Photoinduced ESR Signals from the Primary Electron Donors in Deuterated Highly ^{13}C Enriched Photosynthetic Bacteria and Algae

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The primary photochemical electron donors of photosynthetic organisms eject a single electron when illuminated with light. In purple photosynthetic bacteria such as *Rhodopseudomonas sphaeroides* the oxidized primary donor P865+ exhibits a single Gaussian ESR signal possessing a line width that is narrowed by $1/\sqrt{2}$ relative to that of monomeric BChl a^+ in vitro.[1] The ENDOR spectrum of $P865^+$ yields hyperfine splittings that are one-half those of monomeric BChl a^+.[2,3] The assignment of the ENDOR signals to specific protons in BChl *a* is accomplished by selective biosynthetic deuteration of the chromophore. This assignment is crucial because the observed line width can then be compared with that predicted by simulation using the observed hyperfine splittings. Once this is accomplished the total observed line width of $P865^+$ can be accounted for only if the spin is shared between two BChl *a* molecules.

A similar analysis of the green plant photosystem I donor P700 has not been possible due both to an inability to selectively deuterate the chlorophyll in the organism and to the poor quality of $P700^+$ ENDOR data.[2] On the basis of the fact that the line width of the $P700^+$ ESR signal is about $1/\sqrt{2}$ that of Chl a^+ and that the $P700^+$ ENDOR spectrum does not reveal any large hyperfine splittings, it was concluded that $P700^+$ is a dimer of Chl *a* from which one electron is missing.

We now present data that do not depend on the ambiguous nature of the $P700^+$ ENDOR results. These data show that $P700^+$ is a single oxidized Chl *a* type macrocycle. In addition, using the same methodology and criteria we present new data which confirms that $P865^+$ is a dimer of a BChl *a* type macrocycle.

The central difficulty in using the line width of the $P700^+$ or $P865^+$ ESR signal alone to determine its possible multimeric nature stems from the possibility that the internal spin distribution of a particular chlorophyll in vitro may differ substantially from that in vivo. Moving π spin density away from carbon atoms adjacent to the Chl *a* methyl groups onto carbon atoms with only α protons or with no protons at all will significantly diminish the ESR line width due to a decrease in both the number and the magnitude of hyperfine splittings. It has been shown that the ESR line width of Chl a^+ in vitro is a function of the strength of ligands bonding to its magnesium atom.[4] Molecular orbital calculations predict the presence of a second low-lying doublet state of Chl a^+ that possesses a rearranged spin distribution.[4,5] Recently we have shown that tautomerization of the ring V β-keto ester of Chl *a* to the enol followed by oxidation yields a cation radical which possesses significantly reduced spin density on carbon atoms adjacent to the methyl groups.[6]

Since the ESR signals of chlorophyll cations both in vivo and in vitro are single broad resonances consisting of many overlapping hyperfine split lines and since internal spin redistribution may change the line width of the single broad line without resulting in the appearance of a split signal, a method that accounts for the entire spin regardless of its distribution is needed in order to count the number of spins per macrocycle. The solution to this problem is to make each carbon atom of the π system over which the electron is distributed magnetic resonance active. This requires that each position in the π framework be highly enriched in ^{13}C. Under these conditions the total ESR line width is the sum of the hyperfine lines due to each carbon atom of the π system.[7] In addition, if the chromophores are fully deuterated, contributions to the total ESR line shape from hyperfine splittings other than those of ^{13}C are minimized.

Table I. ESR Data[a]

	ΔH_{pp}, G	$\langle\omega^2\rangle$, G^2
^2H-^{13}C-$P700^+$	12.0	86.6
^2H-^{13}C-Chl a^+	11.5	85.0
^2H-^{13}C-$P865^+$	11.2	63.5
^2H-^{13}C-BChl a^+	15.1	127.0

[a] Radicals in vitro are 10^{-4} M solutions in methylene chloride-0.1 M tetra-*n*-butylammonium perchlorate. For all radicals $g = 2.0025 \pm 0.0001$. All spectra were obtained at 35 K. All values of ΔH_{pp} are ± 0.05 G and all values of $\langle\omega^2\rangle$ are $\pm 10\%$ G^2.

We have grown 99% ^2H, 91% ^{13}C enriched *Scenedesmus obliquus* by methods described earlier[8] and have extracted Chl *a* from a portion of the algae.[9] Algae hydrolysate prepared from these ^{13}C enriched algae was used as a nutrient to grow the bacterium *Rhodospirillum rubrum*. The *R. rubrum* cells were subcultured five times. The final *R. rubrum* cells were 89% enriched in ^{13}C. Typical sample preparation involved centrifuging 25 mL of culture at 3600*g* for 10 min. The resulting wet cells, ~300 mg, were resuspended in 2 mL of centrifugate. The suspension was deoxygenated by bubbling with a gentle stream of N_2. About 0.5 mL of the suspension was placed in a quartz ESR tube. Background spectra were recorded in the dark with the room lights off, while the spectra of the illuminated cells were obtained by irradiation of the sample with light from a Xe arc lamp (300 W) that had been equipped with $\lambda > 600$ nm cutoff filter. Typical intensity ratios of the light to dark ESR signals were greater than 10:1. Light minus dark spectra of the whole cells yielded the data presented in Table I. Bchl *a* and Chl *a* were obtained from their respective organisms in the usual fashion.[9] The chlorophyll cations in vitro were prepared electrochemically.[6,10]

Each ESR line is non-Gaussian with significant intensity appearing in the wings of the spectra. Thus, the line widths of the in vivo and in vitro species are not necessarily related to each other by the $1/\sqrt{2}$ relationship. However, a quantitative and rigorous comparison of the line shapes of these signals is possible by applying the method of moments.[11] The computer calculated second moment of each ESR line shape appears in Table I. Examination of the data reveals that the second moment of ^2H-^{13}C-$P700^+$ and ^2H-^{13}C-Chl a^+ are virtually identical, whereas the second moment of ^2H-^{13}C-$P865^+$ and ^2H-^{13}C-BChl a^+ differ by a factor of 2. These data clearly indicate that the spin in $P700^+$ is located on only one Chl *a* type macrocycle whereas that of $P865^+$ is shared between two BChl *a* type macrocycles. The excellent agreement of the new $P865^+$ data with independent determinations of the dimeric nature of $P865^+$ provides an important test of the ^{13}C method of determining the multimeric nature of the in vivo donors.

Since our data pertain only to the oxidized donors, the nature of the neutral P700 and P865 excitation energy traps remains very much an open question. Optical data indicate that the traps may involve two or more exciton coupled chlorophylls.[12,13] However,

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our data show that once oxidation takes place the resulting cation resides on only one macrocycle in $P700^+$ and on two macrocycles in $P865^+$.

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Heteropolypentatungstobisphosphonates. Cyclopentane-Like Pseudorotation of an Oxometalate Structure

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Heteropoly- and isopolyoxometalate complexes have frequently been described as fragments of close-packed metal oxide lattices, an image which draws attention to their potential catalytic activity but which perhaps overemphasizes the electrostatic contribution to the metal–oxygen bond in these complexes and carries with it a connotation of structural rigidity. That structures of certain polyanions might be fluxional in solution has been suggested only relatively recently.[1] We report here a new series of complexes that are the first heteropolyanions unambiguously to exhibit intramolecular exchange behavior.

The complexes are the tungstate analogues of the pentamolybdobisphosphonates, $(RPO_3)_2Mo_5O_{15}^{4-}$, that have been described earlier.[2] The new tungstates have limited stability in aqueous solution in marked contrast to the molybdates,[3,4] but addition of tri-*n*-butylammonium acetate to an equimolar solution of phenylphosphonic acid and sodium tungstate yields a precipitate of $[(C_4H_9)_3NH]_4[(C_6H_5PO_3)_2W_5O_{15}]$ (I)[5] which may be recrystallized from acetone–benzene (1:1). Potassium, cesium, and guanidinium salts are easily prepared by addition of methanolic solutions of appropriate salts and acetic acid to a solution of I in acetonitrile. A zwitterionic species $Na_2[(H_3^+NCH_2CH_2PO_3)_2W_5O_{15}]\cdot 5H_2O$ has also been prepared and is recrystallizable from hot water.[6] There is no doubt that the molybdates and tungstates are isostructural: IR spectra in the range 1150–600 cm^{-1} (P–O and Mo, W–O vibrations) are very similar for all $(RPO_3)_2M_5O_{15}$ complexes, and the tri-*n*-butylammonium salts of $(C_6H_5PO_3)_2Mo_5O_{15}^{4-}$ and $(C_6H_5PO_3)_2W_5O_{15}^{4-}$ are isomorphous by X-ray powder diffraction. The anion structure deduced from numerous[7] X-ray investigations of molybdo-

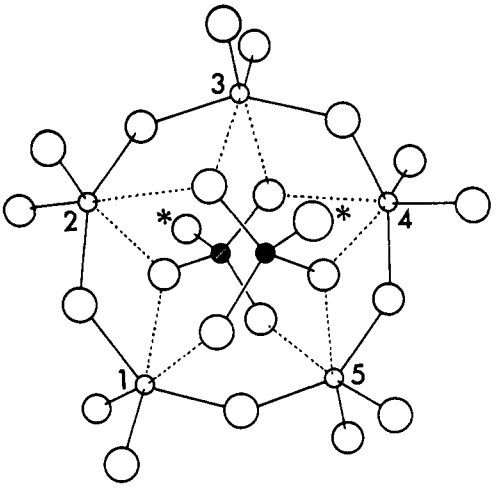

Figure 1. Oxometalate portion (C_2 symmetrized) of the structure of $(RPO_3)_2M_5O_{15}^{4-}$ (M = Mo, W) heteropolyanions. Small numbered circles, metal atoms; small dark circles, phosphorus atoms; large open circles, oxygen atoms. The alkyl, aryl, or oxo-R groups are represented by the starred atoms. Bonds are drawn to emphasize the pseudooctahedral coordination of the M atoms, but as discussed in the text, the M–O(P) bonds, shown as broken lines, are particularly long (typically 2.3 Å) and of low formal bond order.

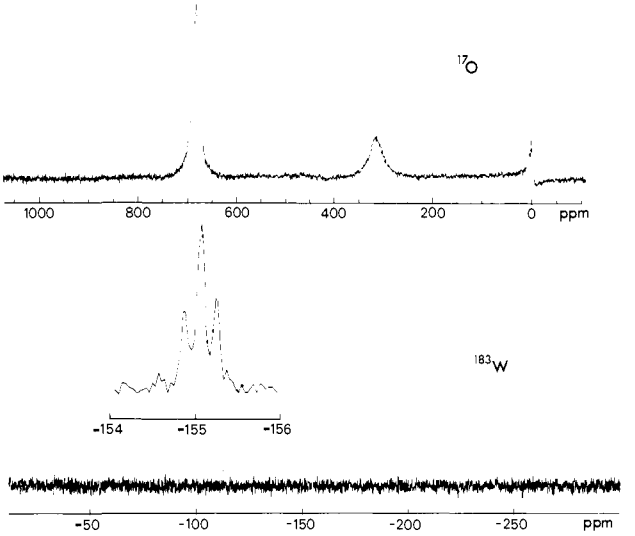

Figure 2. Top: 27.12-MHz ^{17}O NMR spectrum of I (11 atom % enriched), 0.09 M in wet acetonitrile at 299 K. Chemical shifts vs. pure D_2O [line widths, Hz]: δ +677 [780] (terminal oxygens), +311 [390] (tungsten-bridging oxygens), −6 [310] (D_2O). Phosphonate oxygens are not observed due to lack of enrichment. Bottom: 10.42-MHz ^{183}W NMR spectra of I, 0.1 M in CD_3CN at 306 K.[11b] Wide sweep spectrum, 14 000 scans; narrow sweep spectrum, 30 000 scans.

(1) The isomerization and exchange behavior of α-$Mo_8O_{26}^{4-}$ (Klemperer, W. G.; Shum, W. *J. Am. Chem. Soc.* **1976**, *98*, 8291. Day, V. W.; Fredrich, M. F.; Klemperer, W. G.; Shum, W. *Ibid.* **1977**, *99*, 952) appears to be incompletely defined, mechanistically (Masters, A. F.; Gheller, S. F.; Brownlee, R. T. C.; O'Connor, M. J.; Wedd, A. G. *Inorg. Chem.* **1980**, *19*, 3866). Structural rearrangements are also involved in water exchange of $[(RAsO_3)_2M_6O_{18}(OH_2)]^{4-}$ (M = Mo, W) anions. (Wasfi, S. H.; Kwak, W.; Pope, M. T.; Barkigia, K. M.; Butcher, R. J.; Quicksall, C. O. *J. Am. Chem. Soc.* **1978**, *100*, 7786).
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(3) An aqueous solution (0.04 M, pH 3.6) of $K_4[(C_6H_5PO_3)_2W_5O_{15}]$ is ca. 15% dissociated into $C_6H_5PO_3^{2-}$ and isopolytungstate(s) [^{31}P NMR: δ +21.2 (complex), +14.1 (free phosphonate)]. The corresponding molybdate is undissociated at 0.01 M.
(4) The "inorganic" analogue, $(OPO_3)_2W_5O_{15}^{6-}$, has recently been reported as a cesium salt, unstable in solution above 60 °C (Knoth, W. H.; Harlow, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 1865). No other salts of this tungstate are known. The corresponding molybdates, on the other hand, are a major component of weakly acidified molybdate–phosphate solutions (Pettersson, L. *Chem. Scr.* **1975**, *7*, 145) and numerous salts have been characterized.
(5) Anal. Calcd for $C_{60}H_{122}N_4P_2W_5O_{21}$: C, 32.50; H, 5.51; N, 2.53; P, 2.80; W, 41.50. Found: C, 31.83, H, 5.66; N, 2.44; P, 2.65; W, 41.78.
(6) Anal. Calcd for $C_4H_{24}N_2Na_2P_2W_5O_{26}$: C, 3.11; H, 1.56; N, 1.81; W, 59.57. Found: C, 3.46; H, 1.80; N, 2.07; W, 59.70. Details of syntheses and spectral characteristics of the new complexes will be reported in a subsequent paper.
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