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Anion Radicals of Bacteriochlorophyll a and Bacteriopheophytin a. Electron Spin Resonance and Electron Nuclear Double Resonance Studies

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Abstract: Chemical, electrochemical, and photochemical reductions of bacteriopheophytin a (BPh) and deuterated BPh yielded stable anion radicals which were characterized by electron spin resonance and electron-nuclear double resonance techniques. Radicals of the model bacteriochlorin compound, 5,10,15,20-tetraphenyl-2,3,12,13-tetrahydroporphyrin, selectively deuterated, were prepared to guide the interpretation of the BPh data and the experimental results compared with self-consistent field-molecular orbital calculations. ESR and ENDOR results for bacteriochlorophyll a (BChl) and deuterated BChl are also assigned with the aid of a model, zinc tetraphenyltetrahydroporphyrin, and molecular orbital calculations. These data thus help define the electronic profile of the BPh and BChl anions, the radical species presently postulated to result, within picoseconds, from the primary charge separation of bacterial photosynthesis.

Pulsed laser spectroscopy has revealed[2-5] two short-lived intermediates involved in the primary photochemistry of the photosynthetic bacterium, *Rhodopseudomonas spheroides*. The first appears[3-5] in less than 10 ps and is postulated to consist of a dimeric bacteriochlorophyll a cation radical[5,6] and of an anion radical[6] of bacteriopheophytin, a demetallated chlorophyll. This charge separation, caused by the photooxidation, within the reaction center, of a pair of bacteriochlorophylls (BChl) that absorbs at 870 nm (P_{870}) and the concomitant reduction of a nearby bacteriopheophytin (BPh), results[6] in an estimated 1 eV of free energy. For incident light of 870 nm or 1.4 eV, this represents a 70% energy efficiency, an attractive number for a primary biological step.[7] Under physiological conditions, the BPh anion reduces, within 200 ps,[3-5] the next electron carrier (X), a ubiquinone-iron complex,[8-11] whose anion then exists for milliseconds. The electron is eventually returned to the oxidized P_{870} via an established cyclic process which generates the intermediates required for carbon dioxide fixation. If, however, normal chemistry is blocked by poising the reaction center at potentials such that X is reduced, the light-induced charge separation is annihilated in nanoseconds[2] by recombination of the radicals to yield fluorescence[12] and a triplet:[13,14]

$$P_{870}{}^{+}\cdot + BPh^{-}\cdot \xrightarrow{6\text{ ns}} {}^{3}P_{870} + BPh$$

The latter exhibits anomalously polarized ESR spectra probably induced[5,6,15] by a recombination mechanism similar to that which causes chemically induced dynamic electron polarization.[16]

The putative BPh anion can be trapped[17-19] in reaction centers of *Chromatium minutissimum* and *vinosum* in the presence of $X^{-}\cdot$ and ferri- or ferrocytochrome c_{553}. The optical spectra observed are consistent with those expected[6] on reduction of BPh but also include shifts in the bands of the other bacteriochlorophylls (P_{800}) known[11] to be present in the reaction center. The BChl spectral changes may result from delocalization of the reducing electron unto BChl as well as BPh or may simply reflect electrochromic shifts due to the nearby $BPh^{-}\cdot$, $X^{-}\cdot$, and cytochrome.

Although a number of porphyrin, chlorin, and bacteriochlorin anion radicals have been prepared, little detailed information exists about their ESR characteristics (see ref 20-22 for reviews). Few compounds display hyperfine resolution and, of these, even fewer have yielded to hard core analysis of the number, amplitude, and splittings of the hyperfine structure. Among the bacteriochlorins, anions of BPh and BChl have been described.[6,23,24] We have previously assigned[23] the ESR spectrum of zinc tetraphenylbacteriochlorin (ZnTPBC) by selective deuterations. Feher et al.[25] reported a single ENDOR transition for $BChl^{-}\cdot$. Radicals of tetraphenylbacteriochlorin[26] (H_2TPBC), BChl b, and BPh b[27] (found in the bacterium *Rhodopseudomonas viridis*) have also been identified without hyperfine analysis.

We present here ESR and ENDOR data which, combined with model compound studies, isotopic substitution results, and self-consistent field-molecular orbital calculations, help characterize the electronic configuration of the BPh and BChl anions. The line widths, saturation behavior, and *g* values of the monomeric radicals resemble those ascribed[17,18] to the primary electron acceptor in vivo. Added to recent ESR and ENDOR observations[25,28,29] on oxidized bacteriochlorophylls, these results specify the ESR parameters and spin-density profile of the radical species presently thought to exist, within a nanosecond time domain, in the primary charge separation of bacterial photosynthesis.

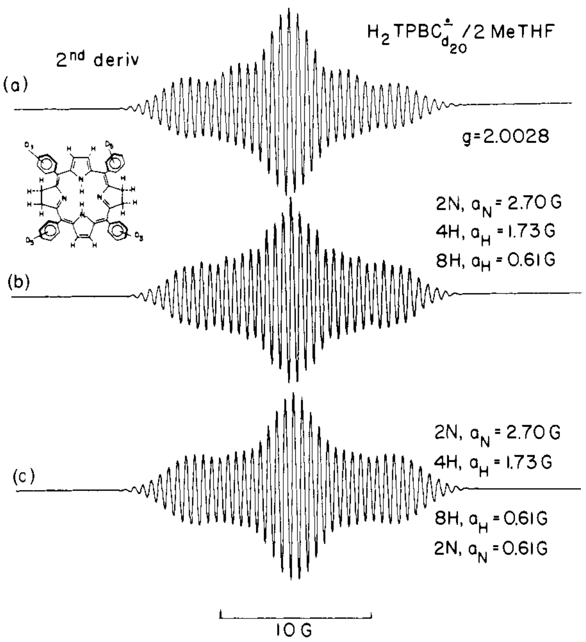


Figure 1. (a) Second-derivative ESR spectrum of H$_2$TPBC-$d_{20}^{-}\cdot$ in MeTHF. (b) Simulation which assumes the splitting constants shown. (c) Simulation which includes two additional nitrogens, a_N = 0.61 G.

Experimental Section

ESR spectra were obtained on a Varian E-12 X-band spectrometer controlled by an SDS Sigma 2 computer. ENDOR spectra were taken with a Varian E-700 high-power accessory using an E-1737 cavity which provides radiofrequency fields of ~40 G in the rotating frame. The low-field side of the power-saturated ESR spectra was monitored. Details of the instrumentation and techniques have recently been described.[29] NMR spectra were recorded at 100 MHz on a Jeolco MH-100 spectrometer.

Computer simulations of the ESR spectra included experimentally determined nitrogen (±5%) and ENDOR proton splittings (±1%) and variable numbers of nuclei assumed to give rise to the hyperfine splittings. Goodness of fit was adjudged by visual inspection of the simulation superimposed on the experimental spectrum on a display screen. Each of the 2000 channels in which the digitized data were stored could be addressed and inspected. Coincidence of the peak positions and intensities and envelopes of the first- and second-derivative ESR spectra were used as criteria.

BChl a and BPh a were extracted from *Chromatium vinosum* and purified by standard techniques.[30,31] Deuterated BChl and BPh were extracted from *Rhodospirillum rubrum* grown[25,28] on deuterated succinic acid and water. The synthesis of the selectively deuterated bacteriochlorins has been described.[31] 18-Crown-6 ether (1,4,7,10,13,16-hexaoxacyclooctadecane) from Aldrich Chemical was recrystallized according to Grokel et al.[32] Ethers were distilled from CaH$_2$ and stored, outgassed, over sodium-potassium alloy. Pyridine and pyridine-d_5 (Aldrich Gold Label), CH$_2$Cl$_2$, HCCl$_3$, and DCCl$_3$ were dried over 4A Linde molecular sieves. All ESR and ENDOR spectra were obtained on samples prepared on a vacuum line using distilled and outgassed solvents with oxygen rigorously excluded. Three reduction methods were used: (i) in situ electrochemical reduction at a platinum electrode using tetrapropylammonium perchlorate as carrier electrolyte;[33] (ii) ether solutions of the pigment were contacted[34] with potassium mirrors or were mixed with separately prepared solutions of solvent, crown ether, and potassium;[35] (iii) solutions of BPh in pyridine containing 10 mM Na$_2$S and 1.5 M water were exposed to white light for a few minutes.[36] The photoreduction could be induced by irradiation in any absorption band of the BPh.

In methods ii and iii, the reactants were prepared in multichambered ampules separated by glass break-seals to allow generation of the radicals on demand (see ref 29 for an example).

NMR spectra of 40 mM BPh solutions in 99.9 atom DCCl$_3$ were recorded using internal Me$_4$Si or HCCl$_3$ as standards. BPh in pyridine-d_5–D$_2$O solutions was photolyzed to generate BPh$^{-}\cdot$ as in method iii and allowed to exchange overnight. The sample was oxidized by exposure to air, diluted with D$_2$O, and extracted with chloroform. (Blank runs, without radical formation, indicate that the extraction procedure itself causes only the C-10 proton to exchange.) After oxidation, the α, β, δ, and C-10 protons were no longer detected. Relative to the C-10 methyl ester group, about 50% of the 1- and 5-methyl protons (or, because their peaks are not well resolved, ~100% of either) as well as >50% of those of the COCH$_3$ group at position 2 had exchanged. Assignments were based on relative intensities of the peaks and the reported spectra of methyl bacteriopheophorbide, BPh, and BChl.[37-39]

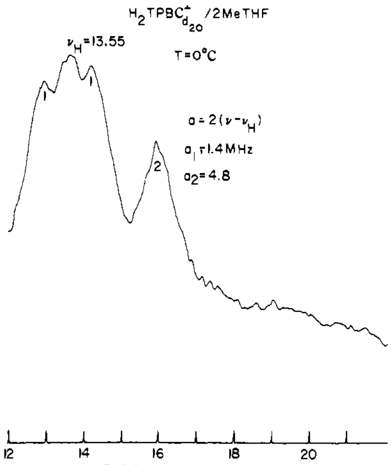


Figure 2. ENDOR spectrum of H$_2$TPBC-$d_{20}^{-}\cdot$ in MeTHF at 0 °C (2.8 MHz = 1 G).

Results and Discussion

(1) Tetraphenylbacteriochlorin. Reduction of H$_2$TPBC in 2-methyltetrahydrofuran (MeTHF) with potassium yields a radical which exhibits a poorly resolved ESR spectrum with a g value of 2.0028 (±0.0001) and a first-derivative peak-to-peak line width (ΔH) of 10.5 G. This compares with $\Delta H \sim$ 9 G reported[26] for the unresolved singlet obtained upon one-electron electrochemical reduction of H$_2$TPBC in dimethylformamide (DMF). A considerable improvement in hyperfine resolution is obtained on potassium reduction of H$_2$TPBC-d_{20} (deuterated phenyl groups) as shown in Figure 1a. The same sample yields, at 0 °C, ENDOR signals (Figure 2) which correspond to proton splitting constants of 1.72 G or 4.8 MHz and ~0.5 G or 1.4 MHz. (The larger error here is due to the overlap of peaks a$_1$ with the matrix or solvent ENDOR signal.) The ESR spectrum obtained from the radical of H$_2$TPBC-d_{28} (all peripheral positions are deuterated except for one proton each at positions 2, 3, 12, and 13) is displayed in Figure 3a. The major five-line pattern can readily be assigned to two nitrogens with a_N = 2.7 G as shown by the simulation in Figure 3b. The 1.7 G proton splitting detected by ENDOR in H$_2$TPBC-$d_{20}^{-}\cdot$ is not observed in the ESR spectrum of H$_2$TPBC-$d_{28}^{-}\cdot$ and, therefore, is attributed to the four protons at positions 7, 8, 17, and 18. The small ENDOR splitting is left, by default, to the eight protons at 2, 3, 12, and 13. (The possibility that the inner protons on the nitrogens were involved was excluded by preparing D$_2$TPBC-$d_{20}^{-}\cdot$. The spectrum of the latter species is essentially that of H$_2$TPBC-$d_{20}^{-}\cdot$.) Simulations which incorporate these assumptions are shown in Figures 1b and 3c (a_D/a_H = 0.1535). Note that the small hyperfine structure of the H$_2$TPBC-$d_{28}^{-}\cdot$ spectrum is not properly simulated. This may be due to incomplete deuteration or alternatively it represents a small interaction of the unpaired electron with the other two nitrogens of the molecule. Addition of two nitrogens with a splitting equal to the small proton hyperfine is shown in Figure 3d. Inclusion of the two nitrogens in the simulation of H$_2$TPBC-$d_{20}^{-}\cdot$ yields a reasonable spectrum of that species as well (Figure 1c). Although the reconstructions (Figures 1b

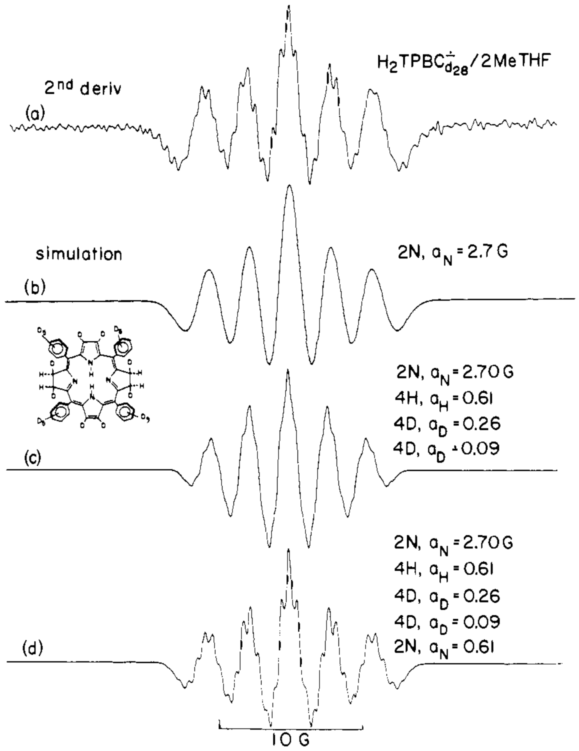


Figure 3. (a) Second-derivative ESR spectrum of $H_2TPBC\text{-}d_{28}^{-}\cdot$ in MeTHF. (b) Simulation which assumes that two nitrogens account for the major spectral features. (c) Simulation which uses the constants shown and is the deuterated equivalent of Figure 1b. (d) Simulation, equivalent to Figure 1c, which includes two additional nitrogens, a_N = 0.61 G.

and c) register properly from the center out to peak 21 on the low-field side of the $H_2TPBC\text{-}d_{20}^{-}\cdot$ spectrum, the visual patterns are critically sensitive to changes as small as 0.5% in the proton splitting constants.

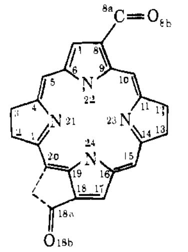
The model compound results are consistent with molecular orbital calculations for the anion radical of a tetrahydroporphyrin. These are computed by the self-consistent field method of Pariser, Parr, and Pople[40,41] applied[33,34] to doublet states in combination with the π orbital model of Weiss et al.[42] Spin density distributions for a $^2B_{2g}$ ground state (D_{2h} symmetry), after configuration interaction, are listed in Table I. They lead to the following predictions: (1) the two nitrogens of the saturated rings will carry large spin densities (a_N(obsd) = 2.7 G); (2) small splittings due to the other two nitrogens of the pyrrole rings are possible (a_N(obsd) = 0.6 G (?)); (3) the eight protons at 2, 3, 12, and 13 will show small splittings (a_H(obsd) = 0.6 G); (4) the four protons at 7, 8, 17, and 18 will exhibit large splittings (a_H(obsd) = 1.7 G); and (5) positions 5, 10, 15, and 20 carry large spin densities which are only indirectly detected by the increased resolutions of $H_2TPBC\text{-}d_{20}^{-}\cdot$ vs. the undeuterated compounds. Splittings of 0.1 G due to the phenyl protons are estimated from computer simulations. Although small, these indicate rather large densities at the meso carbons. Because the phenyl groups lie out of the π plane of the porphyrin ring, the observed hyperfine coupling is therefore a "second-order" effect. (See ref 20 and 33 for details.)

The anion radical of zinc tetraphenylbacteriochlorin (the model for BChl) obtained by electrochemical reduction in butyronitrile exhibits[23] hyperfine structure which, with the aid of selective deuterations, permits the following assignments: four β-pyrrole protons, a_{H_7} = 1.80 G; eight CH_2 protons (saturated rings), a_{H_2} = 0.70 G; two nitrogens, a_N = 2.88 G. Splittings of 0.1 G are also estimated for the phenyl group protons.

The conclusions drawn from the model compounds studies are thus in good agreement with the trend predicted by the molecular orbital calculations (Table I), viz.: (1) the two nitrogens of the saturated rings carry large spin densities; (2) the protons of the saturated rings (2, 3, 12, and 13) show small splittings relative to the protons on the unsaturated rings (7, 8, 17, and 18); and (3) the meso positions (5, 10, 15, and 20) carry large spin densities which are only indirectly observed because the phenyl groups of the model compounds are twisted out of the porphyrin plane.

(2) Bacteriopheophytin. One electron reduction of BPh a in CH_2Cl_2 yields an ESR spectrum (g = 2.0030 ± 0.0002) which

Table I. Calculated Spin Densities (ρ) for Anion Radicals of Free Base (H_2BC) and Metallobacteriochlorins (MBC)[a]



Atom	$H_2BC^{-}\cdot$	$H_2BC^{-}\cdot$ With carbonyls	$MBC^{-}\cdot$ Otten	$MBC^{-}\cdot$	$MBC^{-}\cdot$ With carbonyls
21	0.127	0.101	0.090	0.090	0.081
1	−0.019	−0.018	0.048	0.029	0.022
4	−0.019	−0.005	0.048	0.029	0.027
5	0.118	0.086	0.044	0.059	0.054
22	−0.004	−0.003	0	−0.007	−0.005
6	0.024	0.028	0.068	0.062	0.049
7	0.066	0.078	0.048	0.058	0.080
8	0.066	0.065	0.048	0.058	0.049
8a		0.015			0.011
8b		.045			0.026
9	0.024	−0.010	0.068	0.062	0.028
10	0.118	0.116	0.044	0.059	0.068
23	0.127	0.106	0.090	0.090	0.085
11	−0.019	−0.019	0.048	0.029	0.017
14	−0.019	−0.004	0.048	0.029	0.036
15	0.118	0.090	0.044	0.059	0.056
24	−0.004	−0.003	0	−0.007	−0.006
16	0.024	0.031	0.068	0.062	0.056
17	0.066	0.081	0.048	0.058	0.086
18	0.066	0.061	0.048	0.058	0.048
18a		0.014			0.009
18b		0.042			0.024
19	0.024	−0.007	0.068	0.062	0.037
20	0.118	0.111	0.044	0.059	0.064

[a] To approximate BChl and BPh, carbonyl groups were included at positions 8 and 18 and oriented to maximize the asymmetry of the molecule. Calculations by Otten[43] are displayed for comparison. Semiempirical parameters[33,34,42] used in the computations were: for carbon atoms, core charge (z) = 1, ionization potential (α) = −11.24 eV and repulsion integral (γ) = 9.4915 eV; for oxygen, z = 1, α = −17.25, and γ = 14.670; for nitrogens in metallo complexes, z = 1.5, α = −20.59, and γ = 12.1201; in free bases, for the reduced nitrogens (N−H), z = 1, α = −14.54; and for the other nitrogens, z = 2 and α = −26.64. Observed splitting constants (a) are proportional to the spin densities (ρ): for protons[44] (H_α) directly bonded to carbon atoms (C_α) in the π system, $a_{H_\alpha} \sim 27\rho_{C_\alpha}$; for protons[45] ($H_\beta$) one carbon atom ($C_\beta$) removed from the π system, $a_{H_\beta} \sim \rho_{C_\alpha} B \cos^2 \theta$. The constant B ranges between 45 and 50 for anions[46,47] and θ is the dihedral angle between the plane C_α–C_β–H_β and the p_z orbital of C_α. For rotating methyl groups, $\cos^2 \theta$ averages to $^1/_2$ and $a_{H_\beta} \sim 25\rho_{C_\alpha}$. For rigid protons, β can be estimated by extrapolation of the x-ray data on ethyl chlorophyllides,[48] $\theta \sim 45°$, and on zinc tetraphenylchlorin,[49] $\theta \sim 34°$. For a given spin density at C_α, a_{H_β} in the model should therefore be $^1/_3$ larger than in the natural compound (from $\cos^2 34/\cos^2 45$). Nitrogen splittings a_N, are obtained[50] from $a_N \sim 31\rho_N - 4\rho_C$ where C is the carbon adjacent to the nitrogen.

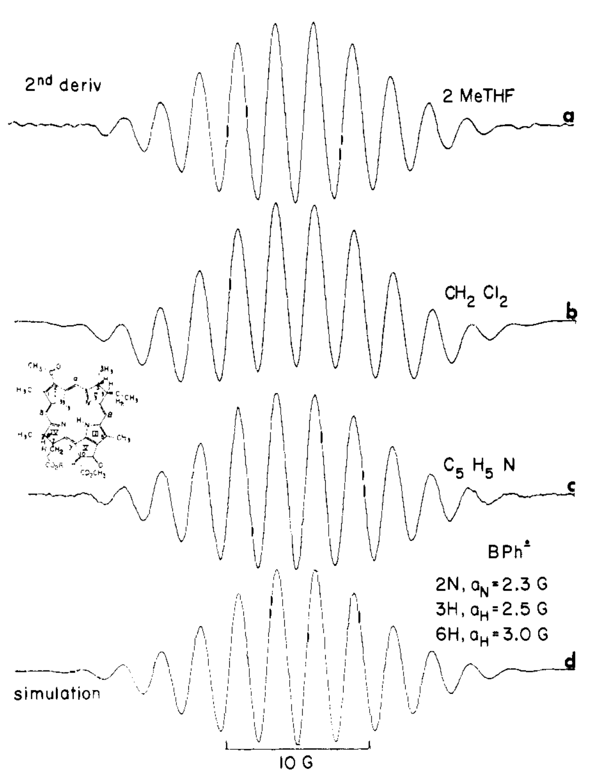


Figure 4. Second-derivative ESR spectra of BPh a$^{-}\cdot$ in: (a) MeTHF, obtained by 18-crown-6/potassium reduction; (b) CH_2Cl_2, generated by electrolytic reduction; (c) C_5H_5N/H_2O, produced by photolytic reduction in the presence of Na_2S; (d) Simulation of c assuming the splitting constants shown.

differs little in resolution from the spectra obtained by reduction of BPh with a potassium/crown ether complex in MeTHF and by photochemical reduction with Na_2S in pyridine (Figure 4). At low temperature, the spectra collapse to a singlet, whose line width is very sensitive to microwave power: at −160 °C, ΔH = 13 G at 1 mW and 12.2 G at 0.01 mW. (By comparison, the presumed primary electron acceptor of *Chromatium* displays,[17,18] in vivo, g values of 2.003 and line widths of 13–15 G.) The room temperature spectra of BPh$^-$· can be reasonably simulated (Figure 4d) by assuming interaction of the unpaired electron with the two nitrogens of rings II and IV (a_N = 2.3 G), the methyl groups 1 and 5 of rings I and III (average $a_H{}^{CH_3}$ = 3.0 G), and with the three meso protons, α, β, and δ (average a_H = 2.5 G). These assignments are in general accord with the results obtained with the model compound[51] H_2TPBC^-·, with molecular orbital (MO) calculations (Table I) and, in addition, are deduced from the following experimental data. In frozen pyridine, at −170 °C, BPh$^-$· exhibits a strong ENDOR signal which corresponds to a_H = 3.1 G. The same sample, in fluid solution at 0 °C, shows two transitions, a_H = 3.0 and 2.5 G of relative intensities ~2 to 1 as determined by computer deconvolution (Figure 5).

Hyde et al.[52] first noted that methyl protons, bonded to an α carbon of a π radical, are easily detected by ENDOR at low temperatures because of the combination of favorable relaxation times and small anisotropies which result from the free rotation of methyl groups even in frozen matrices. The effect has also been demonstrated, and the methyl group assignment verified by deuteration, in the ENDOR spectra of oxidized BChl, even below −200 °C.[25,28,29] By comparison, the rigid β protons of BChl$^+$· are only well resolved in solution.[29] Therefore, we attribute the 3.1 G signal observed at −170 °C to methyl groups 1 and 5 and the 2.5 G transition, found at −30 °C, to the α, β, and δ meso protons. (The approximate 2 to 1 relative intensities of the 3 and 2.5 G peaks support these assignments but strengths of ENDOR signals depend on saturation behavior and are not always reliable criteria of the number of protons involved.[25,53])

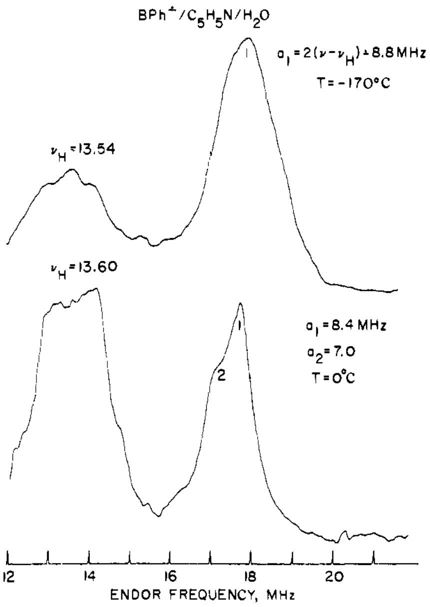


Figure 5. ENDOR spectra of BPh$^-$· in C_5H_5N/H_2O at −170 °C (top) and 0 °C (bottom).

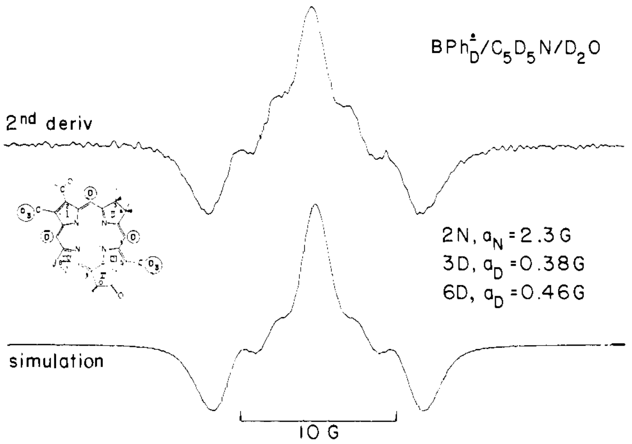


Figure 6. Second-derivative ESR spectrum of perdeuterated BPh$^-$· in C_5D_5N/D_2O and a simulation with the splitting constants shown.

Deuterated BPh$^-$· shows a five-line pattern readily ascribed to two nitrogens, a_N = 2.3 G (Figure 6). The simulations in Figures 4 and 6 reflect these nitrogens and proton assignments. (Since the smallest splittings resolved in the BPh$^-$· ESR spectra are of the order of 2 G, we have not included the small resonances superimposed on the matrix ENDOR signal in the ESR simulations. By analogy with the model compounds (a_{H_7} ~ 0.6 G), these splittings are probably due to the protons of rings II and IV.)

BPh$^-$· is surprisingly stable in pyridine containing Na_2S and up to 2 M H_2O. We therefore considered the possibility that the radical observed results from a proton addition: BPh$^-$· + H^+ → BPhH·. Molecular orbital calculations for such a model (Figure 7) indicate that large spin densities would exist at the site adjacent to the proton addition (presumably at a meso position, as is observed[54] when chlorophyll a is photoreduced in the presence of hydrogen donors, for example) and would result in large couplings with the added proton. We observe no initial difference in the ESR spectrum of BPh$^-$· prepared photochemically in pyridine that contains small amounts of H_2O or D_2O and, conversely, we detect no initial difference

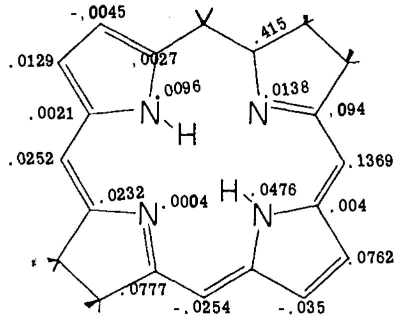


Figure 7. Calculated spin densities for a hypothetical radical $PH_5\cdot$ generated by addition of a proton to the anion radical $PH_4^-\cdot$ of a tetrahydroporphyrin.

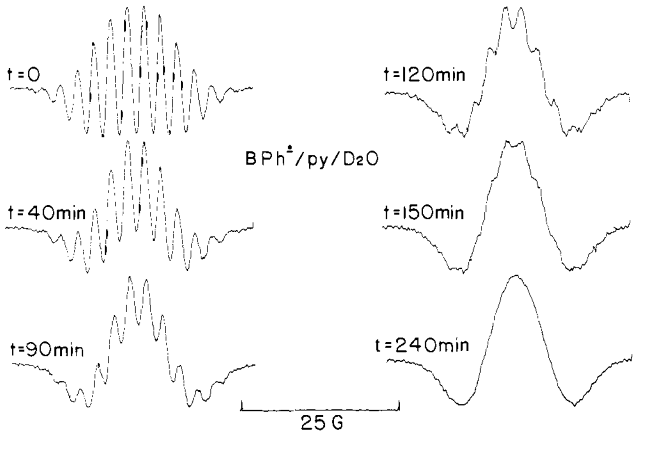


Figure 8. Changes in the second-derivative ESR spectra of $BPh^-\cdot$, prepared in pyridine containing D_2O, which illustrate exchange of deuterium for hydrogen (a_D/a_H = 0.1535).

in the spectra of perdeuterated $BPh^-\cdot$ in deuterated pyridine containing D_2O or H_2O.[55]

$BPh^-\cdot$, in the presence of D_2O, does display a significant collapse of its signal width (Figure 8) as a function of time but neither the number of spins, determined by integration of the ESR signals, nor the optical spectrum of the solution shows any noticeable changes. The anion must therefore be undergoing isotopic *exchange*, not addition. Computer simulations of the ESR spectrum of $BPh^-\cdot$ obtained after 4 h of exchange (Figure 8) indicate that about six protons have been replaced by deuterons. The reverse reaction is more easily followed, because of a kinetic isotope effect. Perdeuterated $BPh^-\cdot$ in deuterated pyridine (C_5D_5N) containing H_2O slowly undergoes exchange to yield, first, ESR spectra consistent with exchange of three deuterons (predominantly the meso positions, Figure 9) followed by exchange of an additional three deuterons (the 1- and/or 5-methyl groups, Figure 10).

These conclusions are also supported by NMR data on BPh exposed to D_2O as the anion and reoxidized. The spectra show that the meso hydrogens as well as the 1- and/or 5-methyl protons have exchanged (Figure 11). The unpaired electron spin distribution of $BPh^-\cdot$, in accord with the model compound studies and with the MO calculations cited above, can be defined as follows. Positions 1, 2, 5, 6, α, β, and δ each carry ~0.1 unit of spin while the spin density in rings II and IV is concentrated on the nitrogens. Smaller splittings, expected from the β protons of rings II and IV, are not resolved in the ESR spectra but are estimated to range between 0.2 and 0.8 G from the ENDOR data. This electronic configuration is very similar to that found for $BChl^-\cdot$.

(3) **Bacteriochlorophyll.** The anion radical of BChl a, generated electrochemically[23] in butyronitrile or by potassium reduction in tetrahydrofuran (THF), displays a g value[56] of 2.0028 (±0.0002) and a 12-line ESR spectrum which collapses upon deuteration of the BChl (Figures 12a and c). In THF, at −140 °C and 0.01 mW microwave power, the line widths for $BChl^-\cdot$ and its deuterated analogue are 12.7 and 4.8 G, re-

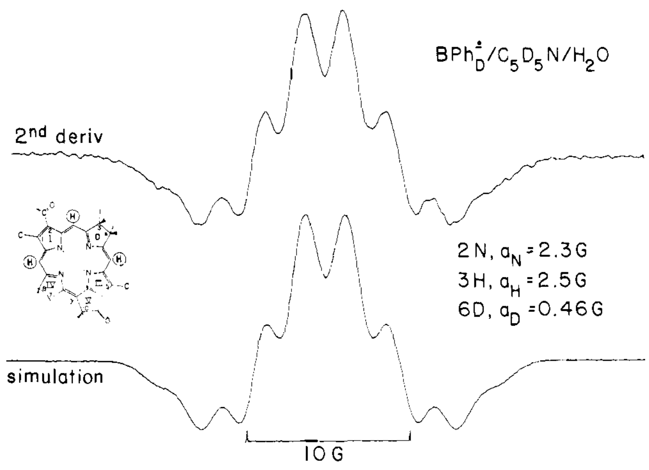


Figure 9. Second-derivative ESR spectrum recorded several hours after generation of the radical of deuterated BPh in C_5D_5N/H_2O. The three meso deuterons are assumed to have exchanged in the simulation.

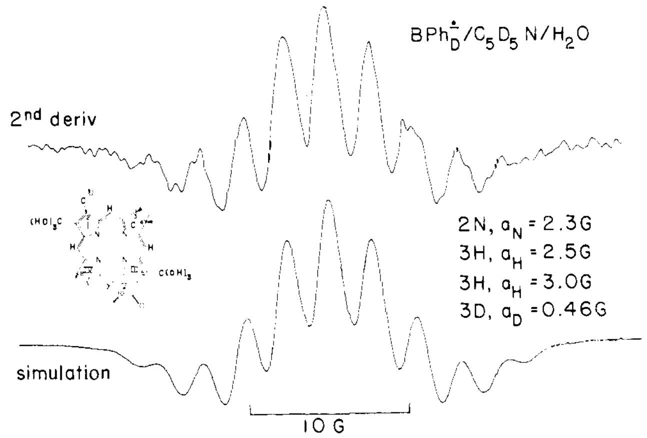


Figure 10. Second-derivative ESR spectrum obtained 2 days after generation of deuterated $BPh^-\cdot$ in C_5D_5N/H_2O. The three meso deuterons and three of the six 1- and 5-methyl deuterons are assumed to have exchanged in the simulation.

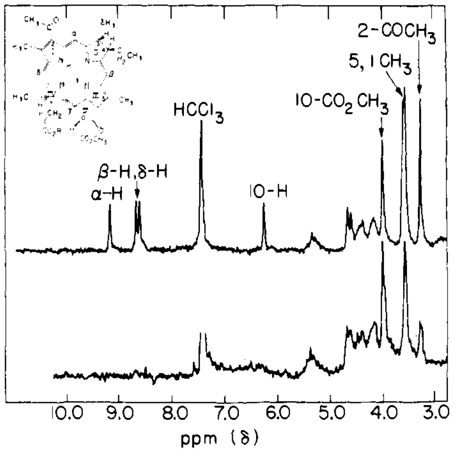
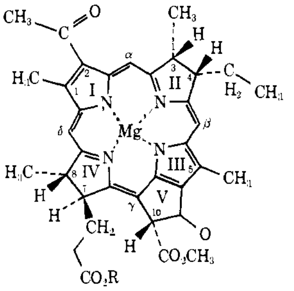
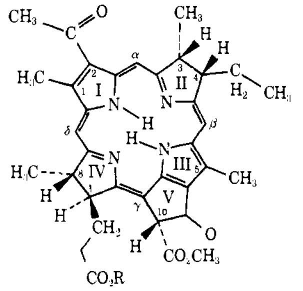


Figure 11. NMR spectra of BPh in $DCCl_3$: (top) before reduction; (bottom) after reduction in C_5D_5N/D_2O followed by oxidation and extraction into $DCCl_3$.

Table II. Splitting Constants, gauss





	1-(CH_3)	5-(CH_3)	3, 4, 7, 8 (H)	α, β, δ (H)	N
$BChl_2^{+\cdot}$	0.9	1.7	2.1, 2.3, 2.4, 2.9	0.3	~0.5 } 4 N
$BChl_2^{+\cdot}$ (normalized, a/1.6)	0.6	1.1	1.3, 1.4, 1.5, 1.8	0.3	~0.5
$BPh^{-\cdot}$ a	3.0[a]	3.0[a]	<1, not resolved	2.5[a]	2.3 } 2 N[b]
$BChl^{-\cdot}$ a	3.2[a]	3.2[a]	<1, not resolved	2.5, 2.0	2.2

[a] Average value. [b] Rings II and IV.

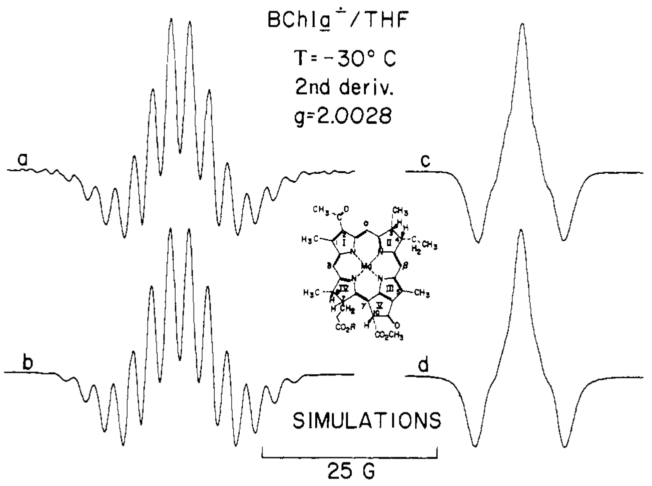


Figure 12. Second-derivative ESR spectra in THF at −30 °C: (a) BChl a$^{-\cdot}$; (b) simulation of a, 2 nitrogens 2.2 G, 6 protons 3.2 G, 2 protons 2.5 G, and 1 proton 2.0 G; (c) deuterated $BChl^{-\cdot}$; (d) simulation of c; all proton splittings of simulation b have been converted to deuteron splittings.

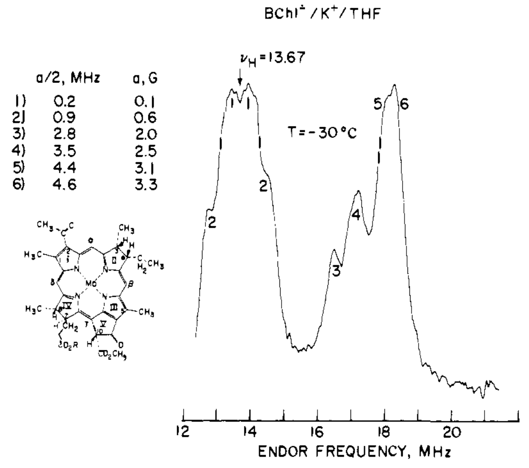


Figure 13. ENDOR spectrum of $BChl^{-\cdot}$ in THF at −30 °C.

spectively. Feher et al. found,[25] for $BChl^{-\cdot}$ in DMF at 80 K, an ENDOR transition which corresponds to a_H = 2.9 G. We similarly find an ENDOR line for a_H = 3.2 G at 130 K in THF and a_H = 3.1 G at ~4 K in MeTHF. Because the relaxation behavior and small anisotropies of methyl groups, discussed above, facilitate their detection by ENDOR at low temperatures, the above transitions are assigned to one or more methyl groups. Several transitions, in addition to the ones attributable to the methyl groups, are detected in fluid THF solution at 240 K (Figure 13). The MO calculations of Table I predict, for $BChl^{-\cdot}$, spin densities of similar magnitude for the meso positions and the methyl groups as well as coupling with only two nitrogens. The anion radical of zinc tetraphenylbacteriochlorin does in fact exhibit[23] resolved ESR spectra which support the MO computations. Extrapolation of the theoretical and experimental results to $BChl^{-\cdot}$ leads to the following assignments: ENDOR peaks 5 and 6 (average a_H = 3.2 G), methyl groups 1 and 5; peaks[57] 3 and 4 (a_H = 2.0 and 2.5 G, relative intensity 1:2), meso protons α, β, and δ; peak 2 (a_H = 0.6 G), β protons 3, 4, 7, and 8; peak 1 ($a_H \sim$ 0.1 G) may be due to a number of sites—C-10, the methyl or methylene groups of rings II and IV, or possibly the methyl group at position 2. These assignments yield reasonable reconstructions of the experimental ESR spectra, recorded at the temperature of the ENDOR measurements, of both protonated and deuterated $BChl^{-\cdot}$ as shown in Figures 12b and 12d. (The small ENDOR splittings are subsumed in the line widths used for the simulations.)

Table II lists experimental hyperfine splittings obtained for $BChl^{-\cdot}$, $BPh^{-\cdot}$, and those calculated for $P_{870}^{+\cdot}$ assuming that it is a dimer cation,[29] $(BChl)_2^{+\cdot}$, or measured by ENDOR.[25,28] The McConnell equation[45] which relates β-proton splittings to spin densities differs for cation and anion radicals. To provide a clearer comparison of the electronic profiles of $BPh^{-\cdot}$, $BChl^{-\cdot}$, and $P_{870}^{+\cdot}$, normalized splitting constants of the latter are also included. For an assumed dihedral angle θ = 45° in chlorophylls, $a_{H_\beta} \sim$ 25 and 40 ρ_{C_α} for anions[47] and cations,[58,59] respectively, i.e., a methyl hyperfine constant of 2.5 G in an anion reflects the same spin density on the adjacent α-carbon atom as does a constant of 4 G in a cation radical.

The ESR and ENDOR parameters of $BPh^{-\cdot}$ and $BChl^{-\cdot}$ do not provide, by themselves, the criteria necessary to identify, unambiguously, the free radical observed[17,18] in vivo and ascribed to these anions. The combination of optical, magnetic, and electrochemical data, however, is most consistent with the properties[6,49] of a monomeric BPh anion.

In addition to $P_{870}^{+\cdot}$, $BPh^{-\cdot}$, and $BChl^{-\cdot}$, other radicals presently assumed to evolve from the primary bacterial photoact have also been characterized by ESR and ENDOR. Within a nanosecond time domain of the incident excitation, these include the quinone anion radicals[8,60-62] which appear in less than 200 ps and the spin-polarized triplets[13-15,18,63] that are detected within nanoseconds and presumably result[5,6,15] from the biradical recombination of $P_{870}^{+}\cdot$ and $BPh^{-}\cdot$ (or $BChl^{-}\cdot$).

The proximity which the reaction center imposes on these primary reactants must facilitate rapid electron transfer and influence their mutual interactions. The splitting constants presented here can be used to assess the extent to which the strikingly different spin density distributions in $P_{870}^{+}\cdot$ and $BPh^{-}\cdot$ contribute to spin polarization in $^{3}P_{870}$. Besides the anomalous triplets which reflect interactions between $P_{870}^{+}\cdot$ and $BPh^{-}\cdot$, magnetic coupling of the latter with $X^{-}\cdot$ (the next electron acceptor) is also evidenced by the ESR spectra elicited[18] at low temperatures (<15 K) from reaction centers poised at low potentials and illuminated to reduce both X and BPh.

A considerable body of information now exists on the identity, electronic configuration, sequence of appearance, kinetics, and energetics of the species involved in the initial stages of photosynthesis. It may provide the guidelines necessary to construct artificial photosynthetic systems that mimic the efficient biological conversion of light into chemical energy but circumvent unwanted physiological reactions.

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