Interpretation of the nitrogen spin densities in the primary donor cation of photosynthetic reaction centers

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Received 7 January 1986

Electron spin echo modulation investigation of the primary donor cation radical (P865$^{\cdot+}$) of photosynthetic reaction centers yields a different set of anisotropic ^{15}N hyperfine coupling constants from the values previously reported by ENDOR measurements [(1985) Proc. Natl. Acad. Sci. USA 81, 7792–7796]. The spin densities on the nitrogens, as reflected by our anisotropic coupling constants, support the special pair model for the primary donor cation, whereas spin densities based on anisotropic ^{15}N hyperfine coupling constants of ENDOR are incompatible with either a monomer or a dimer model.

Photosynthesis *Primary process* (Rhodospirillum rubrum) *Reaction center* *EPR* *Hyperfine coupling constant*

1. INTRODUCTION

ENDOR studies of the bacteriochlorophyll *a* cation (BChl $a^{\cdot+}$) have provided a detailed picture of the spin density distribution in the radical [2,3], while studies of P865$^{\cdot+}$ have established the model for the primary donor cation, namely the special pair model, in which the unpaired electron is delocalized symmetrically over 2 BChl *a* molecules of a dimer [3,4]. Although the high-resolution X-ray structure from *Rhodopseudomonas viridis* [5] clearly shows the existence of the special pair, ESR of the *Rps. viridis* crystal suggests that the triplet state of the donor resides asymmetrically on the special pair [6]. The question therefore arises as to when the special pair behaves like a monomer and when it behaves like a dimer.

The 4 nitrogens near the center of the chlorophyll macrocycle probe a part of the electron distribution which some consider crucial to the development of the special pair model [7]. In ^{15}N ENDOR studies of BChl $a^{\cdot+}$ and of P865$^{\cdot+}$ [1], the 4 isotropic ^{15}N hyperfine coupling constants (hfcs) for P865$^{\cdot+}$ were found reduced relative to BChl $a^{\cdot+}$ by an average factor of 2 supportive of the special pair model. However, reductions of ~5 were reported for the anisotropic coupling constants, an observation inconsistent with the special pair model, especially since the anisotropic, rather than the isotropic, ^{15}N coupling constants are the more direct parameters for probing the monomer vs dimer nature of the primary donor. We have employed the electron spin echo (ESE) spectroscopy [8,9] for measuring hfcs. Our analysis of ESE modulation leads to a different set of anisotropic ^{15}N hfcs for P865$^{\cdot+}$ which have an average reduction factor closer to 2 as required by the special pair model. A detailed analysis of ESE modulation spectra will be published elsewhere [10].

2. MATERIALS AND METHODS

Because of the ^{14}N quadrupole interactions, ^{15}N substituted (>98%) *Rhodospirillum rubrum* was used exclusively in this experiment. P865$^{\cdot+}$ (EPR linewidth ~9.1 G) is generated at ~10 K by continuous irradiation of chromatophores of *R.*

Published by Elsevier Science Publishers B.V. (Biomedical Division)

rubrum with a 300 W xenon arc lamp passing through water and red filters and probed by our X-band ESE spectrometer. For the stimulated echo modulation experiment, the electron spins are excited by 3 short, intense microwave pulses separated by τ and T, respectively, and the echo amplitude at time τ after the third microwave pulse is measured as a function of T.

3. RESULTS

Fig.1a shows several stimulated echo modulation curves taken at various τ. The hfcs of table 1 are obtained by a nonlinear least-squares analysis of the ratios of different modulation curves, thus removing the unknown echo decay function [11]. The modulation is assumed to arise from 8 ^{15}N nuclei (4 pairs of parallel, axially symmetric hf tensors). The 4 isotropic coupling constants are taken from the liquid solution ENDOR measurements [1] while the 4 anisotropic coupling constants [$A_{\parallel}$ components of the traceless, axially symmetric tensors. Throughout this manuscript, $A_{\parallel}$ and $A_{\perp}$ (where $A_{\perp} = -A_{\parallel}/2$) denote components of the traceless dipolar hyperfine tensor A. In [1] the corresponding notations are $A_{\parallel}'$ and $A_{\perp}'$.)] are varied until the best fit is achieved by the least-squares procedure (The procedure is analogous to extracting hfcs by fitting powder spectra in the frequency domain, except that the problem of data truncation inherent in all ESE experiments is considerably less severe in the time domain. For more detail, refer to [10,11].). The average reduction factor for the 4 anisotropic coupling constants (relative to BChl $a^{\cdot+}$) is 2.3. Table 1 also lists the ENDOR results [1], as well as the results for BChl $a^{\cdot+}$ [11].

Fig.1b shows calculated echo modulation with the parameters obtained from the nonlinear least-squares analysis. Calculation with a non-parallel dimer shows little difference from 0 to 30°. Simulations with the ENDOR parameters (fig.1c) failed to match experimental data.

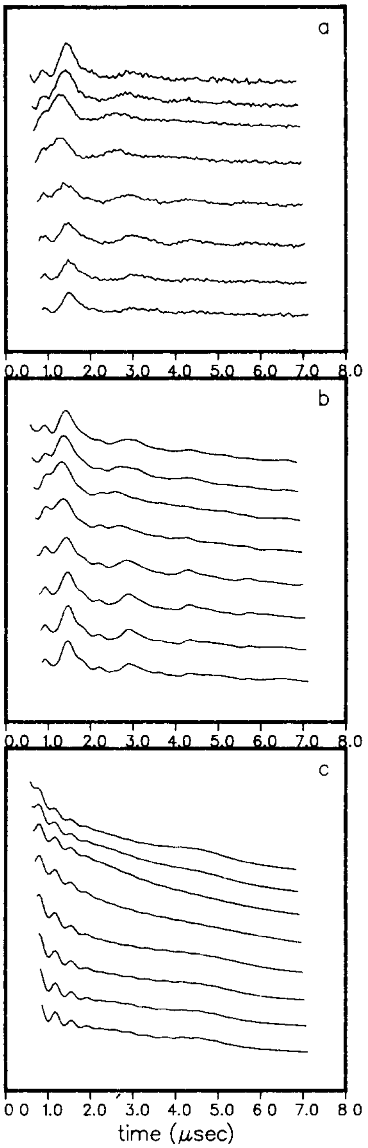

Fig.1. (a) Stimulated echo modulation from the primary donor cation of *R. rubrum*. Traces from top to bottom were taken with τ setting from 0.28 to 0.56 μs. (b,c) Calculated modulation using the above τ settings and the hyperfine coupling constants (table 1) from ESE and from ENDOR, respectively. Each trace is multiplied with an exponential decay constant of 4 μs. The time axis is $\tau + T$.

4. DISCUSSION

The electron-nuclear hyperfine interaction has two contributions, the isotropic hyperfine coupling (the Fermi contact interaction), and the anisotropic coupling (the magnetic dipole-dipole interaction). For an atom bearing unpaired spin density in a simple conjugated π radical, the isotropic hfc has contributions from the central p-orbital as well as from the adjacent carbon p-

Table 1

^{15}N hyperfine coupling constants for BChl $a^{\cdot +}$ and P865$^{\cdot +}$ as determined by ESE modulation and by ENDOR [1] analyses

	BChl $a^{\cdot +}$		P865$^{\cdot +}$		
	a_{iso} (MHz)	$A_{\parallel}$[a] (MHz)	a_{iso} (MHz)	$A_{\parallel}$ (MHz)	RF[b]
ESE[c]	3.16	2.43	1.05[d]	0.57	4.26
	3.41	2.61	1.61	1.34	1.95
	4.10	2.67	2.22	1.77	1.51
	4.45	2.91	2.60	1.96	1.48
ENDOR	3.15	2.3	1.05	–	–
	3.25	2.3	1.61	–	–
	4.05	2.8	2.22	0.67	4.18
	4.40	2.8	2.60	0.53	5.28

[a] $A_{\parallel}$ denotes the largest element of the traceless and axially symmetric dipolar tensor

[b] RF is defined as the ratio $A_{\parallel}$(BChl $a^{\cdot +}$)/$A_{\parallel}$(P865$^{\cdot +}$)

[c] All coupling constants are positive according to [1], although the ESE analysis only determines the absolute values

[d] Isotropic coupling constants in this column are taken from liquid solution ENDOR measurements [1]

orbitals, and in general does not provide directly the nitrogen spin density [12]. Consequently ^{15}N isotropic coupling constants are complicated, multi-atom probes of the monomer vs dimer nature of the primary donor. On the other hand, the magnitude of the anisotropic coupling constant is related primarily to the spin density in the central nitrogen p-orbital because of the r^{-3} distance dependence of the dipolar interaction. Contributions of the adjacent carbon orbitals can be calculated from the classical dipolar interaction formula by approximating each neighboring p-orbitals with two point dipoles at an effective distance above and below the molecular plane and one bond-length away [13]. With 10% spin density in each of the carbon p-orbital adjacent to the nitrogen in the pyrrol ring, this calculation gives the largest dipolar component about -0.2 MHz directed along the line bisecting the C-N-C angle. The component parallel to the nitrogen p-orbital is ~0.1 MHz. (10% spin density on the adjacent carbons represents the upper bound estimate.) Therefore, the measured $A_{\parallel}$ values, which range from ~0.6 to ~2 MHz, reflect mostly the spin density in the nitrogen p-orbital. Also, since a p-orbital has axial symmetry, the assumption of axially symmetric tensors in our analysis is justified.

No existing molecular model, monomer or dimer, for the primary donor of the reaction center can explain reductions of ~5 for the anisotropic coupling constants which would reflect a decrease of 5 in spin density. On the other hand, the average reduction factor of ~2 from our ESE modulation analysis is consistent with the special pair model. The fact that the individual reduction factors deviate from 2 implies that the cation wave function of the special pair is redistributed and the primary donor should be treated as a supermolecule.

ACKNOWLEDGEMENTS

We thank Ms U.H. Smith for providing ^{15}N labelled *R. rubrum* and Mr D.E. Budil for preparation of the chromatophores. This work was supported by the Office of Basic Energy Sciences, Department of Chemical Sciences, US Department of Energy, under Contract W-31-109-Eng-38.

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