# Anion Radicals of Bacteriochlorophylls c, d, and e. Likely Electron Acceptors in the Primary Photochemistry of Green and Brown Photosynthetic Bacteria

**J. Fajer,*[1a] I. Fujita,[1a] A. Forman,[1a] L. K. Hanson,[1a] G. W. Craig,[1b] D. A. Goff,[1b] L. A. Kehres,[1b] and K. M. Smith[1b]**

*Contribution from the Department of Energy and Environment, Brookhaven National Laboratory, Upton, New York 11973, and the Department of Chemistry, University of California, Davis, California 95616.* *Received December 2, 1982*

**Abstract:** Representative redox, optical, ESR and ENDOR data and molecular orbital calculations are reported for the anion radicals of the homologous bacteriochlorophylls and bacteriopheophorbides c, d, and e. The chromophores (*Chlorobium* chlorophylls) have previously been identified or are suspected to exist in the reaction centers of green (c and d) and brown (e) photosynthetic bacteria which appear to straddle green plants and purple bacteria on an evolutionary scale. An early role in the light-driven electron-transport chain of these bacteria is proposed for the *Chlorobium* chlorophylls. Diagnostic optical and electron paramagnetic spectral signatures are presented for the anion radicals which distinguish them from subsequent acceptors and would identify them in vivo.

A combination of optical and paramagnetic resonance spectroscopy has recently led to a generalized photosynthetic scheme by which green plants, algae, and purple bacteria transduce incident photons into the oxidants and reductants that drive the biochemistry of the organisms. The light harvested by antenna pigments is funnelled into a reaction center where a chlorophyll (Chl) or bacteriochlorophyll (BChl) phototrap, P, is raised to its excited singlet state, P*, and transfers an electron to a nearby acceptor, $A_1$, within a few picoseconds. This primary charge separation is then stabilized by the translocation of the electron, within a few hundred picoseconds, to a secondary acceptor, $A_2$, for an overall reaction.[2]

$$PA_1A_2 \xrightarrow{h\nu} P^*A_1A_2 \rightarrow P^+A_1^-A_2 \rightarrow P^+A_1A_2^-$$

In green plants, which function via two photosystems (PS) that cooperatively evolve oxygen (PS II) and fix carbon dioxide (PS I), the following sequences have been proposed.

PS I: P700[3] (Chl a dimer or monomer) $\xrightarrow{e^-}$ Chl a $\rightarrow$ Fe-S (iron-sulfur protein)[2b,f,g]

PS II: P680 (Chl a dimer or ligated monomer) $\xrightarrow{e^-}$ Pheo a (pheophytin, a metal-free Chl) $\rightarrow$ Fe-Q (an iron-quinone complex)[2c,d,f,g]

In purple bacteria, which do not evolve oxygen, a single photosystem comprises the electron-transport chain and is initated by

P870 (BChl a dimer) or P960 (BChl b dimer)[4] $\xrightarrow{e^-}$ BPheo (a or b) $\rightarrow$ Fe-Q[2a,e,g]

An additional short-lived transient ($\sim$1 ps) has also been proposed in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*:[2a]

$$P870 \xrightarrow{\sim 1\ ps} BChl\ a \xrightarrow{\sim 4\ ps} BPheo\ a \xrightarrow{\sim 150\ ps} Fe\text{-}Q^{2a,e}$$

If the secondary acceptors are artifically prereduced, i.e., $PA_1A_2^-$, so as to prevent the normal electron transfer from $A_1^-$ to $A_2$, then the primary charge separation, $P^*A_1 \rightarrow P^+A_1^-$, is annihilated by recombination, and a spin-polarized triplet of P is observed:[2g,5]

$$P^+A_1^-A_2^- \xrightarrow[<10\ ns]{} {}^3PA_1A_2^-$$

The spin-polarized triplet is not detected if $A_1$ is also reduced, i.e., $PA_1^-A_2^- + h\nu \not\rightarrow {}^3PA_1^-A_2^-$, and thus serves as a diagnostic probe of the recombination of $P^+$ and $A_1^-$. The disappearance of the triplet has been used to estimate the midpoint potential of $A_1$.[6] The reported reduction potentials of $A_1$ in purple bacteria and in plants agree within 200 mV with those measured for the same chromophores in nonaqueous solvents by cyclic voltammetry.[2,6,7] Since the potentials for the oxidation of P can be obtained by titration, an estimate of the energy generated by the primary charge separation can be obtained by summing the redox potentials of P and $A_1$. Neglecting entropy terms, 80-90% of the energy of an incident photon reaching the reaction center is converted into chemical form.[2] Conversely, this high percentage of energy conservation can be used to estimate the reduction potential of a putative primary acceptor if the energy of the excited singlet state and the oxidation potential of the donor P are known.

A sizeable effort is presently devoted to extending the above studies to photosynthetic green bacteria.[2g,8-11] The latter are

(1) (a) Brookhaven National Laboratory. (b) University of California.

(2) For recent reviews, see: (a) Parson, W. W. *Annu. Rev. Biophys. Bioenerg.* **1982**, *11*, 57-80. (b) Malkin, R. *Annu. Rev. Plant Physiol.* **1982**, *33*, 455-79. (c) Klimov, V. V.; Krasnovskii, A. A. *Photosynthetica* **1981**, *15*, 592-609. (d) Ke, B.; Dolan, E.; Shuvalov, V. A.; Klimov, V. V. In "Biological Events Probed by Ultrafast Laser Spectroscopy"; Alfano, R. R., Ed.; Academic Press: New York, 1982; pp 55-77. (e) Netzel, T. L. *Ibid.* pp 79-117. (f) Fajer, J.; Fujita, I.; Davis, M. S.; Forman, A.; Hanson, L. K.; Smith, K. M. *Adv. Chem. Ser.* **1982**, *No. 201*, 489-514. (g) Hoff, A. J. *Biophys. Struct. Mech.* **1981**, *8*, 107-150. See also: Clayton, R. K. "Photosynthesis: Physical Mechanisms and Chemical Patterns"; Cambridge University Press: New York, 1980. "Energy Conversion by Plants and Bacteria"; Govindjee, Ed.; Academic Press: New York, 1982.

(3) The notation P*xxx* denotes the maximum, in nanometers, of the lowest energy absorption band of the phototrap P.

(4) Some species of purple bacteria utilize BChl b instead of a as the photoactive chromophore to enable them to function at wavelengths as long as $\sim$1000 nm. BChl b contains an ethylidene group on ring II.

(5) Levanon, H.; Norris, J. R. *Chem. Rev.* **1978**, 185-198.

(6) Dutton, P. L.; Prince, R. C.; Tiede, D. M.; Petty, K. M.; Kaufman, K. J.; Netzel, T. L.; Rentzepis, P. M. *Brookhaven Symp. Biol.* **1976**, *No. 28*, 213-237.

(7) Fajer, J.; Davis, M. S.; Brune, D. C.; Spaulding, L. D.; Borg, D. C.; Forman, A. *Brookhaven Symp. Biol.* **1976**, *No. 28*, 74-104.

(8) Forman, A.; Davis, M. S.; Fujita, I.; Hanson, L. K.; Smith, K. M.; Fajer, J. *Isr. J. Chem.* **1981**, *21*, 265-269.

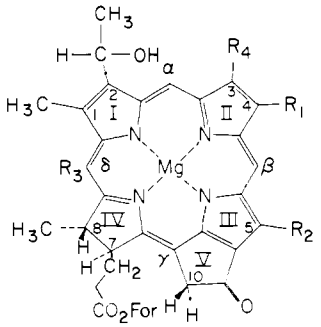
(9) (a) Olson, J. M. *Biochim. Biophys. Acta* **1980**, *594*, 33-51. (b) *Ibid.* **1981**, *637*, 185-188. (c) Olson, J. M.; Prince, R. C.; Brune, D. C. *Brookhaven Symp. Biol.* **1976**, *No. 28*, 237-246. (d) Olson, J. M.; Philipson, K. D.; Sauer, K. *Biochem. Biophys. Acta* **1973**, *292*, 206-217.

(10) Betti, J. A.; Blankenship, R. E.; Natarajan, L. V.; Dickinson, L. C.; Fuller, R. C. *Biochim. Biophys. Acta* **1982**, *680*, 194-201. Bruce, B. D.; Fuller, R. C.; Blankenship, R. E. *Biophys. J.* **1982**, *37*, 222a; *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6532-36.

(11) (a) Swarthoff, T. Ph.D. Dissertation, State University of Leiden, 1982. (b) Swarthoff, T.; Van der Veek-Horsley, K. M.; Amesz, J. *Biochim. Biophys. Acta* **1981**, *635*, 1-12. (c) Swarthoff, T.; Gast, P.; Hoff, A. J.; Amesz, J. *FEBS Lett.* **1981**, *130*, 93-98. (d) Swarthoff, T.; Gast, P.; Van der Veek-Horsley, K. M.; Hoff, A. J.; Amesz, J. *Ibid.* **1981**, *131*, 331-334. (e) Swarthoff, T.; Gast, P.; Hoff, A. J. *Ibid.* **1981**, *127*, 83-86.

0002-7863/83/1505-3837$01.50/0 

Chart I. Structures of Bacteriochlorophylls c, d, and e[a,b]



BChl c,[22] $R_1$ = Et, *n*-Pr, or *i*-Bu
$R_2$ = Me or Et
$R_3$ = $R_4$ = Me

BChl d,[23] $R_1$ = Et, *n*-Pr, *i*-Bu, or neo-Pen
$R_2$ = Me or Et
$R_3$ = H
$R_4$ = Me

BChl e,[15] $R_1$ = Et, *n*-Pr, or *i*-Bu
$R_2$ = Et
$R_3$ = Me
$R_4$ = CHO

pyroChl[c] $R_1$ = Et
$R_2$ = Me
$R_3$ = H
$R_4$ = Me in Chl a, CHO in Chl b
vinyl replaces the 2-(1-hydroxyethyl) group on ring I, the esterifying alcohol is phytol ($C_{20}H_{39}OH$)

[a] Abbreviations used: methyl (Me); ethyl (Et); *n*-propyl (*n*-Pr); isobutyl (i-Bu); neopentyl (neo-Pen); farnesyl (Far). [b] The major esterifying alcohol is farnesol ($C_{15}H_{25}OH$), but several other alcohols have been reported (Caple, M.; Chow, H.-C.; Burns, R. M.; Strouse, C. E. *Brookhaven Symp. Biol.* 1976, *No. 28*, 56-63). [c] Kratky, C.; Dunitz, J. D. *Acta Crystallogr., Sect. B* 1977, *B33*, 547-549.

particularly intriguing because they appear to straddle green plants and purple bacteria on an evolutionary scale.[12,13] Although they do not evolve oxygen, they are capable of direct photoreduction of pyridine nucleotides and contain both BChl a and Chl derivatives (*Chlorobium* chlorophylls) as well as the iron–sulfur proteins found in plants. Such ferredoxins have recently been identified as secondary electron acceptors in the green bacterium *Prosthecochloris aestuarii*.[11a,c] The reaction center of this species contains BChl a, BPheo c (see Chart I for structure), and an unidentified chromophore with an absorption spectrum similar to but not identical with those of BChl and BPheo c.[9b-d,11a,14] The phototrap is comprised of BChl a and exhibits a maximum at 840 nm (P840). The oxidized P840 displays ESR and optical parameters consonant with a dimer of BChl a, as found in the purple bacteria.[2g,9c,11e] However, its oxidation potential of 0.24 V (vs. NHE) is considerably lower than that found in the latter (~0.45 V).[9c] The singlet excited state of P840 (~1.46 eV) should therefore be energetically capable of reducing a primary acceptor with a very negative potential (~−1.2 V). Examination of all the primary acceptors postulated above for green plants and purple bacteria suggests that (bacterio)chlorophylls or -pheophytins may be obligatory to effect the primary charge separation.[2f,8] Extrapolation of these results to green bacteria implies therefore that chlorophyll-like molecules would also act as transient acceptors in these bacteria.[8] Indeed, BChl a has recently been shown to function as an early acceptor in *P. aestuarii*.[11d] However, even when this acceptor was reduced, the spin-polarized triplet of P840 was still detected, suggesting the existence of an earlier intermediate.[11d] We consider here the possibility of such a role for BChl c derivatives and provide diagnostic optical and paramagnetic characteristics of their anion radicals which would help to identify them in vivo. In addition, comparable data are presented for BChl d and e derivatives (Chart I), chromophores found in some species of green and brown bacteria, respectively.[15-17]

Table I. Reduction Potentials, $E_{1/2}$ (V vs. NHE),[a] in DMF

| | |
|---|---|
| BChl a | −0.81 (in $CH_2Cl_2$:[37] monomer, −0.84; aggregate, −0.59) |
| BPheo a | −0.51[18] |
| Chl a | −0.88[20,38] (in $C_3H_7CN$:[38] monomer, −0.94; aggregate, −1.13) |
| Pheo a | −0.64[20,38] |
| pyro Pheo a | −0.72[b] |
| BPheo d | −0.81[b,c] |
| BChl d | −1.00[c] |
| BPheo c | −0.79[b,c] |
| BChl c | −1.03[c] (in $CH_2Cl_2$: aggregate, −1.25) |
| BPheo e | −0.76[b,c] |
| BChl e | −0.95[c] |

[a] Measured by cyclic voltammetry vs. Ag/AgCl or SCE and converted to NHE. [b] Methyl ester derivatives. [c] Homologous mixtures.

## Experimental Section

The methods used for cyclic voltammetry,[18] controlled potential electrolysis,[18] electron spin resonance (ESR), and electron-nuclear double resonance (ENDOR),[19] have been described. The radicals were generated electrolytically in vacuo in dimethylformamide (DMF) containing 0.1 M tetrapropylammonium perchlorate (TPAP).[20] The electrochemical cells used for optical and ESR measurements are detailed in ref 2f.

BChls c, d, and e were obtained from *Prosthecochloris aestuarii* (grown in conjunction with *Desulfuromonas acetoxidans*), *Chlorobium vibrioforme* forma *thiosulfatophilum* (NCIB No. 8327), and *Chlorobium pheovibrioides* (NCIB No. 2631), respectively, and isolated by standard techniques.[15,21] Treatment of the crude chlorophyll extracts with methanol and sulfuric acid yielded the methyl pheophorbides which were then separated into their several homologous components by high-pressure liquid chromatography.[22,23] δ-Methyl, methyl mesopyropheophorbide a, and methyl pyropheophorbide a were prepared as described in ref 24 and 2f.

## Theoretical Methods

(1) Self-consistent field calculations using Pariser–Parr–Pople approximations (PPP) were used to predict optical spectra and unpaired spin densities.[25] A standard chlorin skeleton with a carbonyl group on ring III approximated the BChl c, d, or e structures. The semiempirical parameters used are listed in ref 25b.

(2) The charge-iterative extended Hückel (IEH) programs and parameters are described in ref 26. The calculations used co-

(12) For reviews, see: Clayton, R. K.; Sistrom, W. R., Eds. "The Photosynthetic Bacteria"; Plenum Press: New York, 1978: Pfenning, N., pp 3-8; Olson, J. M., pp 161-178; Pierson, B. K.; Castenholz, R. W., pp 179-197; Dutton, P. L.; Prince, R. E., pp 525-570; Knaff, D. B., pp 629-640; Fuller, R. C., pp 691-705.
(13) Olson, J. M. *Biosystems* **1981**, *14*, 89-94.
(14) Swarthoff, T.; Kramer, H. J. M.; Amesz, J. *Biochim. Biophys. Acta* **1982**, *681*, 354-358.
(15) Gloe, A.; Pfennig, N.; Brockmann, H., Jr.; Trowitzsch, W. *Arch. Microbiol.* **1975**, *102*, 103-109. Brockmann, H., Jr. *Philos. Trans. R. Soc. London, Ser. B.* **1976**, *273*, 277-285.
(16) Purdie, J. W.; Holt, A. S. *Can. J. Chem.* **1965**, *43*, 3347-3353.
(17) Holt, A. S.; Purdie, J. W.; Wasley, J. W. F. *Can. J. Chem.* **1966**, *44*, 88-93.
(18) Fajer, J.; Brune, D. C.; Davis, M. S.; Forman, A.; Spaulding, L. D. *Proc. Natl. Acad Sci. U.S.A.* **1975**, *72*, 4956-4960.
(19) Borg. D. C.; Forman, A.; Fajer, J. *J. Am. Chem. Soc.* **1976**, *98*, 6889-6893.
(20) Fujita, I.; Davis, M. S.; Fajer, J. *J. Am. Chem. Soc.* **1978**, *100*, 6280-6282.
(21) Smith, K. M.; Bushell, M. J.; Rimmer, J.; Unsworth, J. F. *J. Am. Chem. Soc.* **1980**, *102*, 2437-2448.
(22) Smith, K. M.; Kehres, L. A.; Tabba, H. D. *J. Am. Chem. Soc.* **1980**, *102*, 7149-7151.
(23) Smith, K. M.; Goff, D. A.; Fajer, J.; Barkigia, K. M. *J. Am. Chem. Soc.* **1982**, *104*, 3747-3749; *Ibid.* **1983**, *105*, 1674-1676.
(24) Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *Bioorg. Chem.* **1980**, *9*, 1-26.
(25) (a) Fajer, J.; Bielski, B. H. J.; Felton, R. H. *J. Phys. Chem.* **1968**, *72*, 1281-1288. (b) Fajer, J.; Forman, A.; Davis, M. S.; Spaulding, L. D.; Brune, D. C.; Felton, R. H. *J. Am. Chem. Soc.* **1977**, *99*, 4134-4140.

ordinates from the X-ray structures of ethyl chlorophyllide a dihydrate,[27] methyl pheophorbide a[28] and methyl bacteriopheophorbides d.[23] To accommodate the sterically hindered γ-methyl group in BChl c and e derivatives, the Cδ–$CH_3$ bond was set at 1.54 Å and the bonds between Cδ and its neighbors were stretched by 0.03 Å relative to the distances reported for the ethyl chlorophyllide a[27] (referred to in Table III as the Chl a structure). Alternatively, the skeleton of methyl pheophorbide a[28] was distorted by moving the β carbons of rings I and IV up, Cδ down, and lengthening the bonds between Cδ and its neighbors by 0.03–0.06 Å, with Cδ–$CH_3$ = 1.52 Å (referred to in Table III as the distorted Pheo a structure). Three structures of methyl bacteriopheophorbides d were refined during the course of this work,[23] and calculations for BPheo $d^-$ were repeated by using the coordinates of 4,5-diethyl methyl bacteriopheophorbide d for comparison.

## Results and Discussion

The characterizations of the *Chlorobium* chlorophylls that follow are complicated by the arrays of homologues found in vivo (Chart I).[15-17,21-23] Since the specific structures of the chromophores in the reaction centers are not yet known, the data presented should be considered as representative of the general properties of metallo and free-base derivatives of BChls c, d, and e. Note also that the nomenclature BChls c, d and e is misleading: the compounds are derivatives of pyrochlorophylls a and b in which the vinyl group on ring I has been hydrated to a 1-hydroxyethyl group.[29]

Representative reduction potentials for BChls and BPheos c, d, and e are listed in Table I. Comparison of the potentials of Pheo a, pyroPheo a, and BPheo d indicates that removal of the carbomethoxy group at position 10 from Pheo a to yield pyroPheo a renders the latter harder to reduce. Conversion of the 2-vinyl group of pyroPheo a to the 2-(1-hydroxyethyl) group of BPheo d further shifts the reduction potential anodically. Introduction of the δ-methyl groups in BPheo c and e, and of the 3-formyl group in BPheo e, results in small positive shifts relative to BPheo d. Examination of the reduction potentials listed in Table for the *Chlorobium* Chls, Chl a, and BChl a derivatives indicates that the *Chlorobium* Chls are among the hardest to reduce and therefore are likely acceptors in the electron-transport chain of the green and brown bacteria. In the case of *P. aestuarii*, the phototrap P840 acquires ~1.46 eV of energy upon excitation. Since the oxidation of P840 requires 0.24 V,[9c,31] this leaves ~1.2 V of reducing power, of which 10–20% may be lost to effect the rapid reduction of the primary acceptor.[2] If BChl c is present

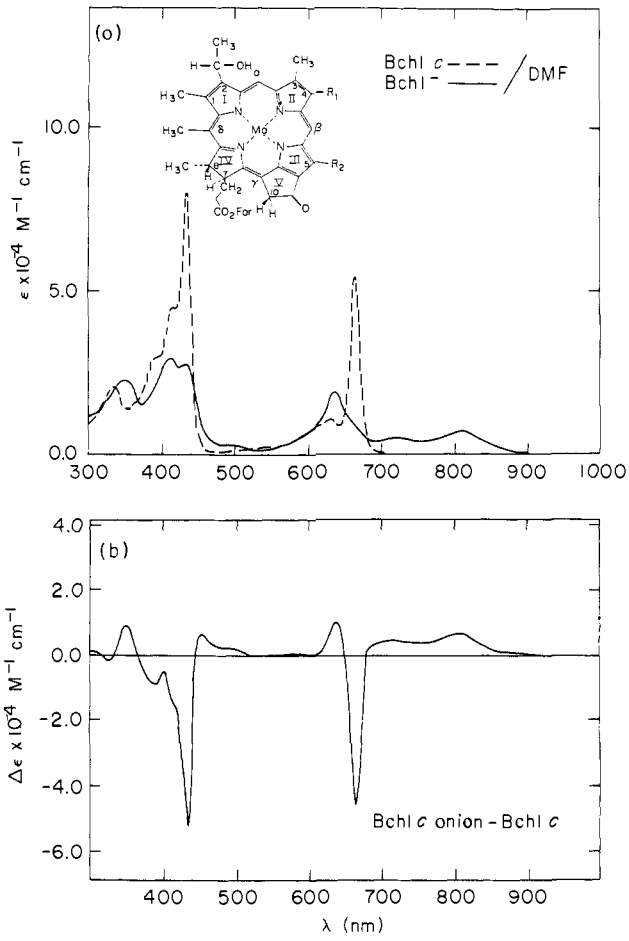



**Figure 1.** (a) Optical absorption spectra of BChl c (---) and its anion radical (—) in DMF at 25 °C (homologous mixture; ε values calculated assuming 4,5-diethyl homologue with farnesyl ester). (b) Difference spectrum, radical minus parent.

in the reaction center,[9,34] the following sequence for electron transfer certainly seems plausible on the basis of the reduction potentials of BChls a and c in DMF: P840 $\xrightarrow{e^-}$ BChl c → BChl a → Fe–S. However, the in vitro results do not definitely exclude BPheo c as the primary acceptor[36] because it and BChl a may be subject to hydrogen bonding, ligand, or charge interactions[2f] that could modify their reduction potentials. In addition, the chromophores may be aggregated with resulting shifts in potential. Olson has in fact suggested that BPheo c is dimerized in the reaction center,[9b] and X-ray structures of two different BPheos d clearly establish the existence of hydrogen-bonded dimers in the crystals.[23] The effects of such aggregation is difficult to predict. For example, aggregates of BChl and Chl a have been shown to be respectively easier[37] and harder[38] to reduce than their mo-

(26) Davis, M. S.; Forman, A.; Hanson, L. K.; Thornber, J. P.; Fajer, J. *J. Phys. Chem.* **1979**, *83*, 3325–3332. Chang, C. K.; Hanson, L. K.; Richardson, P. F.; Young, R.; Fajer, J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2652–2656.

(27) Chow, H. C.; Serlin, R.; Strouse, C. E. *J. Am. Chem. Soc.* **1975**, *97*, 7230–7237.

(28) Fischer, M. S.; Templeton, D. H.; Zalkin, A.; Calvin, M. *J. Am. Chem. Soc.* **1972**, *94*, 3613–3619.

(29) An explanation for the presence of the 2-(1-hydroxyethyl) group in BChls c, d, and e has recently been proposed.[30] The group promotes aggregation by complexing the Mg atom of the chromophores, thereby causing a significant red-shift in their absorption spectra in the antenna system. No rationale for the occurrence of the various hydrocarbon substituents at the 4- and 5-positions has been offered so far. However, when $R_1$ is a bulky group, the chirality of the asymmetric carbon of the hydroxyethyl group switches from *R* to *S*.[22,23]

(30) Smith, K. M.; Kehres, L. A.; Fajer, J. *J. Am. Chem. Soc.* **1983**, *105*, 1387–1389.

(31) The low oxidation potential of P840, compared to those of the phototraps of purple bacteria that also contain BChl a (0.45 V)[2a] or to BChl a in vitro (0.64 V in $CH_2Cl_2$),[18] is in itself intriguing. Wasielewski et al.[32] recently proposed that the low oxidation potential of P700, the Chl a phototrap of PS I, was due to a monomeric enol form of Chl a. Analysis of the ESR spectrum of $^{13}C$-enriched $P700^+$ supported the monomeric assignment. However, similar $^{13}C$ results for $P840^+$,[33] and the ESR line width of natural $P840^+$,[11e] are consistent with an unaltered BChl a dimer.

(32) Wasielewski, M. R.; Norris, J. R.; Shipman, L. L.; Lin, C. P.; Svec, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *87*, 2957–2961. Wasielewski, M. R.; Norris, J. R.; Crespi, H. L.; Harper, J. *J. Am. Chem. Soc.* **1981**, *103*, 7664–7665.

(33) Wasielewski, M. R.; Smith, U. R.; Norris, J. R. *FEBS Lett.* **1982**, *149*, 138–140.

(34) A pigment isolated by Swarthoff et al.[14] from *P. aestuarii* exhibits an optical spectrum similar to a metallo complex of BPheo c. This spectrum is insensitive to acid addition which tends to argue against a Mg complex.. Any other metallo derivative of BPheo c is also expected to be harder to reduce than BPheo c itself because in a wide variety of porphyrins, chlorins, bacteriochlorins, and isobacteriochlorins, a free base is always easier to reduce than its metallocomplexes, if reduction occurs at the macrocycle and not the metal.[7,26,35]

(35) Felton, R. H. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; p 53–125.

(36) The reverse sequence, P840 $\xrightarrow{e^-}$ BChl a → BPheo c → Fe–S, previously appealed to us[8] because the reduction potential of BChl a is somewhat higher than that of BPheo c in vitro *and* because the primary electron transfer would occur between molecules with similar symmetry, the BChl a dimer of P840 and the BChl a acceptor. However, this scheme is inconsistent with the results of Swarthoff et al.,[11d] which indicate that excitation of P840, while BChl a is prereduced, still yields the P840-polarized triplet. Nonetheless, the drastic conditions needed to prereduce the BChl acceptor may have disrupted the *P. aestuarii* reaction center complex and introduced a spurious primary acceptor. The in vitro data presented here combined with in vivo flash photolysis experiments should settle this point.

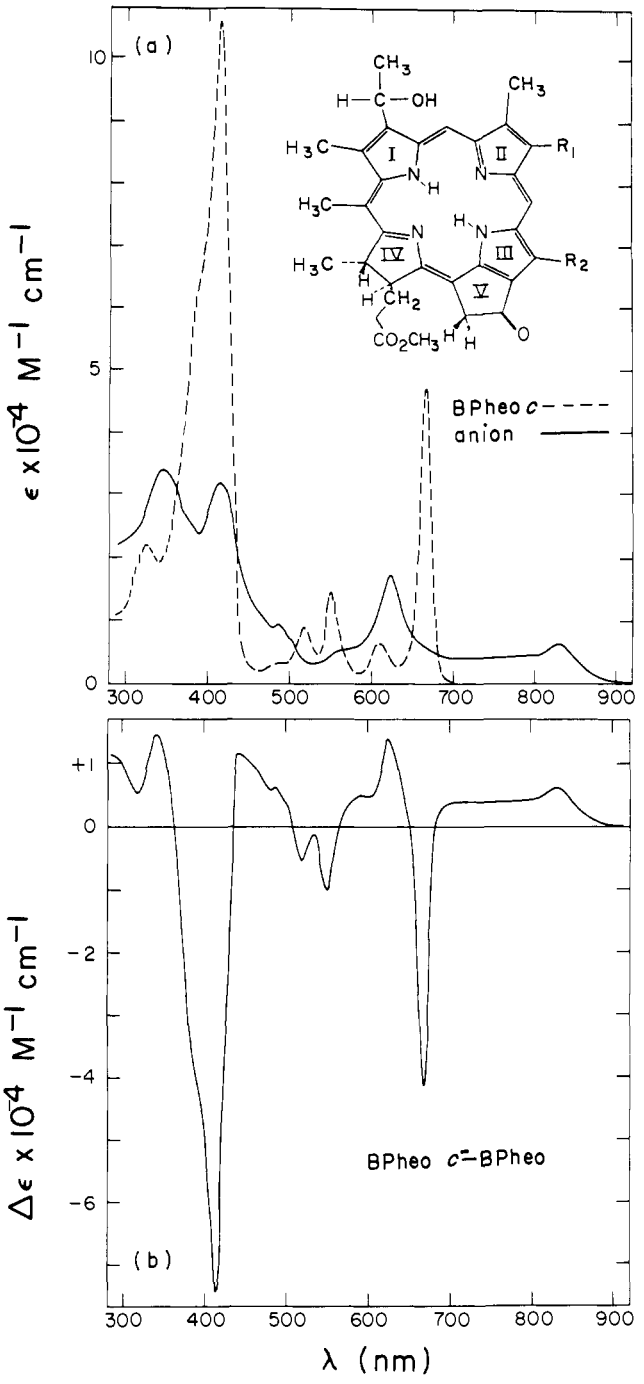



**Figure 2.** (a) Optical spectra of BPheo c (---) and its anion radical (—) in DMF (mixture of methyl bacteriopheophorbides c). (b) Difference spectrum, radical minus parent.

nomeric forms in some organic solvents. As shown in Table I, BChl c in $CH_2Cl_2$, in which optical spectra provide evidence of aggregation,[30] exhibits $E_{1/2}$ values 0.2 V more negative than in DMF (neglecting junction potentials). These possible modifications of the reduction potentials of BChl and BPheo c suggest therefore that either chromophore may well act as acceptor in the primary charge separation of *P. aestuarii*. Extrapolation of these conclusions to the green and brown bacteria that contain BChls d and e suggests similar roles for the d and e pigments in the electron-transport chain of those organisms.

Optical changes induced by oxidation or reduction of Chls and BChls have proven to be particularly useful in identifying transients

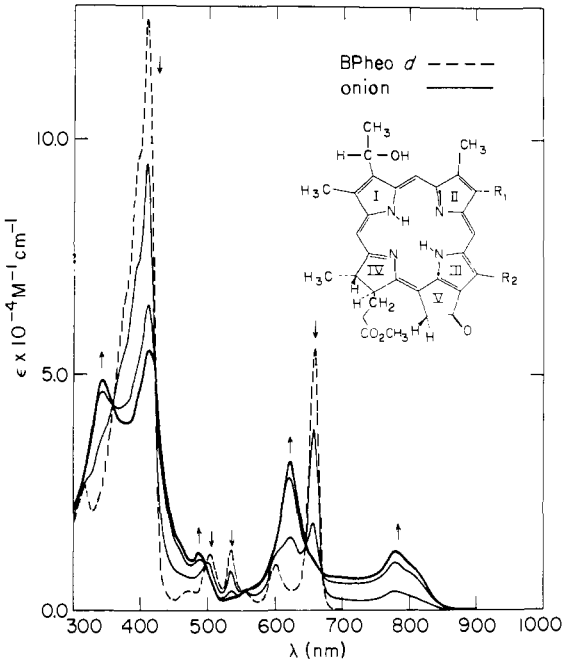



**Figure 3.** Optical changes observed on reduction of BPheo d (---) to its anion radical (—) in DMF (mixture of methyl bacteriopheophorbides d).

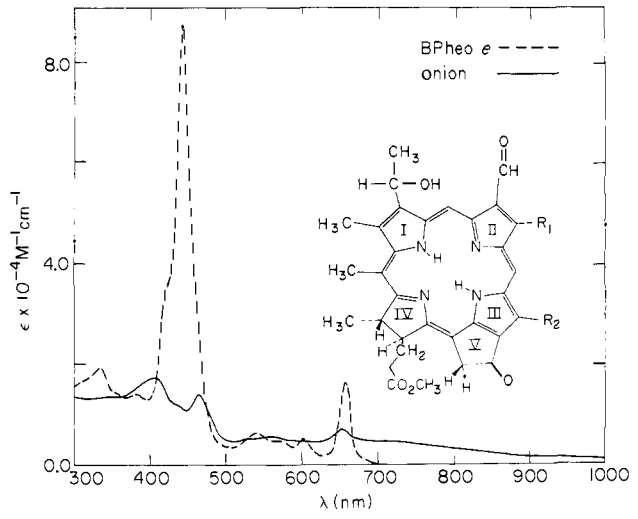



**Figure 4.** Optical spectra of BPheo e (---) and its anion radical (—) in DMF (mixture of methyl bacteriopheophorbides e). Note that the formyl group induces significant changes in these spectra relative to those of BPheos c and d. A similar effect occurs in Pheo b whose optical spectrum resembles that of BPheo e.[15]

observed by picosecond flash photolysis and trapping experiments in reaction centers. Although the spectra obtained in vitro do not match those observed in vivo exactly, the general features are faithfully reproduced.[2,7,8,11d,18,20,26] Figures 1–4 illustrate the effects of reduction on the optical spectra of representative compounds of BChls or BPheos c, d, and e. The electroreductions require 1 (±0.02) electron to yield the spectra shown with the occurrence of sharp isobestic points (displayed in Figure 3 only). Oxidation of the anions at 0.0 V regenerates better than 95% of the starting compounds. In all cases, reduction causes bleaching of the visible band, a significant diminution of the Soret band and the appearance of weak transitions in the near infrared. The optical transitions of BPheo $d^-$ are reasonably well predicted by PPP calculations (Figure 5). However, the intensity of the band at $16 \times 10^3$ $cm^{-1}$ (~625 nm) is not correctly predicted by using the tautomer in which the protons are localized on the nitrogens of rings I and III, as determined by X-ray diffraction for the neutral compound[23] (Figure 5a). A stronger transition in that region is calculated for the tautomer with the protons localized on rings

(37) Cotton, T. M.; Van Duyne, R. P. *J. Am. Chem. Soc.* **1979**, *101*, 7605–7612.
(38) Wasielewski, M. R.; Smith, R. L.; Kotska, A. G. *J. Am. Chem. Soc.* **1980**, *102*, 6923–6928.

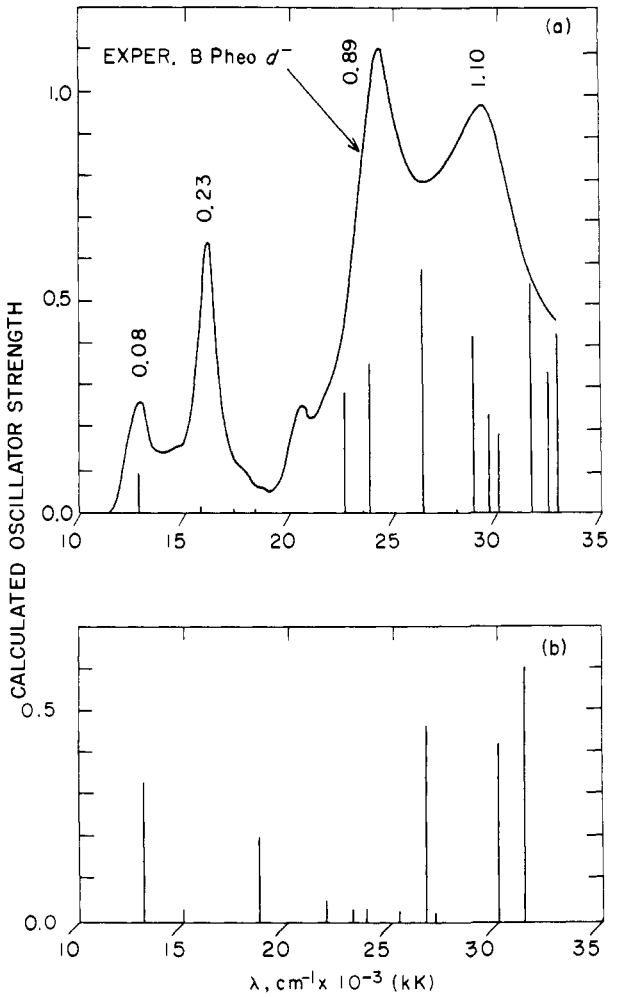



**Figure 5.** Optical transitions predicted for BPheo d⁻ by SCF-PPP-MO calculations using a model chlorin (see Figure 8 for structure). The positions and heights of the bars represent the calculated wavelengths and oscillator strengths of the transitions. The numbers shown above the experimental spectrum of BPheo d⁻ are the oscillator strengths calculated by integrating the major peaks observed. (a) Tautomer with protons localized on nitrogens of rings I and III. (b) Protons on rings II and IV.

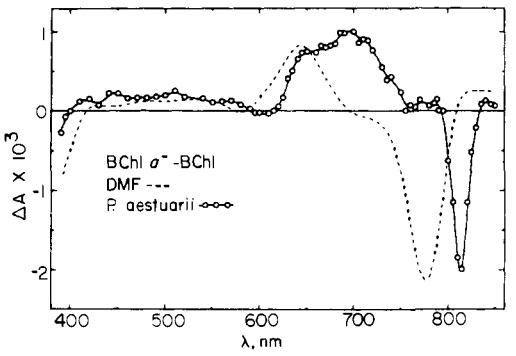



**Figure 6.** Comparison of the difference spectra obtained on reduction of BChl a in DMF (---, from ref 7) and in a reaction center complex of *P. aestuarii* (from ref 11d) (adapted from ref 11d, with permission).

II and IV (Figure 5b) and thus raises the possibility that the N–H protons may be partially delocalized in the radical. Not surprisingly, the features of the c and d derivatives parallel those found previously for the reduction of Chl and Pheo a.[20] However, all the spectra differ sufficiently from those observed on reduction of BChl a (in vivo[11d] and in vitro,[7] see Figure 6 and 1 or 2) so as to clearly distinguish them from the anion of the latter in vivo, if the sequence of acceptors suggested above is correct.

ESR line widths and *g* values, combined with ENDOR coupling constants of model systems, have helped identify primary donors

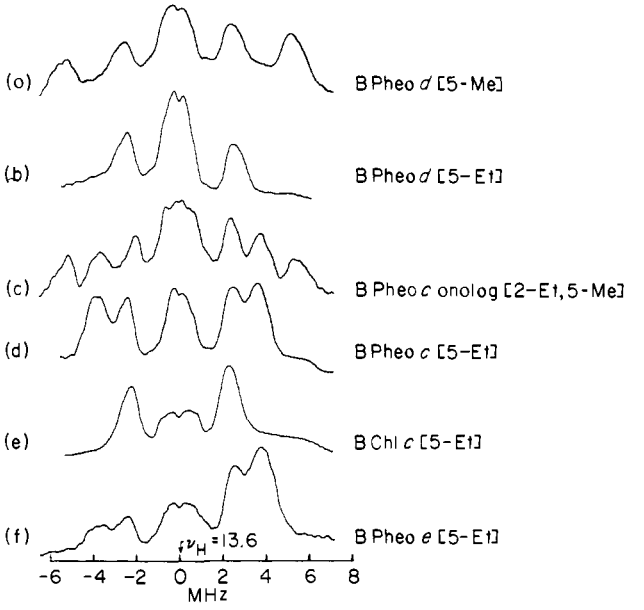



**Figure 7.** ENDOR spectra, in frozen deuterated DMF at −120 °C. The data are presented relative to the free proton frequency, $\nu_H$ 13.6 MHz. Two peaks, corresponding to $\nu_H \pm a/2$ are observed for each proton coupling constant $a$ (1 gauss = 2.8 MHz). (a) Homologous mixture of methyl bacteriopheophorbides d, enriched in 5-methyl. (b) Mixture of BPheo d, enriched in 5-ethyl. (c) Methyl δ-methyl mesopyropheophorbide a (synthetic analogue of BPheo c with 2-ethyl, 5-methyl). (d) Mixture of BPheo c, with 5-ethyl. (e) Mixture of BChl c, with 5-ethyl. (f) Mixture of BPheo e, with 5-ethyl.

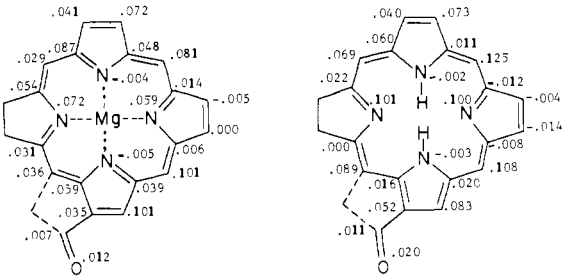



**Figure 8.** Unpaired spin densities, calculated by SCF-PPP-MO theory for free base and metallochlorin models of BPheo⁻ and BChl⁻ d.

and acceptors in a variety of photosynthetic organisms.[2,7,11d,e,19,25,26,32,33,39,40] In addition, in vivo deviations from the parameters of the chromophores in vitro have led to proposals of dimerization,[2g,7,26,32,33,39] conformational changes,[8,26] and specific orientations of substituent groups,[2f,g,8] as well as complexation of the Mg[2f] and/or hydrogen-bonding interactions of the oxygen functional groups[2f,8,32] with protein residues. Although the electronic profiles of the radicals in vitro can be resolved with considerable detail by solution ENDOR,[2f,7,19,25,26,41,42] comparisons with in vivo characteristics are often simplified at low temperatures,[43] where most proton and nitrogen nuclei do not yield ENDOR responses because of anisotropy, and only rapidly rotating methyl groups are readily detected.[20,25,39] ENDOR parameters for derivatives of BChls c, d and e are listed in Table II, along

(39) Katz, J. J.; Norris, J. R.; Shipman, L. L.; Thurnauer, M. C.; Wasielewski, M. R *Annu. Rev. Biophys. Bioeng.* **1978**, *7*, 393–434. Feher, G.; Hoff, A. J.; Isaacson, R. A.; Ackerson, L. C. *Ann. N. Y. Acad. Sci.* **1975**, *244*, 239–259. Lendzian, F.; Lubitz, W.; Scheer, H.; Bubenzer, C.; Mobius, K. *J. Am. Chem. Soc.* **1981**, *103*, 4635–4637.

(40) Feher, G.; Isaacson, R. A.; Okamura, M. Y. *Biophys. J.* **1977**, *17*, 149a.

(41) Hoff, A. J.; Mobius, K. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2296–2300. Lubitz, W.; Lendzian, F.; Mobius, K. *Chem. Phys. Lett.* **1981**, *81*, 235–241; **1981**, *84*, 33–38.

(42) Hoff, A. J.; Lendzian, F.; Mobius, K. *Chem. Phys. Lett.* **1982**, *85*, 3–8. Lendzian, F.; Mobius, K.; Lubitz, W. *Ibid.* **1982**, *90*, 375–381.

(43) The time scale of ENDOR experiments and the short lifetimes of the "primary" acceptors almost dictate that the transients be trapped at low temperatures to be observed.

Table II. Low-Temperature ENDOR Coupling Constants[a]

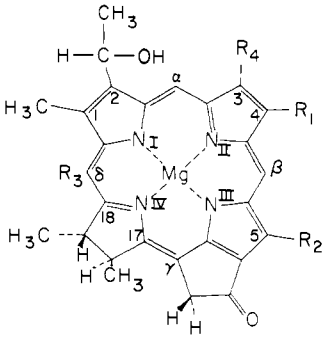
| anion | $a_H$ | assignment |
|---|---|---|
| BChl a | 3.2 | 1-, 5-methyl[25,40] |
| BPheo a | 3.0 | 1, 5[25] |
| Chl a | 1.95, 4.05 | 1, 5[20] |
| Pheo a | 1.95, 3.75 | 1, 5[20] |
| BChl c (5-Et) | 1.63 | 1, δ |
| BPheo c (5-Et) | 1.80, 2.61 | 1, δ |
| BPheo c analogue (2-Et, 5-Me) | 1.61, 2.54, 3.80 | 1, δ, 5 |
| BPheo d (5-Me) | 1.74, 3.71 | 1, 5 |
| BPheo d (5-Et) | 1.76 | 1 |
| BPheo e (5-Et) | 1.80, 2.72 | 1, δ |

[a] $T = -120$ to $-140$ °C. Line widths of the ESR signals of the *Chlorobium* radicals range between 11.8 and 12.7 G at microwave powers of 0.01 mW. The signals saturate easily. $g$ values = 2.0032-34.

with ESR line widths and $g$ values. The observed ENDOR coupling constants (Figure 7) can be assigned to specific sites of the molecules on the basis of MO calculations, and previous studies of pyroPheo, Pheo, and Chl a anions.[2f,8,20,42] The PPP calculations, which treat standardized chlorin skeletons (Figure 8), and the IEH computations, which include the substituents (Table III), yield comparable unpaired spin density profiles for the radicals, with the notable exception of the δ positions of the Mg complexes. Salient features of the calculations are the significant localization of spin at two nitrogens, the methine carbons (α, β, γ, δ), and at positions 1 and 5. Similar profiles[8,20] for Chl⁻ and Pheo⁻ a are in good agrement with both low-temperature and high-resolution ENDOR results for the two anions.[8,20,42] Our previous data for pyroPheo⁻ a[2f,8] are particularly relevant to the present results. Two proton coupling constants of ~2 and 3.8 G were observed at low temperatures in DMF and assigned to the 1- and 5-methyl groups, respectively, on the basis of MO calculations. The identity of the 5-methyl group was confirmed by deuteration experiments. The proton ENDOR spectrum of BPheo d⁻ (homologous mixture, enriched in 5-methyl) reveals two similar constants (Figure 7a) which are also attributed to the 1- and 5-methyl groups, based on the MO calculations. Further support for this assignment derives from the data obtained with (5-ethyl) BPheo d⁻ (Figure 7b) in which the 3.7 G ENDOR transition is lost, probably because the bulkier 5-ethyl group is subject to restricted rotation. A similar effect obtains in (5-ethyl) BPheo c⁻ (Figure 7c), where only a very broad peak is observed in the 4-G region. Replacement of the 5-ethyl by a methyl group in the synthetic compound 2-(dehydroxyethyl) BPheo c (methyl δ-methyl mesopyropheophorbide a)[24] causes the 5-methyl group resonance to reappear (Figure 7d). In BPheo c⁻ and its synthetic analogue an additional peak at 2.6 G is observed which is assigned to the δ- methyl group found in the BChl c derivatives, again on the basis of the MO calculations. (A comparable coupling constant of 2.2 G for the δ proton has been resolved in solution for Pheo a⁻.[42]) Note that the PPP and IEH calculations clearly predict the trend observed in the BPheo anions: $5\text{-}CH_3 > \delta\text{-}CH_3 > 1\text{-}CH_3$.

An interesting result is observed in BChl c⁻ (5-ethyl). Not only is the resonance assigned to the 5-methyl group missing but the δ-methyl is not detected either (Figure 7e). To ensure against possible errors, the same batch of BChl c was converted to the BPheo c derivative. The ENDOR spectrum of the latter again showed the 2.6-G peak assigned to the δ-methyl. The question

Table III. Unpaired Spin Densities for the Anion Radicals of BChls and BPheos c, d, and e Obtained from IEH Calculations[a]



| | BChl c | | BChl d | BChl e | | BPheo c | | | BPheo d | | BPheo e | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| position | *b* | *c* | *d* | *c, e* | *c, f* | *c, g* | *c, h* | *c, i* | *d, i* | *j, i* | *c, e, i, k* | *c, f, i* |
| 1 | 0.037 | 0.038 | 0.039 | 0.039 | 0.038 | 0.032 | 0.040 | 0.036 | 0.037 | 0.043 | 0.035 | 0.037 |
| 2 | 0.034 | 0.035 | 0.035 | 0.033 | 0.033 | 0.034 | 0.037 | 0.036 | 0.036 | 0.037 | 0.028 | 0.033 |
| α | 0.047 | 0.046 | 0.046 | 0.043 | 0.043 | 0.054 | 0.038 | 0.046 | 0.048 | 0.053 | 0.031 | 0.040 |
| 3 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.002 | 0.002 | 0.003 | 0.003 | 0.001 |
| 4 | 0.019 | 0.019 | 0.020 | 0.016 | 0.018 | 0.008 | 0.031 | 0.020 | 0.020 | 0.018 | 0.029 | 0.019 |
| β | 0.102 | 0.103 | 0.106 | 0.099 | 0.102 | 0.103 | 0.093 | 0.098 | 0.104 | 0.105 | 0.108 | 0.097 |
| 5 | 0.094 | 0.092 | 0.094 | 0.090 | 0.091 | 0.091 | 0.088 | 0.090 | 0.093 | 0.092 | 0.096 | 0.090 |
| γ | 0.038 | 0.045 | 0.046 | 0.047 | 0.046 | 0.048 | 0.038 | 0.043 | 0.043 | 0.036 | 0.042 | 0.046 |
| 17 | 0.003 | 0.003 | 0.004 | 0.004 | 0.004 | 0.000 | 0.013 | 0.006 | 0.007 | 0.008 | 0.006 | 0.007 |
| 18 | 0.038 | 0.034 | 0.028 | 0.034 | 0.034 | 0.022 | 0.052 | 0.037 | 0.031 | 0.028 | 0.034 | 0.036 |
| δ | 0.054 | 0.072 | 0.051 | 0.074 | 0.074 | 0.073 | 0.064 | 0.068 | 0.047 | 0.055 | 0.070 | 0.073 |
| $N_I$ | 0.000 | 0.002 | 0.000 | 0.002 | 0.002 | 0.001 | 0.004 | 0.002 | 0.000 | 0.000 | 0.004 | 0.003 |
| $N_{II}$ | 0.095 | 0.089 | 0.092 | 0.091 | 0.090 | 0.131 | 0.051 | 0.091 | 0.095 | 0.101 | 0.071 | 0.086 |
| $N_{III}$ | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.002 | 0.002 |
| $N_{IV}$ | 0.097 | 0.088 | 0.090 | 0.091 | 0.090 | 0.097 | 0.072 | 0.085 | 0.084 | 0.093 | 0.082 | 0.088 |

[a] The structure illustrates the models used in the calculations with the following substitutents: in BChl/BPheo c, $R_1 = R_2 = R_3 = R_4 =$ Me; in BChl/BPheo d, $R_1 = R_2 =$ Et, $R_3 = H$, $R_4 =$ Me; in BChl/BPheo e, $R_1 = R_2 = R_3 =$ Me, $R_4 =$ HCO. Note that the spin profiles, although susceptible to small variations induced by the conformations assumed for the molecules, are all similar and that the positions expected to yield ENDOR signals at low temperatures rank in the order C5 > Cδ > C1. [b] Based on ethyl chlorophyllide a coordinates with Cδ–$CH_3$ = 1.54 A. [c] Distorted methyl pheophorbide a structure to accommodate δ-$CH_3$. Note that the distortion results in increased spin density at the δ-position. [d] Undistorted methyl pheophorbide a coordinates. [e] Formyl group in the porphyrin plane. [f] Formyl group perpendicular to the porphyrin plane. [g] Tautomers with protons on $N_I$ and $N_{III}$ as in crystal structures of BPheos d.[23] [h] Tautomers with protons on $N_{II}$ and $N_{IV}$. [i] Average of two tautomeric forms. [j] Based on the structure of 4,5-diethyl methyl bacteriopheophorbide d. The molecule is quite distorted in the crystal.[23] [k] In these calculations, a π* orbital, which is 45% localized on the C–O atoms of the formyl group, lies just above the normal singly occupied π* orbital and mixes with it, resulting in a somewhat distorted spin distribution.

then arises as to why that group is observed in BPheo c⁻ but not in BChl c⁻. Space-filling models indicate that in BChl c, the δ-methyl is very crowded and cannot rotate freely, whereas in BPheo c, the absence of the Mg atom imparts greater flexibility to the macrocycle thereby allowing conformations in which the δ-methyl group is unhindered. Alternatively, the significant decrease in spin density at the δ-position, predicted by the PPP but not the IEH calculations for the metallo complexes, may account for the missing resonance near 2.6 G, and the peak of the δ-methyl group may be buried under the 1-methyl group or in the peaks with smaller coupling constants centered around $\nu_H$. (Even the IEH calculations lower the spin density at the δ-position if the conformation of BChl c is modified, see Table III.

As expected from the IEH calculations for BPheo⁻ c and e, the ENDOR results for BPheo e⁻ enriched in 5-ethyl resemble those for BPheo c⁻ (Figure 7f). (We have been unable to generate stable radicals of BChl e.)

Low-temperature ENDOR data for BChl a⁻, Chl⁻, and Pheo a⁻ and the results discussed above are compared in Table II. As noted for the optical spectra, the *Chlorobium* Chl anions exhibit properties similar to those of the chlorophylls. Here again, however, their ENDOR parameters differ sufficiently from those of BChl a⁻ so as to be distinctive and allow their identification even in the presence of BChl a⁻.

To account for the differences between Chl⁻ and Pheo⁻ a in vivo and in vitro, we have previously suggested that the reduced acceptors in PS I and II may be hydrogen bonded at the 9-keto group by protein residues[2f,8] The predicted effects of such interactions on the spin density at various positions in the molecules considered here appear to be small enough so as not to cause major changes in the coupling constants,[44] and the distinction between the *Chlorobium* Chls⁻ and BChl a⁻ should still be maintained. The consequence of the BPheo c dimerization suggested by Olson[9b] can also be predicted: if the unpaired electron is equally shared between two molecules on the ESR time scale, then the ENDOR coupling constants would be halved. Again, some of these (the 1- and δ-methyl groups, in particular) would still be clearly distinct from those of BChl a⁻.

In summary, the in vitro redox data for the *Chlorobium* chlorophylls suggest that they are likely candidates as early electron acceptors in green and brown photosynthetic bacteria. To help identify them in vivo and thereby test the electron transport sequence suggested, we have provided here the optical and paramagnetic spectral signatures of the putative acceptors which would clearly differentiate them from other components in the reaction centers. This "primary" transport chain would include components derived from purple bacteria (P840 and BChl a) and from green plants (Chl derivatives and ferredoxins). Thus, the constituents of the reaction center would mirror those found in the antenna system,[9a,10,13] which also contain BChl a and Chl derivatives and therefore further reinforce the hypothesis that green (and brown) bacteria straddle purple bacteria and green plants on an evolutionary scale.[12,13,45]

**Acknowledgment.** We thank Dr. J. Olson for valuable discussions and the authors of ref 11, 14, 33, and 46 for communicating their results prior to publication. This work was supported by the Division of Chemical Sciences, U.S. Department of Energy (Contract No. DE-AC02-76CH00016), at Brookhaven National Laboratory and by the National Science Foundation (Grant No. CHE-81-20891) at the University of California.

**Registry No.** BChl c anion, 85761-74-6; Bchl e anion, 85761-76-8; BPheo c anion, 85761-77-9; BPheo e anion, 85761-79-1; BPheo d anion, 85761-78-0; BChl d anion, 85761-75-7; BChl a anion, 36643-17-1.

(44) IEH calculations were compared for BChl d⁻, BChl d⁻ with the 9-keto group of ring V hydrogen bonded to a hydroxyl function, and BChl d⁻ with the hydroxy group of ring I bonded to a carbonyl function (structural data taken from the X-ray results for the 4,5-diethyl BPheo d dimer).[23] Bonding at the 9-keto position had minimal effect on all positions of interest (maximum changes ≤∼5%, for example C5 increased from 0.092 to 0.097 while C1 remained unchanged). Bonding at the hydroxyl group resulted in maximum changes of ∼10% with C1 intact and C5 decreasing from 0.092 to 0.085. Similarly small effects (≤∼10%) were calculated for BChl e⁻ (formyl group in plane) bonded to hydroxyl functions at the formyl or 9-keto groups.

(45) The reaction center of the green filamentous bacterium *Chloroflexus aurantiacus* appears to be more akin to purple bacteria and contains BPheo a[46] and quinones.[10]

(46) Pierson, B. K.; Thornber, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 80-84.

# Spectroscopic Evidence for Proton Transfer within the Bimolecular Complex HI-$NH_3$ Trapped in Cryogenic Matrices

**L. Schriver, A. Schriver, and J. P. Perchard***

*Contribution from the Laboratoire de Spectrochimie Moléculaire, Université Pierre et Marie Curie, 75230 Paris Cédex 05, France.* *Received July 28, 1982*

**Abstract:** The infrared spectra for the 1:1 complexes of HI and either $NH_3$ or $N(CH_3)_3$ (TMA) isolated in four different matrices (Ar, $O_2$, $N_2$, $C_2H_4$) have been obtained at 10 K. These complexes are mainly characterized by the stretching vibration ($\nu_s$) of the proton along the I-N axis, the frequency of which strongly varies with the nature of the matrix: for HI-$NH_3$, 1255 (Ar), 1310 ($O_2$), 1847-1967 ($N_2$), and 2315 $cm^{-1}$ ($C_2H_4$); for HI-TMA, 1889 (Ar), 1880-1920 ($O_2$), 2100-2132 ($N_2$), and 2315 $cm^{-1}$ ($C_2H_4$). A comparison with the known spectra of HX-$NH_3$ (X = F, Cl, Br) and HX-TMA (X = Cl, Br) (1:1) complexes shows that the hydrogen bond is stronger with HI than with other hydro acids, in which the hydrogen atom of HI has tipped toward nitrogen. Thermodynamic arguments about the enthalpy of reaction of proton transfer within a $NH_3$/HX pair support this conclusion. The matrix frequency shift shows that the rate of proton transfer is strongly matrix dependent and is believed to be complete in an ethylene matrix, for which the $\nu_s$ frequencies are the same for both HI-$NH_3$ and HI-TMA complexes. Such a sensitivity of the proton potential curve to the surroundings had been predicted in recent theories of solvent effects.

The problem of proton transfer within bimolecular hydrogen-bonded complexes has prompted several theoretical (ref 1 and others cited theirein) and experimental studies based on the matrix isolation technique.[2-7] The theoretical approaches deal on the

0002-7863/83/1505-3843$01.50/0 