frequency the principal values of the *g*-tensor are not well resolved. Perdeuteration helps by reducing the hyperfine couplings, but only for the secondary, quinone, acceptor could accurate principal *g*-values in this way be obtained[1]. Recently, the advent of crystals of bacterial reaction centers has allowed a preliminary determination of the principal *g*-values of the oxidized donor of *Rb. spharoides* R-26 using X-band EPR[3]. This work was extended using 3 mm EPR (90 GHz)[4].

In this communication we report on the amxlication of 2 mm (135 GHz) EPR spectroscopy on a number of photosynthetic preparations with the aim to determine the principal values of the *g*-tensor of some co-factors: the primary donor of two purple bacteria and the tyrosyl donor $Y_D^{\bullet}$ located on the D_2 subunit of the reaction center of plant photosystem II. The much enhanced resolution of 2 mm EPR allows such a determination even for protonated, randomly-oriented preparations. It is shown that the two purple bacteria differ considerably in the *g*-anisotropy of their primary donors, while the *g*-anisotropy of the $Y_D^{\bullet}$ radical is appreciately different from that of oxidized tyrosyl in vitro. Since these differences most probably reflect differences in the detailed molecular structure of the co-factors, it is hoped that our data will be an incentive for correlating theoretically the observed *g*-anisotropies and the co-factor structure with the aid of the recently resolved crystal structure of the reaction centers (the purple bacteria[5,6,7]) or the models based on these resolved structures (plant photosystem II[8,9]).

MATERIALS AND METHODS

Reaction centers of *Rb. sphaeroides* R-26 and *Rps. viridis* were prepared as described[10,11]. Photosystem II-containing subchloroplast (so-called BBY) particles were prepared from spinach as described[12]. The primary donor of the bacterial reactions centers was oxized by freezing under illumination. The D_2 tyrosyl donor of the

BBY particles was oxidized by brief illumination and subsequent freezing. The oxidized tyrosyl radical in vitro was generated by UV irradiation of crystals of L-tyrosine (obtained from Fluka, puriss. grade). All biological samples contained 60% v/v glycerol.

2 mm EPR experiments were carried out on an EPR 5-02 2 mm spectrometer produced in the Donetzk Physico-Technical Institute of the Ukrainian Academy of Sciences, equipped with a superconducting solenoid; the cavity (H_{011}) was operated at 135 GHz. Power incident on the cavity was 1-2 mW. The quartz capillaries containing the sample had an inner diameter of 0.3-0.5 mm and a length of 7-9 mm. The magnetic field was calibrated relative to a reference sample (MgO powder doped with Mn^{2+} ions[13]). The accuracy of the determination of g-values was $7x10^{-5}$. The temperature at which the spectra were recorded was 150-210 K. Spectral simulations were carried out by convoluting the calculated stick spectra with a Gaussian spin packet, whose linewidth (representing in part the hyperfine couplings) is largely determined by g-strain.

RESULTS AND DISCUSSION

Bacterial reaction centers

Fig. 1a shows the 2 mm spectrum of the oxidized primary donor, $P865^+$, of reaction centers of *Rps. sphaeroides* R-26. The spectrum is characteristic of a rhombic g-tensor; the simulation shown in Fig. 1b yields the principal values g_{xx} = 2.00159, g_{yy} = 2.00224 and g_{zz} = 2.00402, giving g_{iso} = 2.00262 (all values ± $7x10^{-5}$). The baseline crossing is at g = 2.00236 in Fig.1b (135 GHz); at X-band the baseline crossing of the simulated spectrum (a practically symmetric Gaussian) is at g = 2.00262 (not shown), in agreement with the accepted "g-value" of $P865^+$ (2.0026)[1]. Our values for the principal axes of the g-tensor of $P865^+$ deviate considerably from those given in[3,4], viz. $g_\perp$ = 2.00285(10), $g_\parallel$ = 2.00220(10)[3] and g_{xx} = 2.0021(1), g_{yy} = 2.0025(1) and g_{zz} = 2.0033(1)[4].

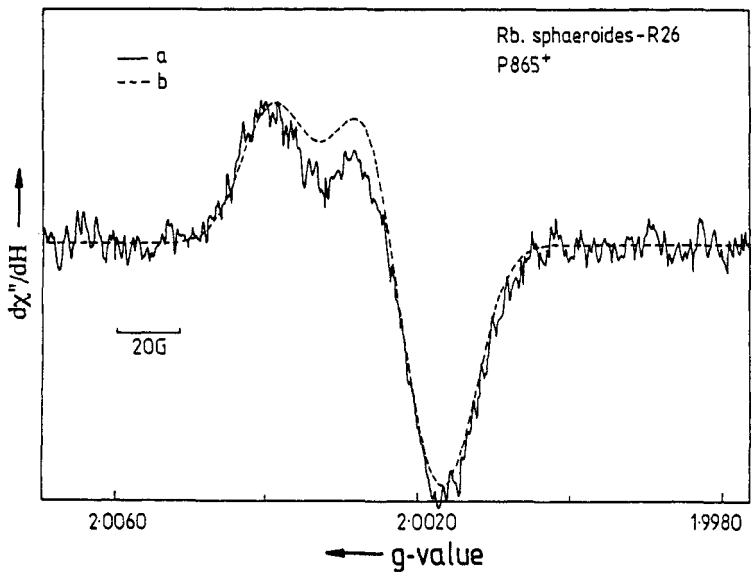

Fig. 1. a. (——) 135 GHz (2 mm) EPR spectrum of the oxidized primary donor, $P865^+$, of RC of *Rb. sphaeroides* R-26. Modulation amplitude, 4 G; temperature, 210 K. b.(- - -) Simulation; spin packet linewidth, 18 G; g-values, see text.

Simulations using these values give an only slightly asymmetric line even at 2 mm, which differs pronouncedly from that depicted in Fig. 1a where the low-field extremum is clearly split. The reason for this discrepancy is not known; we note that a simulation for 3 mm using our g-values yields an unstructured line, which is more difficult to interprete than the 2 mm spectrum (see also below).

Fig. 2a shows the 2 mm spectrum of the primary donor of reaction centers of *Rps. viridis*, $P960^+$. The g-anisotropy is smaller than for $P865^+$; the spectrum is well simulated with a rhombic g-tensor with principal values g_{xx} = 2.00180, g_{yy} = 2.00235 and g_{zz} = 2.00380 (Fig. 2b), giving g_{iso} = 2.00265. The baseline crossing in Fig. 2b is at g = 2.00247; at X-band it is at g = 2.00259 (not shown), in agreement with the accepted "g-value"[11]. The simulation of Fig. 2b is somewhat more ambiguous than that of Fig. 1b because of the absence of a split of the low-field peak. It is not possible, however, to obtain an acceptable fit with an axial g-tensor; each of the principal rhombic values that do give an acceptable fit fall within a range of ± $1x10^{-4}$, with the values quoted yielding the subjective "best" fit.

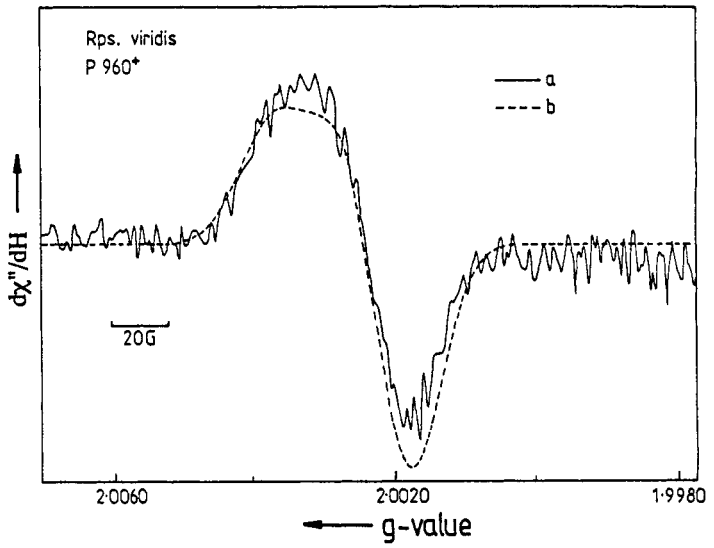

Fig. 2. a. (——) 135 GHz (2 mm) EPR spectrum of the oxidized primary donor, P960$^+$, of RC of *Rps. viridis*. Modulation amplitude, 4 G; temperature, 150 K. b.(- - -) Simulation; spin packet linewidth, 22 G; *g*-values, see text.

The anisotropy of the *g*-tensor of *Rps. viridis*, defined as $[g_{zz} - (g_{xx} + g_{yy})/2] = 3(g_{zz} - g_{iso})/2$, is considerably less than that of *Rb. sphaeroides* R-26, viz. 0.00173 vs 0.00210. In contrast, the rhombicity, defined as $2(g_{yy} - g_{xx})/3(g_{zz} - g_{iso})$, is almost identical, viz. 0.32 vs 0.31.

The smaller *g*-anisotropy for *Rps. viridis* compared to that of *Rb. sphaeroides* R-26 is remarkable, as ENDOR experiments suggest that the BChl dimer of P960 is less symmetric than that of P865 in *Rb. sphaeroides*[14,15]. Possibly, the *g*-anisotropy of monomeric BChl$^+$ in vitro is appreciably smaller than that of the BChl dimer of the primary donors. The more localized spin density for P960$^+$ would than tend to reduce the *g*-anisotropy observed for the delocalized unpaired electron of P865$^+$. An indication for this is the small *g*-anisotropy of BChl *a* in vitro (reported principal *g*-values are $g_{xx} = 2.0022(2)$, $g_{yy} = 2.0026(2)$ and $g_{zz} = 2.0033(2)$[16]) and the virtual absence of *g*-anisotropy for Chl *a*$^+$ in vitro, even at 135 GHz (V.I. Gulin and S.A. Dikanov, unpublished result). Experiments to measure at 2 mm the *g*-anisotropy of oxidized monomeric BChl *a* and *b* in vitro are in progress.

$Y_D^\bullet$ of plant photosystem II

Fig. 3a shows the 2 mm EPR spectrum of the oxidized tyrosyl donor of the D_2 subunit of the reaction center of photosystem II, $Y_D^\bullet$. The spectrum has the shape of the undifferentiated, χ'' vs field, spectrum due to passage effects, which are persistently found at high microwave frequencies and low measuring temperatures, due to the long T_1 of the tyrosyl radical (M. Brok, unpublished results). The spectrum is typical for a rhombic *g*-tensor, the principal values of which are $g_{xx} = 2.00212$, $g_{yy} = 2.00426$ and $g_{zz} = 2.00752$ (Fig. 3b).

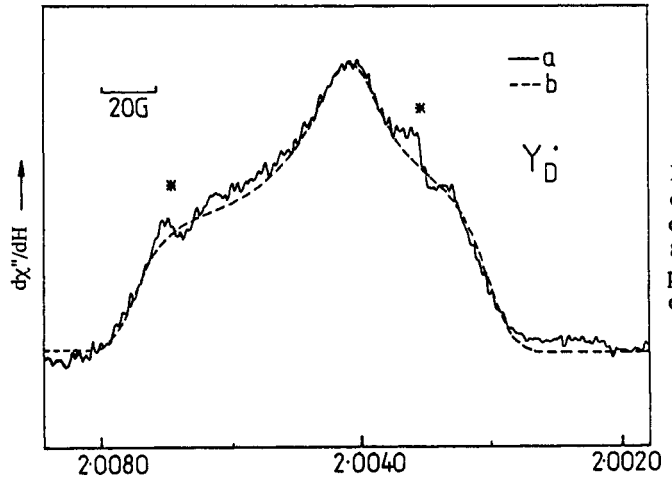

Fig. 3. a. (——) 135 GHz (2 mm) EPR spectrum of the oxidized tyrosyl donor, $Y_D^\bullet$, of the D_2 subunit of RC of photosystem II. Modulation amplitude, 8 G; temperature, 150 K. b.(- - -) Simulation; spin packet linewidth, 16 G; *g*-values, see text. *: lines of an indigenous Mn-hexaquo complex.

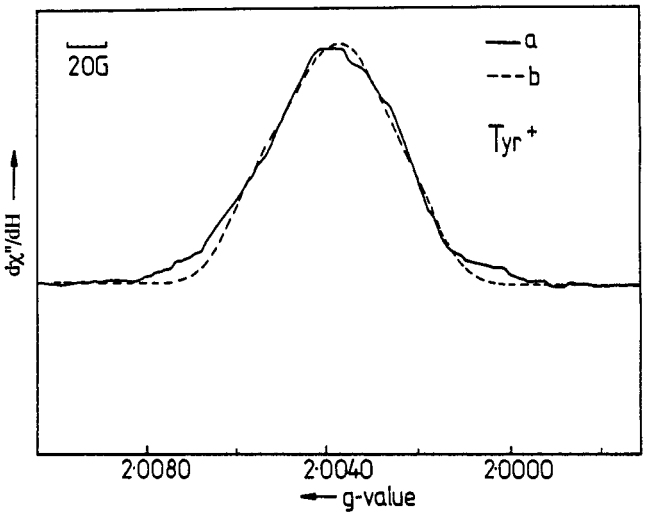

Fig. 4. a. (——) 135 GHz (2 mm) EPR spectrum of microcrystals of tyrosine oxidized with UV light. Modulation amplitude, 8 G; temperature, 150 K. b.(- - -) Simulation; spin packet linewidth, 30 G; *g*-values, see text.

The *g*-anisotropy of $Y_D^{\bullet}$ and the spin packet linewidth are considerably more pronounced than that of oxidized tyrosine in vitro (Fig. 4a; the spectrum is somewhat distorted, especially in the wings, presumably due to different environments of the radicals in the microcrystals of the powdered sample), whose principal *g*-values are approx. g_{xx} = 2.00181, g_{yy} = 2.00381 and g_{zz} = 2.00613 (Fig. 4b). The isotropic *g*-values are 2.00463 and 2.00392 for $Y_D^{\bullet}$ and Tyr^+ in vitro, respectively. Apparently, both the *g*-anisotropy and the average *g*-value are influenced by the protein matrix. This is in line with the rather different hyperfine couplings measured for the in vivo and in vitro tyrosine radical[17,18], which difference has been attributed to different values for the dihedral angle of the methylene β-protons of the tyrosine[17].

Summarizing these first results of the application of 2 mm spectroscopy to photosynthetic systems, we conclude that the high resolving power of this technique makes it possible to extract accurate principal *g*-values from spectra of protonated, randomly-oriented samples. There are clear differences between the *g*-tensors of the primary donors of the two bacterial reaction centers investigated, and between those of oxidized tyrosyl in plant photosystem II and in vitro. More work needs to be done to correlate these differences to structural features.

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