β-Mercuri bromo peroxides **2a** and **3a** were independently subjected to demercuration with basic $NaBH_4$,[25] a reaction also known to involve alkyl radical intermediates.[26] **2a,** the equatorial isomer, leads to a 50:50 mixture of peroxide **2d** and epoxy alcohol **4**, the S_Hi product. In contrast, the axial isomer **3a** leads cleanly to peroxide **3d** with no epoxy alcohol, **4,** being observed. The presumed radical intermediates **2c** and **3c** thus show the same reaction pathway preferences in these reactions as were observed in the tin hydride reaction of the β-bromoperoxides **2b** and **3b.**

Inspection of molecular models, as well as the X-ray crystal data, for equatorial **2a** suggest that a colinear radical-peroxide bond arrangement is easily achieved for this radical isomer, **2c.** In fact, the $\cdot CH_2$-C-O-O torsion angle for the equatorial isomer is 176 (2)°, not significantly different from the ideal value of 180°. Thus, the geometry of this isomer is fixed in an array that favors back-side radical substitution on the peroxide bond. The axial radical **3c** is constrained to attack the peroxide bond by a side-on pathway. The crucial $\cdot CH_2$-C-O-O torsion angle for this isomer is 60°, a geometry which apparently does not offer the possibility of substitution.

We conclude that carbon radical substitution on the peroxide bond does, indeed, follow a colinear approach pathway. The S_H2 and S_N2 pathways for reaction on first-row elements are thus apparently similar, with back-side displacement being the rule.

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Supplementary Material Available: Tables of atomic positional and thermal parameters, interatomic distances and angles, and a list of observed and calculated structure amplitudes (16 pages). Ordering information is given on any current masthead page.

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(12) ^1H and ^{13}C NMR spectra, as well as C and H analyses, were consistent with the structures of all new compounds reported.
(13) *trans*-2-Vinylcyclohexanol was prepared by $LiAlH_4$ reduction of *trans*-2-vinylcyclohexanecarboxaldehyde. The aldehyde was prepared by addition of lithium divinylcuprate to 1-cyclohexenecarboxaldehyde.
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(15) LC was carried out on a Whatman "Magnum 9" μ silica column with 5% CH_2Cl_2, 25% $CHCl_3$, 70% C_6H_{14} solvent.
(16) $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
(17) Intensity data were recorded on an Enraf-Nonius CAD-3 automated diffractometer (Ni-filtered Cu Kα radiation, λ = 1.5418 Å; θ–2θ scans) by use of procedures detailed elsewhere; see, e.g., C. D. Garner, N. C. Howlader, F. E. Mabbs, A. T. McPhail, R. W. Miller, and K. D. Onan, *J. Chem. Soc., Dalton Trans.,* 1582 (1978). Although the colorless lathlike crystals are stable over long periods when exposed to ordinary light, they darken and deteriorate fairly rapidly in the X-ray beam. Consequently, it proved necessary to use three different crystals to obtain a complete data set; measurements from different crystals were put on a common scale by comparison of intensities from a number of common reflections. Data from the individual crystals were corrected for crystal deterioration by remeasuring the intensity of a strong reflection after each batch of 99 measurements and scaling the data appropriately. Absorption corrections were also applied to the intensity data.
(18) The C(1)-O(2)-O(3)-C(4) torsion angle is −75 (3)°. With a mean endocyclic torsion angle of 64° the 2,3-dioxacyclohexane ring is overall more puckered than the cyclohexane ring in **2a** where the corresponding value of 58° is close to normal.
(19) Displacements of atoms from the O(3'), Br'', Br''' plane follow: Hg, −0.083; C(11), −2.176; Br, 2.343; O(2), −2.511; O(3), −3.060.
(20) Angles subtended at O(3') follow: O(2')-O(3')-Hg, 115 (2); C(4')-O(3')-Hg, 131 (2)°.
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(22) **4** was independently prepared by epoxidation of *trans*-2-vinylcyclohexanemethanol.
(23) Greater than 90% product accountability was attained in all the tin hydride reactions reported here.
(24) The trace amounts of **4** found in the axial isomer reaction may possibly be formed from traces of **2b** present in **3b.** The LC separation of **2a** and **3a** had an α value of 1.15 and it seems likely that trace **2b** (undetectable by ^{13}C NMR and LC) was present in **3b.**
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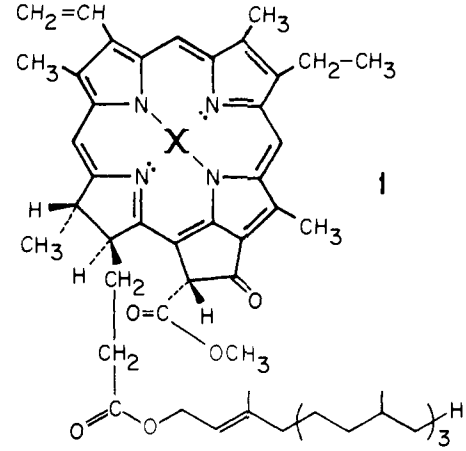
Hydration Behavior of Chlorophyll *a*: A Field Desorption Mass Spectral Study

Sir:

In vivo and in vitro studies of chlorophylls have indicated that chlorophyll-chlorophyll[1,2] and chlorophyll-water[3,4] interactions are central to an understanding of chlorophyll function in photosynthesis.[5,6] This study examines in more detail the nature of chlorophyll-chlorophyll and chlorophyll-water interactions by use of field desorption mass spectrometry.[7]

The literature contains conflicting reports concerning the possibility of obtaining anhydrous chlorophyll *a*[1a] in vitro and the stoichiometry of in vitro chlorophyll hydrates. One group has reported a structure and spectrum for anhydrous chlorophyll *a* dimers,[8a] a photoelectron spectrum for anhydrous chlorophyll *a*,[8b] and has subsequently stated "We have been unable to observe anhydrous chlorophyll."[8c] The data presented below indicate that (1) it is possible to obtain anhydrous chlorophyll *a* under mild conditions; (2) it is possible for chlorophyll *a* to exist with only tetracoordinate magnesium; and (3) field desorption data appear to favor a 1:1 stoichiometry for hydrated chlorophyll *a*.

Chlorophyll *a* (**1a**) and pheophytin *a* (**1b**) were prepared by standard procedures;[9] anhydrous chlorophyll *a* was obtained by dissolution in carbon tetrachloride followed by evaporation



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1a) – chlorophyll a; X = Mg^{II}
1b) – pheophytin a; X = H,H

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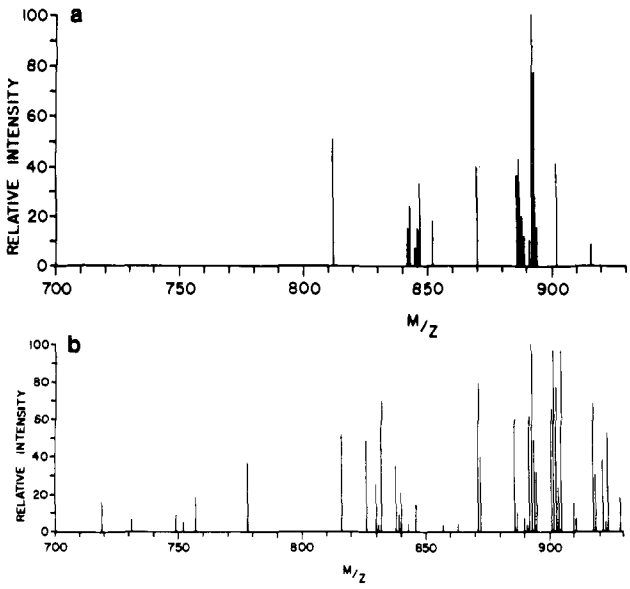

Figure 1. (a) High mass region of the FD spectrum of anhydrous chlorophyll *a* (summed over 0–24-mA emitter heating current). (b) High mass region of the FD spectrum of hydrated chlorophyll *a* (summed over 0–24-mA emitter heating current).

of the solvent on a vacuum line. By general chemical standards this is a mild procedure. Chlorophyll *a* prepared in this way has a visible absorption maximum at 680 nm when dissolved in dry carbon tetrachloride. Although other authors have claimed that chlorophyll preparations that absorb in the 680-nm region are oligomers of chlorophyll *a* monohydrate,[8] independent analytical evidence indicates that there was <1 water molecule/100 chlorophyll molecules in chlorophyll samples prepared as described above.[10] The field desorption data presented below are completely consistent with this being a dry chlorophyll preparation.

Field desorption (FD) mass spectra were obtained with a Varian CH-5DF double focussing mass spectrometer: extraction voltage, −(8–10 kV); filter, 1000 Hz; SEM, −2.0 to −2.2 kV; scan rate, 6 s/decade; resolution, 1000. All data were collected with a Varian MAT SS-100 computer. Emitters were activated with a Varian activation chamber and only emitters with a uniformly dense microneedle growth produced satisfactory results. Emitter heating was controlled manually because the 0.1–0.2-mA heating bursts obtainable in this way produced the greatest response for molecular ions in all cases. The best anode temperature for chlorophyll *a* was obtained with slightly less than 24 mA; for pheophytin *a* the best emitter heating current was 15.2 mA, corresponding to an emitter temperature of ~260 and 188 °C, respectively.[7d,e]

Figure 1a presents a summed spectrum (over emitter heating currents of 0–24 mA) for the molecular ion region for anhydrous and nucleophile-free chlorophyll *a* that was applied to the emitter in dry carbon tetrachloride solution. In our experience summed spectra are considerably more reproducible than individual spectra which are much more susceptible to variations in intensity caused by emitter hotspots. The molecular ion, *m/z* 892, is the base peak in this region of the spectrum and is assigned to chlorophyll $a^{+\cdot}$ in which the central Mg atom has a coordination number of 4. The isotope ratio for *m/z* 893 to *m/z* 892 was 76.9% vs. a theoretical value for chlorophyll *a* of 77.2%. This agreement is remarkably close for FD spectra. The ions for which probable structures can be assigned in this region of the spectrum include *m/z* 870, the molecular ion of pheophytin *a*; *m/z* 852, pheophytin less water; *m/z* 902, pheophytin plus methanol; *m/z* 916, chlorophyll *a* plus magnesium; and *m/z* 886, chlorophyll *a* less magnesium plus water. The ion at *m/z* 902 seems certain to be associated

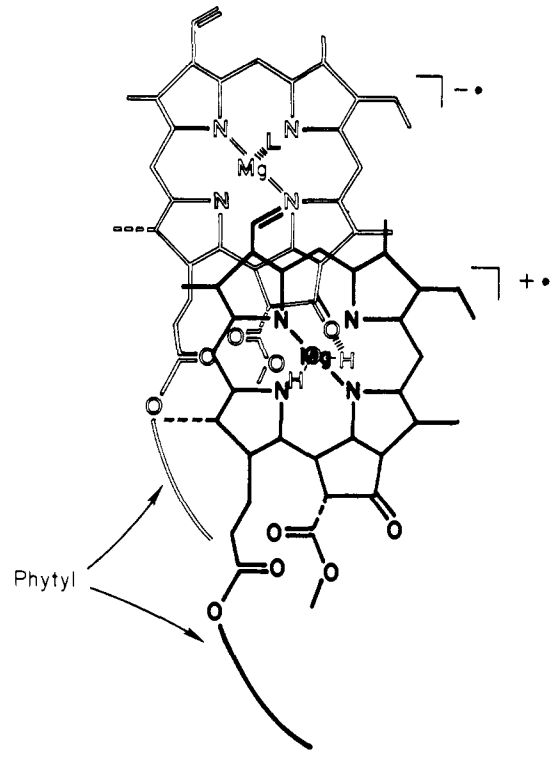

Figure 2. Postulated charge-transfer excited state in the photosynthetic reaction center chlorophyll *a* special pair. Electron transfer to the primary electron acceptor in photosynthesis, L (at the back of the figure), would produce the known[1,3] special pair radical cation.

with pheophytin *a* as the intensity of this peak varies with the intensity of the pheophytin ions in the spectrum. This ion is most probably formed by transfer of the carbomethoxy group and a hydrogen from one pheophytin to another.

The high mass portion of the FD spectrum of anhydrous pheophytin *a* contained only three ions: *m/z* 870 (100%, $M^{+\cdot}$), 811 ($M - CH_3CO_2$, 24%), and 759 (25%). When pheophytin *a* was hydrated by shaking the CCl_4 solution with water prior to application to the emitter, intense ions appeared at *m/z* 901, 902, and 903. Methanol transfer from one molecule to the next may have been involved in the formation of these ions.

Hydration of chlorophyll *a* by shaking a carbon tetrachloride solution against water prior to application to the emitter dramatically increased the intensity of the resulting FD mass spectrum. The absolute intensity of the chlorophyll *a* molecule ion (all instrument parameters held constant) for the anhydrous chlorophyll *a* was 0.058 V while the corresponding ion in the spectrum of hydrated chlorophyll *a* had an intensity of 0.17 V. The roughly threefold increase in spectral intensity indicated by this comparison is considerably less than the actual intensity increase as the number of ions above threshold more than doubled on hydration of the chlorophyll sample (Figure 1b).

The presence of the water altered the FD mass spectrum and produced large numbers of even electron ions rather than odd electron ions as seen in Figure 1a. The amount of pheophytin *a* and the pheophytin adducts at *m/z* 902 and 903 substantially increased relative to the intensity of the protonated chlorophyll and the chlorophyll molecular ion. The pheophytin concentration in the original chlorophyll sample was <0.1% as shown by chromatography and visible spectroscopy. The pheophytin is an artifact, produced by reaction with water on Lewis acid sites of the emitter.

The base peak from the anhydrous chlorophyll sample, *m/z* 278 (the hydrocarbon portion of the phytyl side chain), did not appear in the spectrum of the hydrate. The ion at *m/z* 910 in the FD mass spectrum of hydrated chlorophyll *a* may corre-

spond to the molecular ion of the chlorophyll *a* monohydrate, Chl $a \cdot H_2O^+ \cdot$. It seems unlikely that this ion is the result of adduct formation after ionization of the chlorophyll because postionization adduct ions are generally even electron species.[11a,b] In cases where molecules specifically interact with water, e.g., when crystals contain stoichiometric amounts of water, then $MH_2O^+ \cdot$ ions are seen in FD spectra presumably as a result of ionization of the associated adduct.[11c] The fact that the relative intensity of the ion at *m/z* 910 was less than one third the intensity of the molecule ion (*m/z* 892) and less than one fifth the intensity of the protonated molecule (*m/z* 893) suggests that the coordinate-covalent bond in the hydrate is not unusually strong, as molecules with single bond strengths in excess of 30 kcal/mol routinely survive FD analysis.[11c]

The ion at *m/z* 929 in the hydrate spectrum (Figure 1b) corresponds to the protonated dihydrate of chlorophyll *a*. This ion, being an even electron ion, is probably a postionization adduct ion.

The results reported above support the view that, contrary to assertions in the literature,[1b,8c] it is possible (under admittedly unusual circumstances) to obtain chlorophyll *a* without coordinated water or any additional ligand on the central magnesium atom. The fact that the intensity of the mass spectrum substantially increased as did the intensity of the chlorophyll *a* molecular ion when the chlorophyll was hydrated prior to FD analysis suggests that carbonyl groups can effectively compete with water for coordination to the central magnesium of chlorophyll. The results indicate that the lattice energy of chlorophyll *a* hydrate is lower than that of anhydrous chlorophyll *a* in which the chlorophylls are associated by carbonyl-magnesium coordination.[1]

The anhydrous chlorophyll lattice is held together by magnesium-chlorophyll carbonyl coordination interactions.[1] The hydrated chlorophyll lattice is held together by coordination of water with the central magnesium atom and hydrogen bonding between the water and an adjacent chlorophyll carbonyl function. Since magnesium-oxygen coordination bonds are generally stronger than hydrogen bonds, the increase in intensity of the FD spectrum on hydration of the chlorophyll is to be expected. The anhydrous chlorophyll lattice is an interesting model for antenna chlorophyll in vivo.[1b,5d]

Water serves a role in the organization of the photoreaction center chlorophyll special pair[3b,5a] because it can bind chlorophylls by both magnesium-oxygen coordination and hydrogen bonding to carbonyl groups. The direction of water coordination in an unsymmetrical chlorophyll *a* special pair[3b,5a] would direct formation of a charge-transfer excited state of the special pair (Figure 2)[12] as the first step in photosynthesis. Removal of one electron by the primary electron acceptor in photosynthesis (L in Figure 2) would give the radical-cation dimer which is observed in EPR and ENDOR experiments[1,3] and would have a greater energy economy than a one-electron oxidation of a special pair which had been charge polarized by proton transfer.[8c] This model is compatible with the radical-pair mechanism for the primary light conversion event in photosynthesis.[13] We expect that continued investigation of the desorption behavior of chlorophyll *a* and its hydrates will shed light on this electron-transfer process.

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Natural Abundance ^{13}C NMR Spectroscopy of Double-Stranded DNA

Sir:

Although ^{13}C NMR spectroscopy has already proved extremely useful in studies of biopolymers,[1] including t-RNA's,[2] and single-stranded polynucleotides,[3] no successful study of native double-stranded DNA has been reported.[4] This failure is mainly due to extremely unfavorable ^{13}C spin relaxation parameters (very short T_2's and long T_1's) expected for the long and relatively rigid chains of typical DNA preparations (length >10 000 Å; weight $>2 \times 10^6$ Daltons).

Standard hydrodynamic calculations of rotational correlation times of rigid rod models suggested that the spectrum of short DNA molecules, on the order of 100 nucleotide pairs (NP), could be observed with modern instrumentation. DNA near this size can be obtained from native chromatin digested extensively with micrococcal nuclease. Histone binding to DNA in chromatin restricts access of the nuclease to specific sites. As a result, digestion yields DNA that is mostly near 140 NP in length along with some smaller fragments.[5,6] Unlike sonication, this well-studied method of reducing the size of DNA is very gentle and yields fragments that are almost entirely doubly stranded, with few unpaired nucleotides extending from the ends.[7]

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