methyl hyperfine coupling constants of dimer cations.[18,19]

As yet, we have not explained the significant intensity in the wings (17–19 MHz) of the *in vivo* spectrum. If a small amount of monomeric BChl$\cdot^+$ were present in the "*in vivo*" systems, it could account for these wings. An alternate interpretation seems more tenable. *R. rubrum* cultured in ^2H_2O with [^{1}H]succinic acid as substrate gives similar endor spectra to those in Figure 1, except that the wings are absent. The bacteriochlorophyll isolated from organisms grown in this way contains no ^{1}H at the methine positions.[20,21] The endor spectra from bacteriochlorophyll of this unusual isotopic composition indicate that only methyl groups give rise to the peaks associated with a_1 and a_2. In addition, [^{2}H]BChl in which the α- and β-methine protons have been exchanged for ^{1}H[22] gives weak endor resonances between 16 and 20 MHz. Furthermore, a weak endor signal can be observed from [^{1}H]BChl itself from 18 to 21 MHz when signal-to-noise is optimal. These observations support the view that the weak broad wings in the endor spectra arise in part from anisotropic methine protons and the intense narrow peaks arise from isotropic methyl groups. The special pair model predicts sharpening of these broad weak peaks such that they would be more intense for *in vivo* systems.

As we have been unable to devise any other simple model that appears to be consistent with both the endor and epr spectra, we conclude that the 9-G epr signal associated with photosynthesis in purple bacteria has a line width consistent with delocalization of the unpaired electron over two active-center bacteriochlorophyll molecules.[23] We have carried out similar experiments on chlorophyll a free radicals in algae and have arrived at essentially similar conclusions.

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Electron Spin Resonance of [^{25}Mg]Chlorophyll a[1]

Sir:

The electron spin resonance (esr) signal from oxidized chlorophyll is widely accepted to originate in a π-cation radical.[2,3] The details of the π cation are still obscure. While it has been assumed that the unpaired electron is fully delocalized over the entire chlorophyll macrocycle as a true π-cation radical, the possibility that spin on ^{25}Mg (10.13% natural abundance)[4] might contribute to the esr spectrum has not been previously investigated. Esr studies of *in vivo* chlorophyll systems[2,5] or some fraction of the photosynthetic apparatus[6–11] have been interpreted in terms of photooxidation of chlorophyll in the photosynthetic reaction center.[12] Whereas the reversible photo-esr signal from green plants,[13] signal I, is characterized by a Gaussian peak, a g value of 2.0025, and a line width of about 7 G, the signals recorded from *in vitro* chlorophyll a monomer systems are approximately 9 G in width.[3] The long-known discrepancy between the signal from Chl a$\cdot^+$[14] and the *in vivo* signal has recently been explained as arising from spin delocalization over two adjacent chlorophyll molecules positioned in a special way.[15]

Analysis of esr spectra of π-electron radicals is facilitated by application of the theory of second moments,[16,17] particularly if applied to radicals of different isotopic composition. Since in our experiments we encounter only Gaussian line shapes, it is proper to apply eq 1 to obtain the second moment, $\langle \Delta H^2 \rangle$, where

$$\langle \Delta H^2 \rangle = {}^1/_4(\Delta H_{pp})^2 \quad (1)$$

ΔH_{pp} is the first derivative, peak-to-peak, absorption line width. Second moment analysis of esr signals from chlorophyll of unusual isotopic composition may then be formulated as follows[15]

$$\langle \Delta H^2 \rangle_{^1H} = 15.91 \langle \Delta H^2 \rangle_{^2H} \quad (2a)$$

$$\langle \Delta H^2 \rangle_{\text{total},\,^1H} = \langle \Delta H^2 \rangle_x + (0.1013)\langle \Delta H^2 \rangle_{^{25}Mg} + \langle \Delta H^2 \rangle_{^1H} \quad (2b)$$

$$\langle \Delta H^2 \rangle_{\text{total}\,^2H} = \langle \Delta H^2 \rangle_x + (0.1013)\langle \Delta H^2 \rangle_{^{25}Mg} + \langle \Delta H^2 \rangle_{^2H} \quad (2c)$$

$$\langle \Delta H^2 \rangle_{\text{total},\,^1H,\,^{25}Mg} = \langle \Delta H^2 \rangle_x + (0.99)\langle \Delta H^2 \rangle_{^{25}Mg} + \langle \Delta H^2 \rangle_{^1H} \quad (2d)$$

where $\langle \Delta H^2 \rangle_x$ is the contribution to the second moment from atoms other than hydrogen and ^{25}Mg, *i.e.*, ^{14}N, ^{13}C, and g anisotropy. The other subscripts identify

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the second moment contributions of each particular nuclear species. Equations 2b and 2c are for natural abundance ^{25}Mg and eq 2d applies to 99% enriched ^{25}Mg. Because g anisotropy typically broadens lines asymmetrically, and even in spectra of fully deuterated algae,[15] which give very narrow Gaussian esr lines, the spectrum is highly symmetric, we assume g anisotropy is negligible here. With this premise, and using the ΔH_{pp} values of ^2H and ^1H Chl a$\cdot^+$ of 4.2 and 9.3 G,[15] respectively, solution of eq 1, 2a, 2b, and 2c yields a value of about 15% for the contribution to the line width from sources other than protons.

We expect an increase in line width for highly enriched ^{25}Mg Chl if the unpaired electron of the free radical interacts with Mg. An increase of 0.5 G in line width would be detectable. Since the line shape of Chl a$\cdot^+$ is Gaussian and we have only a single ^{25}Mg atom per unpaired spin, second moment theory can be applied rigorously to determine the minimum coupling constant observable for ^{25}Mg. By definition the second moment for a stick spectrum of ^{25}Mg (spin $^5/_2$) is

$$\langle \Delta H^2 \rangle_{^{25}Mg} = {}^{35}/_{12} A_{^{25}Mg}{}^2 \qquad (3)$$

where $A_{^{25}Mg}{}^2$ is the root-mean-square ^{25}Mg hyperfine coupling constant. We assume that the minimum line-width change that we could reliably detect would result in a 9.8-G line width, *i.e.*, $\langle \Delta H^2 \rangle_{total,\,^1H}$ is $^1/_4$(9.3 G)^2 and $\langle \Delta H^2 \rangle_{total,\,^2H,\,^{25}Mg}$ is $^1/_4$(9.8 G)^2. Thus, eq 1, 2c, 2d, and 3 indicate that an increase of 0.5 G in line width would be produced by a 0.9-G ^{25}Mg hyperfine coupling constant.

We have grown *Phormidium luridum* on a medium in which the magnesium (obtained from Oak Ridge National Laboratory) was 99% ^{25}Mg, according to the procedures of DaBoll, *et al.*[18] Chlorophyll was extracted by the method of Strain and Svec.[19] Preparation of chlorophyll samples for esr was performed as previously described.[15] Spectra were recorded on a Varian E9 spectrometer with 100-kHz modulation and a maximum modulation amplitude of one-third of the line width in a TE_{104} mode cavity equipped with a low-temperature quartz dewar insert. Microwave power was 2 mW. Calibration procedures were those of Norris, *et al.*[15] For increased precision, data were acquired with a Fabritek time-averaging device (Model 1072) on-line to the Argonne Chemistry Division central Sigma V computer.

Esr spectra of monomeric Chl a oxidized with I_2 in CH_3OH–CH_2Cl_2 (1:1) at both room temperature and $-160°$ consisted of single Gaussian lines, whether the chlorophyll contained ^{24}Mg or ^{25}Mg. At $-160°$, the line width for [^{25}Mg]Chl a was found to be 9.3 ± 0.5 G. [^{24}Mg]Chl a yields a line width of 9.3 ± 0.3 G at that temperature.[15]

Esr spectra of aggregated Chl a hydrates[20,21] containing ^{24}Mg or ^{25}Mg were also found to be indistinguishable. [^{25}Mg](Chl a$\cdot H_2O)_n$ oxidized by exposure to red light or chemically with I_2 has a line width of ~1.2 G (based on two determinations). [^{24}Mg](Chl a$\cdot H_2O)_n$ under the same conditions has the same line width for the photosignal.

The esr spectra of oxidized Chl a with natural abundance Mg and those of 99% [^{25}Mg]Chl a are thus indistinguishable. If significant spin density were in the magnesium 3s orbital, a difference in spectra from the two isotopic species should have been observed. Our data indicate that the ^{25}Mg coupling constant is less than 1 G; how much less is not known. The free ion coupling constant of ^{25}Mg(II) is 247.2 G.[22] Thus, our data indicate that the unpaired electron spin has little magnesium s character (less than 1/250). To relate this figure to another example of an unpaired electron interacting with ^{25}Mg, we note that the isotropic coupling constant in F centers with ^{25}Mg (4 G)[23] is consistent with an electron with very little s character (~1/60). Our data confirm the π nature of the Chl a free radical, and are thus consistent with conclusions arrived at in other recent studies on porphyrin π cations of biochemical significance.[24]

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Nuclear Magnetic Resonance Studies of a Polypeptide in a Nonprotonating Solvent System

Sir:

Many polypeptides undergo a helix to coil transition as the solvent composition is varied, and trifluoroacetic or dichloroacetic acids (TFA, DCA) are the most commonly used helix breakers for inducing the transition. A strong solvation of the peptide groups by hydrogen bonding with acid in the coil form is the usual explanation offered for breakdown of the helix.[1] However, it has been proposed that the acid protonates the amide groups and the resulting electrostatic repulsions result in helix breakdown[2] and a coil form at least partially protonated. Nmr spectra in this journal and elsewhere[3] have been adduced as supporting protonation, although the spectral interpretations have been questioned.[4] The evidence opposing the idea of protonation is considerable.[1,5] However, none has been unequivocal and this communication presents nmr data that prove beyond doubt that protonation is not an essential step in helix breakdown.

It is now well established[6] that for low molecular

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