coupling of C-H_β protons in the substituted alkoxy radicals and proposed that $B_0 = 5$ G and $B_2 = 94$ G for the $a_\beta = B_0 + B_2 \cos^2 \theta$ rule. These constants give $a_\beta = 52$ G for a freely rotating methyl group in an excellent agreement with our value for $CH_3O\cdot$. In addition, if the contribution from the anisotropic coupling is neglected, the E line splitting is expected to be 47 G from these constants in agreement with the observed splitting of 47 G. These results indicate that substituted alkoxy radicals found in very different crystals such as nucleosides and nucleotides have essentially the same electronic structure as that of the most fundamental species $CH_3O\cdot$ in methanol crystals.

$CH_3O\cdot$ in polycrystalline methanol is unstable at 77 K and disappeared after annealing for a few minutes at 77 K. During the annealing at 77 K, $\dot{C}H_3$ radicals also disappeared with a concommittant increase of $\dot{C}H_2OD$ radicals, suggesting that both the $CH_3O\cdot$ and $\dot{C}H_3$ radicals were converted into $\dot{C}H_2OD$. Besides these isolated radicals, we have also found from the $\Delta M_s = \pm 2$ spectra that radical pairs between $\dot{C}H_3$ and $\dot{C}H_2OD$ are formed. After annealing at 77 K, $\dot{C}H_3$ in this radical pair was converted into $\dot{C}H_2OD$ forming a pair between two $\dot{C}H_2OD$ radicals. This is consistent with the radical conversion of isolated $\dot{C}H_3$ radicals. The details of the results obtained from the species other than $CH_3O\cdot$ will be reported elsewhere.

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^{25}Mg Hyperfine Coupling in the Bacteriochlorophyll Radical Cation

Sir:

Much of our understanding of the nature of the reactants which take part in the primary electron-transfer steps in photosynthesis has come from applications of magnetic resonance techniques (EPR and ENDOR). For instance, it was shown that the reversible photo-EPR signal observed in green plants and photosynthetic bacteria (signal I) arises from photooxidation of a pair of chlorophyll molecules known as the "special pair".[1,2] Subsequently, much effort has been spent on elucidating the electronic structure of the π-cation radicals of chlorophyll and related systems.[2-5] The possibility that spin density on ^{25}Mg (10.05% natural abundance) might contribute to the EPR spectrum of the chlorophyll a radical cation was recently investigated.[6] No difference was observed in comparing EPR line widths of the chlorophyll a radical cation

containing the natural abundance of ^{25}Mg with chlorophyll a highly enriched in ^{25}Mg. It was concluded that the ^{25}Mg hyperfine coupling constant was <1 G. It was shown in that work that the contribution to the second moment of the EPR line from sources other than protons is ~15%. On the other hand, a similar analysis shows that the contribution to the second moment of the EPR line from sources other than deuterons in fully deuterated chlorophyll a radical cations is ~74%! Thus, by using fully deuterated chlorophyll highly enriched in ^{25}Mg it should be possible to detect any contributions of a ^{25}Mg coupling to the second moment. We have carried out this experiment using perdeuterobacteriochlorophyll a (^{2}H Bchl) and ^{2}H Bchl enriched to 92% in ^{25}Mg, and have found that there is indeed a small interaction with the magnesium.

The techniques for growing fully deuterated *Rhodospirillum rubrum* have been described.[7] For isotopic enrichment the growth medium contained 97.9% ^{25}Mg as MgO[8] as the sole magnesium source. Bacteriochlorophyll was extracted by the method of Strain and Svec.[9] It was found to contain 92% ^{25}Mg by mass spectrometric techniques.

Bacteriochlorophyll cations were produced by I_2 oxidation in methanol-methylene chloride (1:1). Spectra were recorded on a Varian E9 spectrometer on line with the Argonne Chemistry Division Sigma 5 computer. Modulation amplitudes did not exceed 0.1 of the line width, and microwave powers were 0.5 mW at room temperature and 2 μW at 77 K. Temperature regulation was achieved with an Air Products Heli-Tran system used with an Oxford Instruments temperature controller.

Samples of the fully deuterated bacterium *R. rubrum* containing either natural abundance or 92% ^{25}Mg were made either in 0.1 M tris-DCl (pH 7.4) or 0.01 M glycylglycine buffer (pH 7.4) or 50% buffer–50% glycerol. Light of wavelength greater than 530 nm was used to produce signal I.

For ^{2}H Bchl radical cation one expects the contribution to the second moment from sources other than deuterons to be ~66%. These other contributions are the source of slight asymmetries observed in the low-temperature EPR signal of this species so that in these experiments it is necessary to carry out a numerical integration[10,11] in order to determine the second moments of the EPR spectra.

To obtain the ^{25}Mg hyperfine coupling we have

$$\langle \Delta H^2 \rangle_{^{25}\text{Mg}} = (1/0.8195)(\langle \Delta H^2 \rangle_{^2\text{H},^{25}\text{Mg}(92\%)} - \langle \Delta H^2 \rangle_{^2\text{H},^{25}\text{Mg}(10\%)})$$

and

$$\langle \Delta H^2 \rangle_{^{25}\text{Mg}} = (35/12) A^2_{^{25}\text{Mg}}$$

where $\langle \Delta H^2 \rangle_{^2\text{H},^{25}\text{Mg}(x\%)}$ is the total second moment observed for ^{2}H Bchl cation containing the given percent of ^{25}Mg, and $\langle \Delta H^2 \rangle_{^{25}\text{Mg}}$ is the calculated contribution of the ^{25}Mg coupling to the second moment. $A_{^{25}\text{Mg}}$ is the root-mean-square ^{25}Mg hyperfine coupling constant.

Furthermore

$$[(12/35)\langle \Delta H^2 \rangle_{^{25}\text{Mg}}^{300\text{K}}]^{1/2} = (a^2)^{1/2}$$

and

$$[(12/35)\langle \Delta H^2 \rangle_{^{25}\text{Mg}}^{77\text{K}}]^{1/2} = \left(a^2 + \tfrac{1}{3} \sum_{\substack{i=x, \\ y,z.}} b_{ii}^2 \right)^{1/2}$$

where a is the ^{25}Mg isotropic hyperfine coupling constant and b_{ii} represents the components of the dipolar coupling tensor. Measurements made at the two temperatures allow one to determine both a and b_{ii}. The results are given in Table I.

Using the mean values in Table I the analysis yields

$$|a_{^{25}\text{Mg}}| = 0.23 \text{ G}$$

$$T = \pm(0.69, 0.69, -0.69 \text{ G}) \text{ or } \pm(0.23, 0.23, -1.15 \text{ G})$$

Table I. Total Second Moments Obtained for ^2H Bchl ($X\%$ ^{25}Mg) at Two Temperatures

$X\%$ ^{25}Mg	300 K, G^2	77 K, G^2
10% ^{25}Mg	6.30 ± 0.23^a	7.93 ± 0.13
92% ^{25}Mg	6.43 ± 0.08	9.05 ± 0.23

^{a} Standard deviation evaluated from several determinations. For 300 K the difference in second moments is not statistically significant; however, the difference is always in the same direction, and we believe is indicative of a small coupling.

where G = gauss and T is the hyperfine coupling tensor assuming axial symmetry.

In comparing the second moments of the light-induced signal I in fully deuterated *R. rubrum* with those produced in fully deuterated *R. rubrum* enriched to 92% in ^{25}Mg there was no change within experimental error. In fact, we have calculated that this is the expected result for a monomeric hyperfine splitting of <0.3 G. Likewise, no change is expected in the low-temperature dimer second moments when we consider the observed monomer differences (Table I). This is yet another indication of the "special pair" nature of signal I in bacteria since this shows a reduction in the ^{25}Mg hyperfine coupling compared with the value determined for monomeric bacteriochlorophyll.

We can compare our determination of the ^{25}Mg isotropic hyperfine coupling in bacteriochlorophyll with values obtained for metal couplings in the related radicals of zinc *meso*-tetraphenylporphyrin (ZnTPP$^+$), ^{67}Zn coupling of 1.22 G,[12] and cobalt(III) tetraphenylporphyrin (Co^{III}TPP^{2+}), ^{59}Co coupling of 5.7 G,[13] and cobalt(III) octaethylporphyrin (Co^{III}OEP^{2+}), ^{59}Co coupling of 1.4 G.[13] The free ion isotropic coupling constant of ^{25}Mg is 692 MHz,[14] of $^{67}Zn^{II}$ is 1686 MHz,[15] and of $^{59}Co^{III}$ is 3666 MHz.[16] Thus the degree of metal s character is <1% in all three cases and may reflect a correlation of the ground-state wave functions for the three cations.[4,12] The relatively small coupling in Co^{III}OEP^{2+} has been shown by Fajer to arise from a change in the ground-state wave functions relative to that of Co^{III}TPP^{2+}.[12]

It is significant that the result presented here provides a new probe of the nature of the primary reactants in bacterial photosynthesis. Although we cannot distinguish between signal I in the bacteria enriched in ^{25}Mg to that in bacteria containing the natural abundance of ^{25}Mg by conventional EPR techniques, we are using electron spin echo techniques to make this comparison.[17]

From MO calculations on the bacteriochlorophyll radicals, we expected[2] and have found[18] a considerably larger ^{25}Mg hyperfine coupling in the bacteriochlorophyll radical anion. We are using this to identify the first electron acceptor in bacterial photosynthesis.

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Dioxetanone Chemiluminescence by the Chemically Initiated Electron Exchange Pathway. Efficient Generation of Excited Singlet States[1]

Sir:

Many recent studies of chemiluminescence from organic molecules have centered on the reactions of the strained four-membered-ring peroxides known as dioxetanes.[2] These molecules have been identified or implicated in many of the most efficient chemi- and bioluminescent reactions. In particular, a carbonyl substituted dioxetane (dioxetanone) has been suggested as the key high energy molecule responsible for light production in the firefly.[3] In this paper we report our findings on the mechanism of light production from simply substituted dioxetanones. Our studies show that the most important light forming path for reaction of dioxetanone **1** is the bimolecular route which we have recently identified as chemically initiated electron-exchange luminescence (CIEEL).[4] Moreover, we have found that under conditions favoring CIEEL the fraction of reacting dioxetanone molecules that generate a photon of light approaches the most efficient bioluminescent reactions known. In addition, by analogy, it appears that the initiating reaction in firefly bioluminescence is an intramolecular electron transfer akin to the observed intermolecular reaction reported herein.[5]

Dimethyldioxetanone (**1**) was prepared and purified according to the procedure of Adam.[6] Thermolysis of **1** in
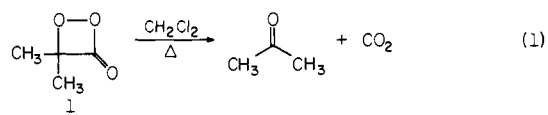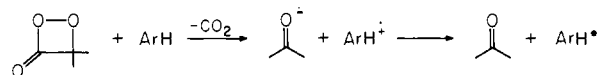
CH_2Cl_2 at 24.5 °C leads to the quantitative generation of acetone and to light emission.[7] The observed chemiluminescence under these conditions is a result of acetone emission. It has been reported previously that the addition of certain aromatic hydrocarbons to solutions of dioxetanone **1** results in the generation of hydrocarbon luminescence and markedly increases