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Hyperfine Coupling in Chlorophyll Radical Cations. A Nuclear Magnetic Resonance Approach

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Abstract: Most of the relative proton hyperfine coupling constants in the radical cations of chlorophyll a, chlorophyll b, and bacteriochlorophyll a have been determined using electron transfer NMR line broadening in the fast exchange limit. The results have been used to confirm ENDOR assignments for some methyl groups and to give a detailed spin density distribution for many positions exhibiting no ENDOR signals. Agreement with ENDOR is qualitatively good for Chl a and Bchl a, but is poor for Chl b.

In photosynthesis, light energy from the sun ·is trapped as chemical energy in the form of reducing power. In the first few nanoseconds after light absorption an electron is ejected from a reaction-center chlorophyll–protein complex and a chlorophyll radical cation is produced.[1,2] The reaction center chlorophyll of plant photosynthesis is chlorophyll a (**1**), which may be accompanied by the accessory chlorophyll b (**2**); pho-

1 R = Me
2 R = CHO
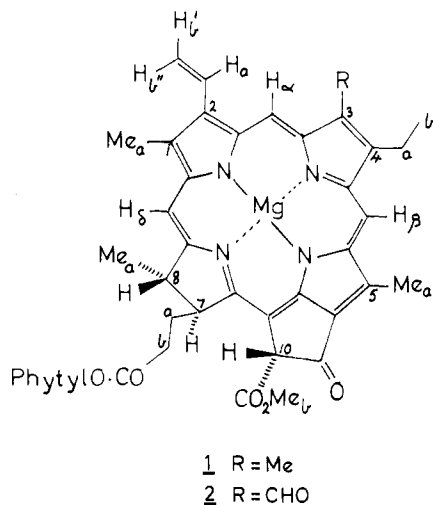
tosynthetic bacteria employ bacteriochlorophyll a (**3**) or bacteriochlorophyll b ($\Delta^{4,4a}$**3**).

Although chlorophyll radical cations play a central role in photosynthesis, elucidation of their properties is made difficult by their chemical instability and the limitations of the techniques normally used to study them. Thus, their ESR spectra generally consist of a single Gaussian signal containing over 10^9 unresolved lines[3], and at the outset of this work ENDOR was giving only rudimentary information.[4] Progress has been rapid in both techniques recently[5-7] but each suffers a fundamental limitation: assignment of hyperfine coupling constants to individual protons requires deuteration, chemical modifi-


3
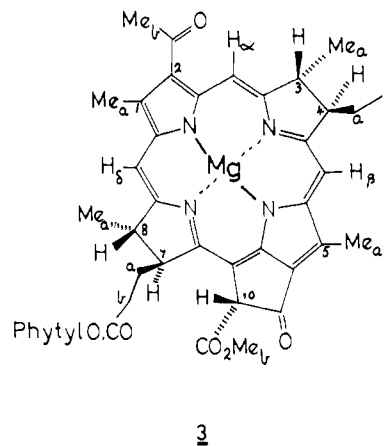
cation, or fitting observed couplings to those predicted by MO theory.

In contrast, the NMR spectra of chlorophylls are rich in information, can be assigned *spectroscopically* without recourse to modification,[8] and can yield relative hyperfine coupling constants via electron transfer broadening. This technique has been known for many years,[9] the theory is well established,[10] and its use in assigning ESR spectra has been advocated,[11] but it has been largely ignored. We show here[12] that the method can be used successfully in systems which are much more complex than those[13] previously studied. It should be applicable also to the recently synthesized "reaction-center" dimers,[14-17] and the equally significant radical anions.[18] A similar technique is being used to study chlorophyll triplets.[19]

Theory

Spin densities (ρ_c) on carbon atoms in a π radical can in principle be determined from proton hyperfine coupling constants (a_H) via the McConnell relation (eq 1) where Q is a parameter which reflects the efficiency of spin transmission
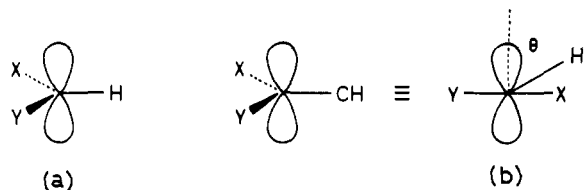
Figure 1. Spin transmission from a π system.

to the proton.

$$a_H = Q\rho_c \qquad (1)$$

For protons α to the π system (Figure 1a), Q_{CH} is 76 MHz.[20a] We have presented evidence elsewhere[20b] that for protons β to the π system (Figure 1b), $Q_{CCH} = -15 + 224 \cos^2 \theta$ MHz, giving $Q_{CCH_3} = 97$ MHz.[20c]

The hyperfine coupling can be obtained by NMR: the theory of the exchange broadening and contact shift of a proton exchanging between diamagnetic and paramagnetic sites (Figure 2 and eq 2) was developed by de Boer and MacLean.[10,21]

$$\text{Chl} + \text{Chl}^{+\cdot} \xrightleftharpoons{k_{ex}} \text{Chl}^{+\cdot} + \text{Chl} \qquad (2)$$
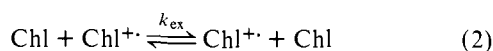
The exchange contribution to the line width, $\Delta\nu_{ex}$, is given by

$$\Delta\nu_{ex} = (\pi T_{2ex})^{-1} = \frac{f_P \tau_P a_H^2 / 4\pi}{1 + (f_D \tau_P^2 a_H^2 / 4) + 2\tau_P T_{1e}^{-1}} \qquad (3)$$

where T_{2ex} is the exchange contribution (in seconds) to the proton transverse relaxation time, f_P and f_D are the fractions paramagnetic and diamagnetic, T_{1e} is the electron spin–lattice relaxation time, and τ_P and τ_D are respectively the lifetimes in the paramagnetic and diamagnetic states, in seconds:

$$\tau_P = (k_{ex[D]})^{-1} \qquad (4)$$

$$\tau_D = (k_{ex[P]})^{-1} \qquad (5)$$

where [D] and [P] are respectively the concentrations of diamagnetic and paramagnetic in moles/liter.

The contact shift depends on the Boltzmann distribution between the electron spin states which is approximated by

$$\frac{\text{fraction in lower state}}{\text{fraction in upper state}} = 1 + \frac{g_e \beta_e H}{4kT} \qquad (6)$$

Assuming that the shift in the paramagnetic state in the absence of hyperfine coupling effects is given by the diamagnetic chemical shift, then the contact shift in hertz, δ_c, is given by

$$\delta_c = -a_H f_P \frac{g_e \beta_e H}{4kT} \frac{1 + 2\tau_P T_{1e}^{-1}}{1 + (f_D \tau_P^2 a_H^2 / 4) + 2\tau_P T_{1e}^{-1}} \qquad (7)$$

where $g_e = 2.0026$, $\beta_e = 9.27 \times 10^{-21}$ erg G^{-1} (Bohr magneton), H = magnetic field in gauss, $k = 1.38 \times 10^{-16}$ erg deg^{-1}, and T is the absolute temperature.

The direction of the contact shift is generally the best method for determining the sign of a_H, although *triple* techniques can be used in favorable cases.[22]

For eq 3 and 7 there are three limiting cases, each of which we have observed experimentally in this work.

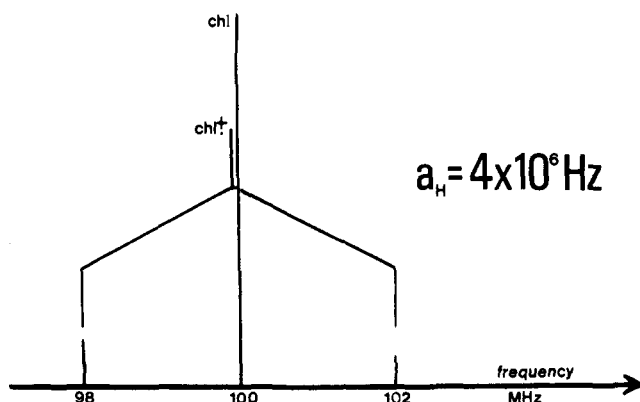**(1) Fast Exchange, Type 1.** If electron spin–lattice relaxation is so fast that

$$2\tau_P T_{1e}^{-1} \gg 1 + (f_D \tau_P^2 a_H^2 / 4) \qquad (8)$$

then

$$\Delta\nu_{ex} = f_P T_{1e} a_H^2 / 8\pi \qquad (9)$$

and

$$\delta_c = -a_H f_P \frac{g_e \beta_e H}{4kT} \qquad (10)$$

Figure 2. Hypothetical ^{1}H 100-MHz NMR spectrum for a proton with $a_H = 4$ MHz, long T_{1e}, and small k_{ex}.

(2) Fast Exchange, Type 2. If electron transfer is very fast, then τ_P is small and

$$1 \gg 2\tau_P T_{1e}^{-1} + (f_D \tau_P^2 a_H^2 / 4) \qquad (11)$$

then

$$\Delta\nu_{ex} = f_P \tau_P a_H^2 / 4\pi \qquad (12)$$

and δ_c is the same as for fast exchange type 1.

In each type of fast exchange, broadening is proportional to a_H^2 so that relative hyperfine coupling constants within a molecule can be determined from relative broadenings without a knowledge of τ_P, T_{1e}, or f_P. Type 1 and 2 can be experimentally distinguished by the concentration dependence of broadening: Type 1 is concentration independent (apart from any dependence of T_{1e}) but type 2 shows a strictly bimolecular dependence via τ_P and eq 4.

(3) Slow Exchange. Where electron and spin–lattice relaxation are both sufficiently slow that

$$f_D \tau_P^2 a_H^2 / 4 \gg 1 + 2\tau_P T_{1e}^{-1} \qquad (13)$$

then

$$\Delta\nu_{ex} = f_P / \pi\tau_P = (\pi\tau_D)^{-1} \qquad (14)$$

All resonances in slow exchange have identical broadenings. It is possible for a proton with large a_H to satisfy eq 13 and be in slow exchange while another proton in the same molecule with small a_H satisfies eq 8 or 11 and is in fast exchange.

Both τ_P and T_{1e} increase on cooling so that in fast exchange, broadening will increase until a coalescence point is reached. On further cooling the resonances will pass into slow exchange. For type 2 exchange, dilution will have the same effect. If two resonances with different hyperfine couplings are in fast exchange, cooling will leave the ratio of the broadenings unchanged until the signal with the largest hyperfine passes into slow exchange. Further cooling should cause the ratio to decrease monotonically until the second resonance passes into slow exchange and the ratio reaches unity. Thus, our criterion for fast exchange in this work has been the temperature independence of broadening ratios. Note that changes in f_P will change the absolute size of broadenings (and shifts) but not the ratio of broadenings. Changes in k_{ex} will change the broadening but not the shift.

Inspection of eq 9, 10, and 12 shows that contact shifts are most likely to be observed at high magnetic field (since δ_c is field dependent but $\Delta\nu_{ex}$, the result of coupling, is not), high temperature and concentration (to minimize $\Delta\nu_{ex}$), and where a_H is small (since δ_c is proportional to a_H but $\Delta\nu_{ex}$ is proportional to a_H^2).
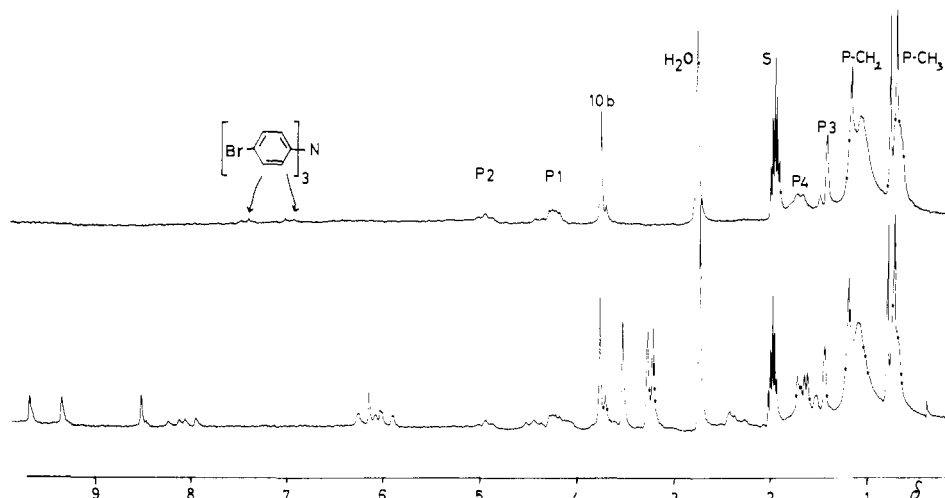
Figure 3. 100-MHz ^{1}H NMR spectra of **1**, 20 mM in acetone-d_6 at 290 K. Below, normal spectrum; above, broadened spectrum after partial oxidation with **5**.

Experimental Section

Chlorophylls a and b were isolated from leaf beet perpetual spinach (*Beta vulgaris* var. *cicla*) and bacteriochlorophyll a from *Chromatium D*. They were partially purified by dioxane precipitation, and chromatographed on sucrose to analytical and spectroscopic purity. Details are being published elsewhere.[8b]

Zinc tetraphenylporphyrin perchlorate (**4**) was a gift from Dr. J. Fajer. The tris(bromophenyl)aminium salts **5** and **6** were prepared by Ledwith's procedure.[23]
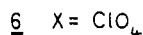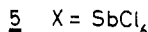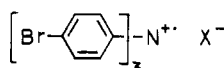
5: mp 189–194 °C dec (lit. 141–142 °C).[23] Anal. Calcd for $C_{18}H_{12}NBr_3SbCl_6$: C, 26.5; H, 1.50; N, 1.7. Found: C, 26.7; H, 1.55; N, 1.7.

6: mp 120–123 °C (lit. 129 °C).[23]

^{1}H NMR spectra were obtained at 80 (Varian CFT 20), 100 (Varian XL100), or 270 MHz (Bruker WH270) in the Fourier transform mode. Five to one hundred free induction decays were collected and summed before transformation. In time-dependent experiments care was taken to ensure that spectral change during spectral accumulation was small. Line broadening experiments were generally carried out in acetone-d_6 as solvent by directly adding oxidizing agent. Bacteriochlorophyll solutions were always thoroughly degassed to minimize the steady-state concentration of radical cation (see Results). Experiments with the other chlorophylls were run both with and without degassing.

Line widths were determined directly from the width at half-height and, in the case of very broad lines, the peak height. A computer program was used to simulate the broadening of overlapping lines,[24] assuming a Lorentzian line shape. Exchange line broadenings were determined from the line width by subtracting the unbroadened line width, determined under the same conditions of temperature and concentration. Uncertainties in the line-broadening ratios represent the spread of values obtained from different spectra and are partly subjective.

The ESR experiments were performed using an extensively modified Varian V4500 X-band spectrometer.[25] The near-infrared spectra were obtained with a Pye Unicam SP500, Series 2 visible spectrometer.

Results

Acetone-d_6 was found to be an ideal solvent as it is sufficiently basic to disaggregate the chlorophylls but is too weak a nucleophile to attack the radical cations rapidly. The NMR spectra in this solvent were unambiguously assigned spectro-

scopically using T_1, NOE, and coupling arguments.[8b] Radical cations were generated by the addition of any of the crystalline salts **4–6**, denoted A^+:

$$Chl + A^+ \cdot \rightarrow Chl^+ \cdot + A \qquad (15)$$
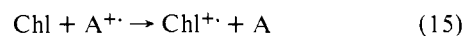
ESR spectra of the chlorophyll solutions after addition of small quantities of $A^+ \cdot$ showed the presence of only $1^+ \cdot$–$3^+ \cdot$ with the line widths expected for monomeric species.[2,3,26] A solution of **1** in acetone treated with **5** showed the characteristic 800-nm absorbance of $1^+ \cdot$ in the near IR.[27]

When small quantities of solid **5** were added to solutions of Chl a in acetone-d_6, selective NMR line broadening was observed. If sufficient **5** was added, resonances from all the protons α, β, and γ to the conjugated system were broadened so much as to be unobservable (Figure 3) and the NMR spectrum showed only signals from the phytyl group and the 10b ester methyl, together with solvent and water peaks and the two doublets from neutral tris(p-bromophenyl)amine. Under the conditions of the experiment the broadenings slowly decreased and disappeared and the NMR spectrum of Chl a was restored unchanged, except that the water peak moved slightly downfield; perhaps this indicates that small quantities of HCl are formed when the anion of **5** decomposes. The signals from the neutral amine were never observed to vary in height or width during such a series of spectra; they were always completely sharp. From this observation we conclude that eq 15 is obeyed and electron transfer to **5** from chlorophyll is rapid and irreversible.

During this decay, spectra of Chl a in the presence of progressively smaller concentrations of Chl $a^+ \cdot$ could be obtained. From such a series of spectra all the relative broadenings from the largest to the smallest could be determined. At 310 K with Chl a around 25 mM, half-times for this decay were typically around 5 min in undegassed solutions, somewhat longer degassed. Although the radical cation was more stable at lower temperatures, and below about 250 K was stable over a period of hours, it was not possible to determine hyperfine couplings since the fast exchange conditions were not satisfied. Dilute *degassed* solutions at 310 K were also not in fast exchange for the protons with larger hyperfine couplings.

Table I gives our observed relative broadenings, for **1** in fast exchange, together with derived hyperfine couplings and unpaired spin densities. The broadenings represent the combined results of several different experiments. Values for H_7 and H_8 are less precise than for other protons as the signals are ill-defined multiplets which overlap with a phytyl resonance. H_{4a} could only be observed using a $(180°-t-90°)_n$ pulse sequence

Figure 4. Partial 100-MHz ^{1}H NMR spectra of **2**, 55 mM in acetone-d_6 at 270 K. Bottom, normal spectrum; above, spectra with successively increasing concentrations of **2**$^{+\cdot}$.
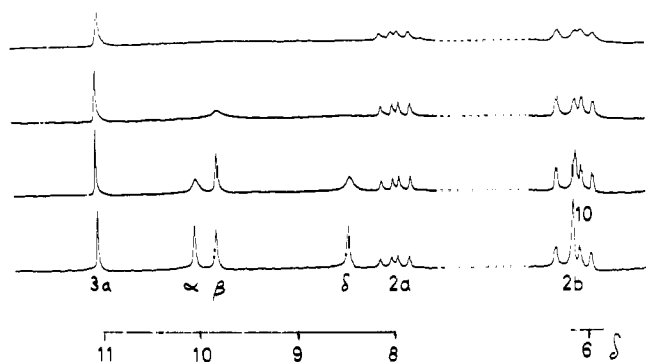

Figure 5. Partial 100-MHz ^{1}H NMR spectra of **3**, 32 mM in acetone-d_6 at 310 K. Bottom, normal spectrum; above spectra with successively increasing concentrations of **3**$^{+\cdot}$.

Table I. Broadenings and Derived Parameters for Chlorophyll a and Its Radical Cation[a]

Proton	Rel broadening ($\Delta\nu_{ex}$)	Absolute a_H, MHz[b]	Q, MHz	ρ_c
α	0.095	2.30 (±0.11)	76	0.030
β	0.010	0.75 (±0.08)	76	0.010
δ	0.310	4.20 (±0.21)	76	0.055
2a	0.017	0.98 (±0.14)	76	0.013
2b',2b''	0.023	1.10 (±0.16)	76	0.014
1a	0.29	4.1 (±0.2)	97	0.042
3a	0.29	4.1 (±0.2)	97	0.042
4a	0.03	1.3 (±0.3)	41	0.032
5a	1.000	7.5[c]	97	0.078
7	>1.4	>9.0	100	>0.090
8	>1.4	>9.0	120	>0.075
10	0.025	1.2 (±0.2)	153	0.008
4b	0.005	0.5 (±0.1)		
7a,7b	0.005	0.5 (±0.1)		
8a	0.001	0.24 (±0.05)		
10b	<0.0001	<0.075		
Phytyl	<0.0001	<0.075		

[a] [1] ≤ 80 mM in acetone-d_6; 270-310 K; fast exchange; **5** as oxidant. [b] $a_H = 7.5(\Delta\nu_{ex})^{1/2}$ MHz. [c] ENDOR value.[2,6]

Table II. Broadenings and Derived Parameters for Chlorophyll b and Its Radical Cation[a]

Proton	Rel broadening ($\Delta\nu_{ex}$)	Absolute a_H, MHz[b]	Q, MHz	ρ_c
α	1.3	8.60 (±0.43)	76	0.113
β	0.33	4.30 (±0.21)	76	0.056
δ	1.2	8.30 (±0.42)	76	0.109
2a	0.01	0.75 (±0.11)	76	0.098
2b',2b''	0.02	1.10 (±0.14)	76	0.014
3a	0.02	1.10 (±0.14)	76	0.014
1a	0.36	4.50 (±0.23)	97	0.046
4a	0.20	3.5 (±0.5)	41	0.085
5a	1.000	7.5[d]	97	0.077
7	>0.02[c]	>1.1	100	>0.011
8	1.5[c]	9.2 (±2.1)	120	0.077
10	1.60	9.5 (±1.3)	153	0.062
4b	0.010	0.75 (±0.17)		
7a,7b	0.005	0.53 (±0.12)		
8a	0.005	0.53 (±0.12)		
10b	<0.001	<0.24		
Phytyl	<0.001	<0.24		

[a] [2] ≤ 60 mM in acetone-d_6; 270-300 K; fast exchange; **5** as oxidant. [b] $a_H = 7.5(\Delta\nu_{ex})^{1/2}$ MHz. [c] The resonances from H$_7$, H$_8$, P$_1$, and H$_{4a}$ overlap. [d] ENDOR value.[26]

to eliminate H$_{10b}$, which has a longer T_1.[8,28] The absolute hyperfine couplings in Table I have been calculated using the reasonably unambiguous ENDOR[2,6] result a_{5a} = 7.5 MHz to calibrate our relative values. Q values for β protons have been calculated using our equation (see Theory), assuming free rotation for methyl groups and the following values of θ taken from the crystal structure[29] of ethyl chlorophyllide a: 4a (60°), 7 (44°), 8 (39°), 10 (30°). However, Q is extremely sensitive to θ and is therefore less reliable than Q_{CCH_3}.

Essentially identical experiments were carried out with Chl b (**2**) and Bchl a (**3**), and these are illustrated in Figures 4 and 5. We observed that **2**$^{+\cdot}$ decayed much more rapidly and **3**$^{+\cdot}$ much more slowly than **1**$^{+\cdot}$.

Whenever a solution of Bchl a in acetone-d_6 was prepared, line broadening was seen. This line broadening was observed even when the solution was flushed with nitrogen. As the solution stood in the probe of the spectrometer, the broadening disappeared; this decay was accelerated if the temperature was raised. The broadening was restored when air was admitted to the sample, and is accentuated by light. The line broadening was identical with that observed when solid **5** was added, and we interpret these observations as indicating that in acetone, air or light oxidation of bacteriochlorophyll a creates small steady-state concentrations of the radical cation, sufficient to induce line broadening. The ESR spectra of these solutions indicate the presence of radical cation, but with our equipment
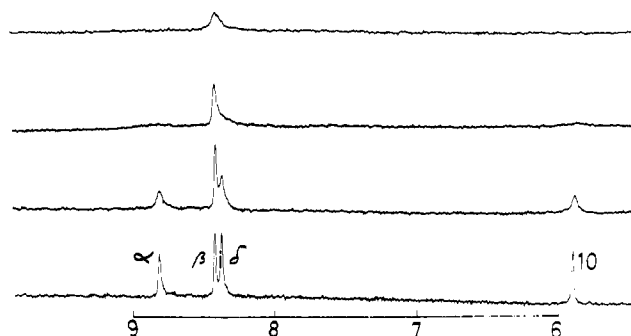
the NMR line broadening is more sensitive, being able to detect radical cation concentrations down to ca. 10^{-8} M.

The observed line broadenings and derived parameters for **2**$^{+\cdot}$ and **3**$^{+\cdot}$ are listed in Tables II and III. The Q values follow those for **1** except that in **3** we assume ring B to be less distorted than ring D.[29] Despite much effort at 100 and 270 MHz we were unable to accurately determine the broadenings of the methine protons H$_7$, H$_8$, and (in Bchl a) H$_3$ and H$_4$, except to say that they are very large. Possibly the problems caused by overlapping of peaks could be resolved by high-field studies on the respective methyl chlorophyllides.

For both **2** and **3**, we have to admit considerable experimental uncertainty in the ratio of the smaller to the larger broadenings, since there are no convenient resonances with intermediately sized broadenings. We are thus forced to compare the broadenings of very broad and fairly sharp lines.

In order to ascertain the counterion, oxidant, and solvent dependence of our broadenings we have carried out the sets of experiments listed in Table IV. No evidence of significant redistribution of the unpaired spin density was found in any of them. The only experimentally measurable variation in hyperfine coupling is for H$_\beta$ in chlorophyll a, which is estimated to be 0.6 MHz in expt 4 and 1.3 MHz in expt 3. These variations need not concern us unduly since the amount of spin density at C$_\beta$ is minute and small changes in distribution can lead to very large changes in a_β.

Table III. Broadenings and Derived Parameters for Bacteriochlorophyll a and Its Radical Cation[a]

Proton	Rel broadening $(\Delta\nu_{ex})$	Absolute a_H, MHz[b]	Q, MHz	ρ_c
α	0.20	4.30 ($\pm$0.43)	76	0.057
β	0.01	0.95 ($\pm$0.13)	76	0.013
δ	0.15	3.70 ($\pm$0.37)	76	0.049
1a	0.45	6.40 ($\pm$0.64)	97	0.066
2b	0.0008	0.27 ($\pm$0.05)	97	0.003
3	>1.0[c]	>9.5	136	>0.07
4	>1.0[c]	>9.5	136	>0.07
5a	1.000	9.5[d]	97	0.098
7	>1.0[c]	>9.5	100	>0.095
8	>1.0[c]	>9.5	120	>0.079
10	0.11	3.1 ($\pm$0.3)	153	0.02
3a	0.005	0.67 ($\pm$0.15)		
4a,7a,7b	0.002	0.43 ($\pm$0.10)		
4b	0.002	0.43 ($\pm$0.10)		
8a	0.003	0.52 ($\pm$0.12)		
10b	<0.0001	<0.1		
Phytyl	<0.0001	<0.1		

[a] [3] ≤ 70 mM in acetone-d_6; 270–325 K; fast exchange; **5** as oxidant. [b] $a_H = 9.5(\Delta\nu_{ex})^{1/2}$ MHz. [c] Not measurable due to overlapping. [d] ENDOR value.[5]

Table IV. Conditions for Line Broadening Experiments

Expt no.	Chlorophyll	Oxidant	Solvent
1	1	5	Acetone-d_6
2	1	6	Acetone-d_6
3	1	5	Acetone-d_6-pyridine (500:1)
4	1	5	CD_3OD–CD_2Cl_2 (1:10)
5	2	5	Acetone-d_6
6	2	6	Acetone-d_6
7	2	5	CD_3OD–CD_2Cl_2 (1:10)
8	2	4	CD_3OD–CD_2Cl_2 (1:10)
9	3	5	Acetone-d_6
10	3	O_2	Acetone-d_6

Table V. Apparent Pyrrole β-Carbon Spin Densities[a]

Carbon	1	2	3
1	4.2	4.6	6.6
3	4.2		
4	3.2	8.5	
5	7.8	7.7	9.8

[a] Percentage of one electron.

With the exception of H$_\beta$ in **1**, there is no experimentally accessible combination of concentration, temperature, and magnetic field which will allow measurement of contact shifts before the resonances are too broad to observe. We have observed small (<0.2 ppm) upfield shifts of H$_\beta$ in **1** at 270 MHz which are consistent with a contact mechanism, but other experiments also indicate a possible pseudocontact contribution.[30]

A few results on the concentration dependence of the broadening apparently indicate type 1 fast exchange for **1**-**1**$^{+\cdot}$ (undegassed) and type 2 fast exchange for degassed solutions.

From solutions of **3**$^{+\cdot}$ in acetone-d_6 we have isolated $\Delta^{3,4}$-bacteriochlorophyll a (2-acetyl 2-devinyl-**1**; R. G. Brereton and J.K.M.S., unpublished results). **1**$^{+\cdot}$ apparently gives "allomerized" products, oxidized at C$_{10}$.[31]

Table VI. Apparent Meso Carbon Spin Densities[a]

Carbon	1	2	3
α	3.0	11.3	5.7
β	1.0	5.6	1.3
γ	0.8	6.2	2.0
δ	5.5	10.9	4.9
Total	10.3	34.0	13.9

[a] Percentage of one electron.

Table VII. Hyperfine Coupling Constants (MHz) Determined by ENDOR and NMR

	Proton	This work	ENDOR Katz[6]	ENDOR Fajer[2]	ENDOR Feher[7]
1	1a, 3a	4.1	2.83, 3.72	2.8	
	5a	7.5	7.56	7.4	
	7,8	>9.0	11.0	11.8	
3	1a	6.4	5.3	4.78	5.0
	5a	9.5	9.8	9.44	9.2
	3, 4, 7, 8	>9.5	14.0	16.38, 13.48 12.92, 11.48	16.0

Discussion

The couplings to the methine protons on the reduced rings are rather large, inasmuch as they can be determined by NMR. They reflect spin densities of around 10% on the pyrrole α carbons. The ring methyl couplings range from 4 to 9.5 MHz, indicating 3–10% spin density on the pyrrole β carbons (Table V); the measured values for H$_{4a}$ in **1** and **2** support these indications but both the observed broadening and Q are less reliable.

In Chl a and Bchl a the coupling constants to the meso protons, and also to H$_{10}$ which senses the unpaired spin density at C$_\gamma$, are comparatively small (Table VI); however, they are relatively much larger in Chl b. In each case H$_\beta$ has much the smallest of the three meso proton hyperfine couplings. Lastly, we can observe that little spin density leaks out onto the proton-bearing substituents: the 2-vinyl group in Chl a and Chl b, the 3a-formyl group in Chl b, and the 2b-acetyl in Bchl a all show only small couplings.

Our results must be compared with those determined by ENDOR (Table VII), remembering that our *absolute* values are derived using the ENDOR values for a_{5a} as a standard to calibrate our *relative* hyperfines. The general pictures produced by NMR and ENDOR are in good agreement for Chl a and Bchl a. Indeed the NMR method provides good assignments for the ENDOR signals. However, in both compounds we find a_{1a} to be larger than ENDOR does.[32] Whether this is a solvent/temperature/concentration effect or a fundamental problem in comparing dissimilar techniques is not clear, but the HOMO is clearly identified as the expected[2,33] one. For Chl b$^{+\cdot}$ we find strong indications of a different spin density distribution, consistent perhaps with the slight occupancy of a thermally accessible empty orbital,[2,33] but ENDOR experiments have not confirmed these results.[2,26] The possibility remains that part of our observed line broadening arises from additional unidentified exchange processes.

In no case is a specific meso, H$_{10}$, or vinyl hyperfine coupling observed by ENDOR. This is unsurprising for the frozen samples, but even in the solution spectra of **3**$^{+\cdot}$ when the methine protons on rings B and D give well-resolved signals[5] no couplings to H$_\alpha$, H$_\beta$, H$_\delta$, or H$_{10}$ greater than 1.52 MHz are seen. However, ENDOR intensities are unreliable and an apparent absence of couplings is not uncommon. On theoretical grounds, ENDOR intensities are expected to decrease as the hyperfine coupling decreases.[34]

Recent MO studies on **1**$^{+\cdot}$ include simple Hückel,[35] self-

consistent Pariser–Parr–Pople,[36] and 340 electron ab initio calculations.[37] Each predicts for the experimentally found orbital virtually no spin density at the meso positions and none predicts the remarkable differences between H_{1a} and H_{5a}.

In the very dilute solutions used in this work fast exchange is difficult to achieve solely through electron transfer, particularly where large a_H are involved. The presence of dissolved oxygen acting as an electron "relaxation reagent" is then beneficial, although it masks the electron transfer kinetics.

Conclusions

The NMR and ENDOR approaches to hyperfine coupling constants in large molecules are complementary. NMR provides a detailed pictorial determination of a large number of relative hyperfine coupling constants without recourse to chemical modification or biosynthetic manipulation. It is particularly good for small couplings, and holds the promise of insight into the dynamics of the system, but its precision is relatively poor. ENDOR provides precise data on an absolute scale and is applicable to intact reaction centers or organisms.

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