# Communications to the Editor

## Optical and Paramagnetic Identification of a Primary Electron Acceptor in Bacterial Photosynthesis

*Sir:*

Light harvested by photosynthetic organisms is funneled into a phototrap or reaction center (RC), where the absorbed photons are converted into chemical energy in the form of oxidizing and reducing species.[1] A mechanism for this transduction in purple photosynthetic bacteria has recently evolved[2-7] whereby photoexcited bacteriochlorophylls transfer an electron to an intermediate (I) in <10 ps, and the back-reaction of the resulting radicals is prevented by the rapid reduction (~200 ps) of a second electron acceptor (X):

$$PIX \xrightarrow{h\nu} P^*IX \xrightarrow{<10\ ps} P^+I^-X \xrightarrow{\sim 200\ ps} P^+IX^-$$

where P is a pair of bacteriochlorophylls (BChl) absorbing at 870 nm in bacteria that contain BChl a and at 960 nm for those with BChl b, P* is an excited singlet, $P^+$ a cation radical, and X an iron-quinone complex. The nature of I is in question; it has variously been postulated to be the anion radical of BChl,[8] of bacteriopheophytin (BPh), a demetallated BChl,[3,4a,5,6,9] or of a complex of both.[4d,10,11]

We present here electron spin resonance (ESR) and electron nuclear double resonance (ENDOR) results which establish that, in reaction centers of *Rhodopseudomonas viridis* (which contain BChl b), the reducing electron is not shared between BChl b and BPh b on the ESR time scale but, rather, that $I^-$ exhibits the characteristics of a radical analogous to the monomeric anion of either BChl b or BPh b in vitro. Reduction of I to $I^-$ in isolated reaction centers of *R. viridis* causes optical changes which parallel most of the features observed on reduction of BPh b in vitro. In addition, the midpoint potentials recently reported[5,11] for the reduction of I bracket those found for the one-electron reduction of BPh b in organic solvents. We conclude that, on a picosecond time scale, BPh b is the most likely candidate for the primary electron acceptor of the charge separation induced by light in this bacterium.

One-electron electroreduction[12] of BPh b in dichloromethane yields[4a] a radical species which displays the optical spectrum shown in Figure 1a and a 12-line ESR signal with $g = 2.0033$ (±0.0002). Reduction[12] of BPh b with a potassium-18-crown-6 complex in ethers or photoreduction[12] in pyridine with sodium sulfide results in similarly resolved ESR signals. The resolution decreases upon cooling until it is barely perceptible in frozen solutions (Figure 2) when the signal is nearly a gaussian singlet whose line width is very susceptible to microwave power saturation: in pyridine, at −140 °C, the first derivative peak to peak line width, $\Delta H = 12.2$ G at 0.01 mw and 13 G at 0.1 mw.

Electroreduction of BChl b in dimethylformamide (DMF) or tetrahydrofuran (THF) generates species with the absorption spectra shown in Figure 1b. Electrolytic or potassium reductions[12] yield radicals of BChl b with ESR characteristics similar to those of BPh $b^-$: $g = 2.0033$, and 12-line spectra in fluid solution. The resolution persists even at −140 °C in glassy matrices of 2-methyl-THF (see Figure 2) but merges into overall gaussian singlets in frozen solvents: $\Delta H = 12.8$–13 G at 0.01 and 0.1 mw power in THF at −140 °C. The easily saturated ESR signals of BChl $b^-$ and BPh $b^-$ result in strong ENDOR responses: proton hyperfine splitting constants of 0.3, 2.6, and 3.0 (±0.1) G and 0.3, 2.5, 2.9, and 3.1 G are obtained for $BChl^-$ and $BPh^-$, respectively, at −140 °C in THF.[13]

The intermediate $I^-$ was trapped in reaction centers of *R. viridis* (extracted[14] with lauryldimethylamine oxide (LDAO)) by a technique[9-11] which takes advantage of the presence of cytochromes (Cyt) in the reaction center. Under continuous illumination, and at redox potentials low enough to reduce X, the rapid, reversible photooxidation of P

$$Cyt_{II}PIX^- \overset{h\nu}{\rightleftharpoons} Cyt_{II}P^+I^-X^-$$

is terminated by the eventual reduction of $P^+$ by a ferrocytochrome oxidation:

$$Cyt_{II}P^+I^-X^- \rightarrow Cyt_{III}PI^-X^-$$

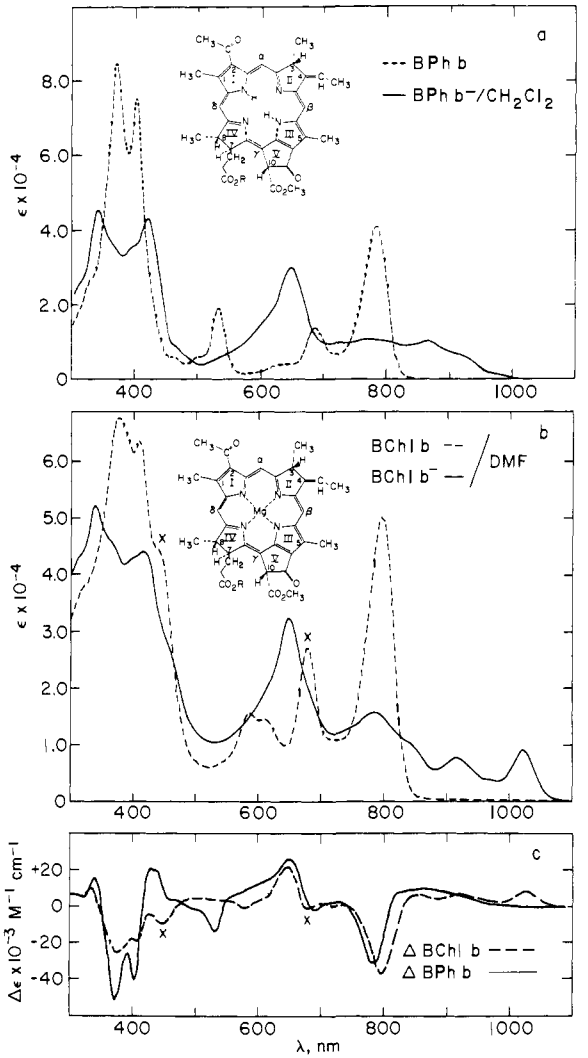


**Figure 1.** Absorption spectra of (a) BPh b (- - -) and its anion radical, BPh $b^-$ (—) in dichloromethane and (b) BChl b (- - -) and BChl $b^-$ (—) in dimethylformamide. (c) Difference spectra, radical minus parent compound, ΔBChl (- - -), ΔBPh (—). Radicals were generated[12] electrolytically at platinum electrodes in solutions containing 0.1 M tetrapropylammonium perchlorate. × indicates absorption bands due to a chlorin impurity.



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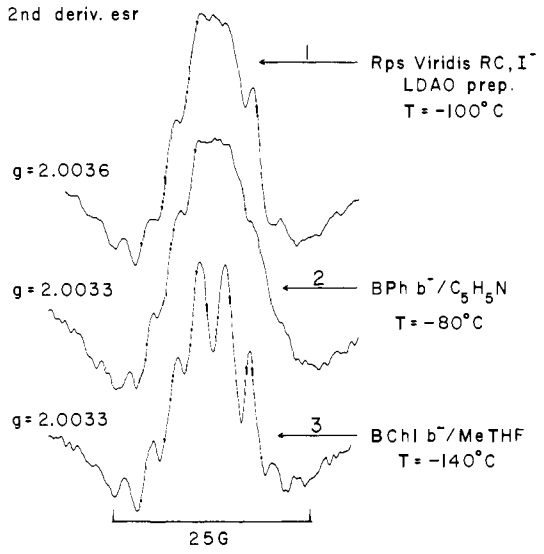


**Figure 2.** Second derivative ESR spectra of (1) $I^-$, generated by continuous illumination of LDAO preparations of *R. viridis* reaction centers containing 50% glycerol and cooled to −100 °C (the RC was poised at ~−300 mV vs. NHE with sodium dithionite at pH 8); (2) BPh $b^-$, prepared photolytically in solutions of pyridine containing 0.1 M $Na_2S$ and 1 M $H_2O$, at −80 °C; and (3) BChl $b^-$, obtained by potassium reduction in 2-methyltetrahydrofuran, at −140 °C.

At ~−300 mV vs. NHE the $Cyt_{III}$ is reduced by the medium with the net photoreaction[15]

$$Cyt_{II}PIX^- \xrightarrow{h\nu} Cyt_{II}PI^-X^- + \text{oxidized medium}$$

When cooled under continuous illumination, the *R. viridis* reaction centers display the ESR signal shown in Figure 2 with a *g* value[16] of 2.0036 (±0.0002). At −100 °C, the signal exhibits vestiges of hyperfine resolution, saturates easily ($\Delta H$ = 12.2 and 14 G at 0.01 and 1.0 mw) and yields ENDOR resonances at 0.3, 2.7, and 3.2 (±0.1) G.

The $I^-$ species thus displays ESR parameters such as *g* value, line width, saturation behavior, similarity of resolution, and ENDOR responses which are clearly characteristic of a monomeric anion radical of BPh b or BChl b. (Electron sharing, on the ESR time scale, between two or more molecules such as $(BChl)_2^-$, $(BPh)_2^-$, or (BChl-BPh)$^-$ would reduce the line width and the hyperfine splittings observed by ENDOR.) $I^-$ must therefore be BPh $b^-$ or BChl $b^-$ (or possibly a heterogeneous array of the two) but *not* a dimeric complex. Comparison of the optical difference spectra obtained on reduction of BPh and BChl with those found[11,14,16-19] for the reduction of I in reaction centers and chromatophores of *R. viridis* indicates that many of the optical changes observed in vivo mirror those found upon reduction of BPh b in vitro (Figure 3). The optical changes at ~830 nm which apparently implicate BChl b are then attributable to electrochromic shifts of the BChl absorption bands caused by the nearby $I^-$ and $X^-$. (The 830-nm band also shifts on oxidation[14] of the reaction center.)

We further note that the midpoint potentials estimated[4d,5,18] for the reduction of I in chromatophores and reaction centers of *R. viridis* by two independent titration techniques, $E_m$ = −400 and −620 mV, bracket the half-wave potentials found[4a] for the reduction of BPh in organic solvents: $E_{1/2}$ = −500 in DMF and −560 mV in $CH_2Cl_2$, whereas reduction of BChl b in DMF occurs at $E_{1/2}$ more negative then −700 mV (vs. NHE). The combination of ESR, ENDOR, optical, and redox data thus leads to the conclusion that $I^-$ in *R. viridis* exhibits many of the properties of a monomeric anion radical of BPh b.

Kinetic, optical, and paramagnetic results obtained[4-7,11,14,17-19] for the species involved in the initial stages of photosynthesis of *R. viridis* parallel those found[1-5,8-10,20-23] in *Rhodopseudomonas Sphaeroides* and *Chromatium vinosum* which contain BChl a and BPh a. These data imply that a common molecular architecture controls the primary events in photosynthetic bacteria that contain BChl a or b. It is thus likely that BPh functions as the prime acceptor in both types of organisms.

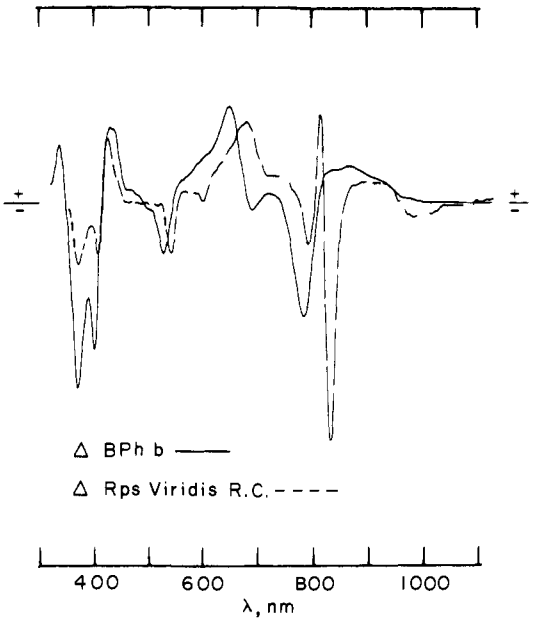


**Figure 3.** Comparison of the difference spectra induced by the reduction of BPh b in $CH_2Cl_2$ ($\Delta$BPh = $BPh^-$ − BPh) and by the photoreduction of I in reaction centers of *R. viridis* as reported by Shuvalov et al.[11] Spectra have been normalized at ~540 nm. Chromatophores, sodium dodecyl sulfate, and LDAO RC preparations yield similar results.[14,17-19]

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(13) Molecular orbital calculations predict small hyperfine splitting constants for the protons on rings II and IV and large splittings for the methyl groups of rings I and III, the $\alpha$, $\beta$, and $\delta$ protons, and the nitrogens of rings II and IV.
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(15) Support for the assumption that this technique generates $I^-$ comes from picosecond flash experiments[5-7] which indicate that, in *R. viridis* reaction centers so treated, photooxidation of P is effectively prevented because all electron acceptors have been reduced:

$$P^*I^-X^- \rightarrow P^+I^-X^-$$

and

$$P^*I^-X^- \xrightarrow{20\ ps} PI^-X^-$$

(16) This value is clearly distinct from that found[4a] for $P^+$, $g = 2.0026$. The small variation in the anion values, 2.0033–2.0036, may reflect the tightness of the ion pairs formed by the anion radicals and their gegenions. For a discussion of mechanisms which influence $g$ values of porphyrins, see J. Fajer and M. S. Davis in "The Porphyrins", D. Dolphin, Ed., Academic Press, New York, N.Y., in press.
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## Products of Reaction of Superoxide and Hydroxyl Radicals with $Mn^{2+}$ Cation

*Sir:*

We would like to report the absorption spectra of $MnO_2^+$ and $Mn(OH)^{2+}$ which are the products of the interaction of the $Mn^{2+}$ cation with superoxide and hydroxyl radicals:

$$Mn^{2+} + O_2^- \rightarrow MnO_2^+ \quad (1)$$

$$Mn^{2+} + OH \rightarrow Mn(OH)^{2+} \quad (2)$$

Although the formation of these species had been correctly diagnosed in an earlier pulse radiolysis study,[1,2] the reported spectra were not well resolved (see Figure 2 in ref 1) and hardly differed from each other. Because of the importance of these species in biological reactions,[3,4] where it had been erroneously suggested that $O_2^-$ is capable of oxidizing $Mn^{2+}$ to $Mn^{3+}$, we investigated the system using the stopped-flow radiolysis technique[5] by which reactions 1 and 2 can be studied separately and in the absence of interfering reactions.

**Reaction 1.** The superoxide radical was generated at 23.5 °C by a 2-MeV electron beam impinging on an air-saturated 1.0 mM sodium formate solution pH 10.0:[5]

$$H_2O \xrightarrow{O_2,\ HCOO^-} O_2^-, H_2O_2, H_2 \quad (3)$$

The $O_2^-$ solution (6 $\mu$M) was rapidly mixed with a 5 mM $MnSO_4$ solution of such acidity ($H_2SO_4$) that the final mixture was at pH 6.0. Effective scavenging of $O_2^-$ was monitored at 270 nm; it was found to be independent of $Mn^{2+}$ concentration in the range studied (0.5–50 mM). With the stopped-flow technique one can use relatively low formate concentrations for the conversion of the primary radicals into $O_2^-$, because the $Mn^{2+}$ cation is added ~15 ms (dead time between radiation zone and mixer) after irradiation and does not enter into competition reactions with the primary radicals. Also, since in the final solution-mixture the total amount of formate is only 0.5 mM, effects due to $Mn^{2+}$-$HCOO^-$ complex formation are minimized. All chemicals and the water were of highest purity;[5] manganese(II) sulfate (99.999% purity) was an Apache Chemical Inc. product. The upper limit for scattered light was of the order of 3% at 210 nm.

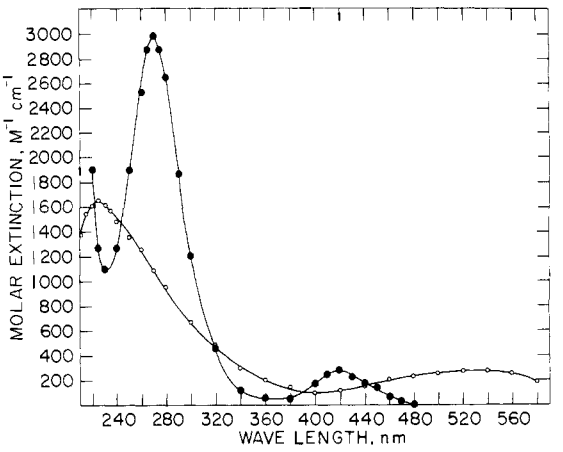


**Figure 1.** Absorption spectrum of $MnO_2^+$ (●) at pH 6.0 and 23.5 °C in an air-saturated 0.5 mM sodium formate solution containing a small amount of phosphate buffer. Absorption spectrum of $Mn(OH)^{2+}$ (○) at pH 6.3 and 23.5 °C in an $N_2O$-saturated solution containing sodium phosphate and perchloric acid.

The absorption spectrum of $MnO_2^+$ (Figure 1) was obtained by monitoring the absorbance at various wavelengths under conditions of constant energy input. The spectrum has been corrected for fluctuations in the beam current which were of the order of 5%. The corresponding molar extinction coefficients are normalized values based upon experiments in which the absorbance of $MnO_2^+$ at 270 nm gave a linear plot as a function of beam current (which is proportional to the energy input) and was compared with a similar plot for $O_2^-$ at 245 nm. Hence the molar extinction $\epsilon_{MnO_2^+}^{270nm} = 3000 \pm 150\ M^{-1}\ cm^{-1}$ is based upon the molar absorbance of superoxide radical,[6] $\epsilon_{O_2^-}^{245nm} = 2350 \pm 120\ M^{-1}\ cm^{-1}$ at 23.5 °C. Control experiments carried out in presence of a wide concentration range of $MnSO_4$ (2.5–100 mM) suggest that neither $Mn^{2+}$ nor $SO_4^{2-}$ affects the molar absorbance of $MnO_2^+$. Absence of absorbance above 480 nm in the system indicates the absence of reaction 2.

**Reaction 2.** The spectrum of the reaction product of OH with $Mn^{2+}$ was determined in the same apparatus as the spectrum of $MnO_2^+$. A 1 mM $MnSO_4$ solution, pH 6.3, saturated with $N_2O$ was irradiated, mixed with nonirradiated solution, and monitored for absorbance at the various wavelengths:

$$H_2O \xrightarrow{N_2O} OH, H_2O_2, H_2 \quad (4a)$$

$$Mn^{2+} + OH \rightarrow Mn(OH)^{2+} \quad (4b)$$

The molar absorbance of $Mn(OH)^{2+}$ was determined from a linear plot of the absorbance at 225 nm as a function of energy input and compared with an $O_2^-$ absorbance vs. energy input curve at 245 nm. All other points in the spectrum were normalized in terms of the molar absorbance $\epsilon_{Mn(OH)^{2+}}^{225nm} = 1640 \pm 80\ M^{-1}\ cm^{-1}$ at 23.5 °C. The numerical values of the extinction coefficients of $Mn(OH)^{2+}$ reported here are based upon the assumption that $G(OH)_{N_2O} = G(O_2^-)_{O_2,HCOO^-} = 6.05$, where $G$ is the number of primary radicals formed per 100-eV energy dissipated. Since the fate of the H atom is unknown in this system, $G_H = 0.55$, the values of $\epsilon_{Mn(OH)^{2+}}$ given in Figure

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