# A NOVEL ELECTRON PARAMAGNETIC RESONANCE SIGNAL ASSOCIATED WITH THE 'PRIMARY' ELECTRON ACCEPTOR IN ISOLATED PHOTOCHEMICAL REACTION CENTERS OF *RHODOSPIRILLUM RUBRUM*

Roger C. PRINCE

*Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19174*

and

J. Philip THORNBER

*Biology Department, Brookhaven National Laboratories, Upton, Long Island, NY 11973, USA*

Received 11 July 1977

## 1. Introduction

It is now generally accepted that the initial photo-activated electron transport steps occurring in the photochemical reaction center of purple bacteria involve the oxidation of a bacteriochlorophyll dimer (abbreviated to $(BChl)_2$) with the concomitant reduction of an intermediary electron carrier (I) (see ref. [1] for a recent review). The photo-reduced I in turn reduces what has classically been termed the 'primary acceptor'; this latter step effectively renders the light reaction irreversible under physiological conditions [1]. The chemical nature of I has not been unequivocally determined, but current indications are that it involves a single bacteriopheophytin molecule [2] in close association with other components (e.g., bacteriochlorophyll and iron) of the reaction center [1,3]. The chemical nature of the 'primary acceptor' has been the subject of some controversy in the past, but the present general consensus is that it involves a quinone in close association with an iron atom [4].

A portion of this work was carried out at Brookhaven National Laboratories, under the auspices of the Energy Research and Development Administration. JPT's permanent address: Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024, USA

In *Chromatium vinosum* [5], *Rhodopseudomonas sphaeroides* [6,7] and *Rps. viridis* [3,8] this quinone–iron complex (abbreviated to QFe) is characterized by an electron paramagnetic resonance (EPR) signal at $g_y$ 1.82 and a $g_x$ band which varies from $g$ 1.68 to $g$ 1.72 in the different species.

In this paper we present the EPR spectrum of the 'primary acceptor' of *Rhodospirillum rubrum*; it is quite distinct from that reported in the other organisms, exhibiting a prominent signal centered close to $g$ 1.87. In addition we also examine some other spectroscopic properties of an isolated reaction center preparation from *R. rubrum*, and demonstrate that the optical absorbance attributed to bacteriopheophytin in the isolated reaction center can also be observed in whole cells, which is a strong indication that bacteriopheophytin is a bona fide component of the reaction center, and not an artefact introduced by the detergent treatment used during the isolation of the reaction center.

## 2. Materials and methods

*Rhodospirillum rubrum* strain S-1 was grown photosynthetically, and reaction centers were prepared by a modification of the method of Noel et al. ([9] cf. also Gingras [10]) using lauryl

dimethylamine oxide (LDAO). LDAO-solubilized chromatophores (LDAO/BChl, 20/1, w/w) were either diluted with Tris buffer to give a final concentration of 0.1% LDAE or were dialyzed overnight against 50 mM Tris–0.1% LDAO, pH 7.8, and then loaded onto a DEAE-cellulose column. After washing the column with 50 mM Tris–0.1% LDAO, a reaction center-enriched fraction was eluted by 50 mM Tris–0.1% LDAO–100 mM NaCl. Further purification was achieved by hydroxylapatite chromatography, from which a reaction center preparation was eluted by 0.2 M sodium phosphate, pH 7.0 (cf. [3]).

Optical spectra were recorded on a Cary 14R spectrophotometer, while ERP spectra were recorded on Varian E-4 or E-9 spectrometers equipped with flowing helium cryostats (5, 7, 8).

## 3. Results

Figure 1 shows optical spectra of the isolated reaction center preparation as isolated (in the $((BChl)_2I)QFe$ state), and during illumination (in the $((BChl)_2^+ I)Q^-Fe$ state); the preparation is free of cytochromes, and both spectra are very similar to those reported by Noel et al. [9]. The two bands in the 1000–1400 nm region observed in the oxidized preparation are attributed to the optical absorbance of $(BChl)_2^+$, the oxidized form of the primary electron donor (P870). The molar extinction coefficient of the 1248 nm band is approx. 14 000 $cm^{-1}$, a value which is identical to that determined for *Rps. sphaeroides* (cf. [11]).

In the past there have been some suggestions that the bacteriopheophytin *a* which gives rise to the absorbance peak at 760 nm may be an artefact of the preparative procedure. Figure 2 shows the optical spectrum of intact cells of *R. rubrum*. This species, unlike many purple bacteria, lacks antenna pigments absorbing around 800 nm, and the small absorbance in this region in whole cells is almost certainly due to that of the pigments contained in the photochemical reaction center. The inset of fig.2, which shows the spectrum of a more concentrated cell suspension in the 800 nm region, demonstrates that the 760 nm band which is seen in the isolated reaction centers is clearly present in unfractionated whole cells, and that the relative absorbances of the 760 and 800 nm peaks are the same as occurs in the isolated reaction center. This strongly suggests that the 760 nm-absorb-

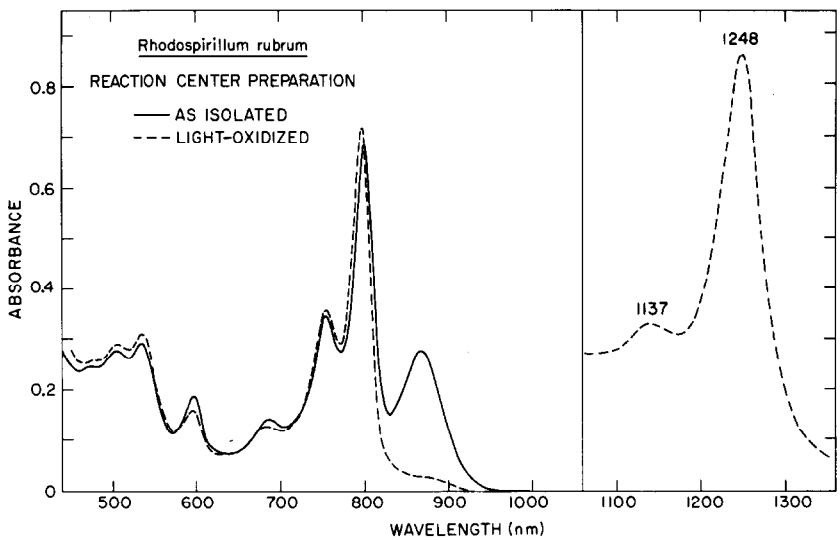


Fig.1. Optical spectra of the reaction center of *R. rubrum*. Reaction centers (~ 2 μM) were suspended in 50 mM Tris–HCl, pH 8.0, and their spectrum recorded in a Cary 14R spectrophotometer in the IR1 (solid line) and IR2 (dashed line) modes. In the IR2 mode the cuvettes are illuminated with intense white light. The spectrum of the infrared bands due to $(BChl)_2^+$ (see [1]) shown on the right was obtained with a more concentrated (~ 60 μM) sample.

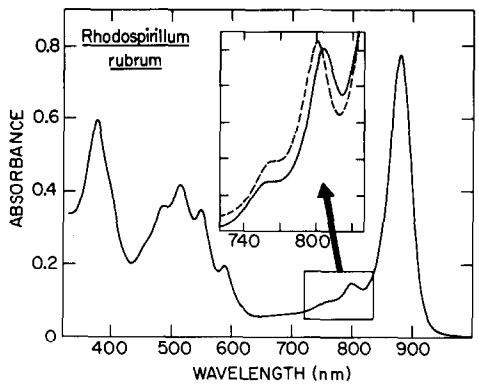


Fig.2. Optical spectra of whole cells of *R. rubrum*. The inset shows the 800 nm region prior or (solid line) and during (dashed line) illumination (IR1 and IR2 modes of the Cary 14R spectrophotometer, respectively). The shift of the 800 nm band on oxidation of the reaction center, which also occurs in the isolated reaction center (fig.1) and the 760 nm band due to reaction center bacteriopheophytins, are clearly resolved. The spectra were recorded using opal glasses to correct for light-scattering.

ing bacteriopheophytins are bona fide members of the reaction center.

Figure 3 shows the EPR characteristics of the bacteriochlorophyll dimer $(BChl)_2$ of the reaction center. On the left is the light-induced signal seen when normal photochemistry gives rise to $(BChl)_2^+$. The signal has a gaussian line shape, and is centered close to $g$ 2.0025. It has a peak-to-peak line width of about 9.7 G and is essentially identical to the signal measured in whole cells [12], which is $\sqrt{2}$ narrower than that of the cation radical of monomeric bacteriochlorophyll *a* [12]. This difference between the in vivo and in vitro EPR spectra provided the first definitive evidence that the primary electron donor in bacterial photosynthesis was a bacteriochlorophyll dimer or 'special pair' [13].

On the right of fig.3 is the light-induced triplet or biradical signal of the $(BChl)_2$ [14], which is observed at low temperatures if the 'primary acceptor' is reduced prior to illumination. The triplet signal is also indistinguishable from that detected in whole cells [8,15,16], having $D$- and $E$-values of $188 \times 10^{-4}$ $cm^{-1}$ and $33 \times 10^{-4}$ $cm^{-1}$.

Figure 4 shows the EPR spectrum of the reduced 'primary acceptor' of *R. rubrum* with that of *Rps. sphaeroides* for comparison. The acceptor was reduced by an identical procedure in both organisms. Figure 4 shows spectra of dithionite reduced reaction centers; however, very similar spectra are obtained when reaction centers, poised at an ambient potential so that they are in the $((BChl)_2I)QFe)$ state, are illuminated in the EPR cavity. Although the two spectra shown in fig.4 are distinctly different in appearance, they do

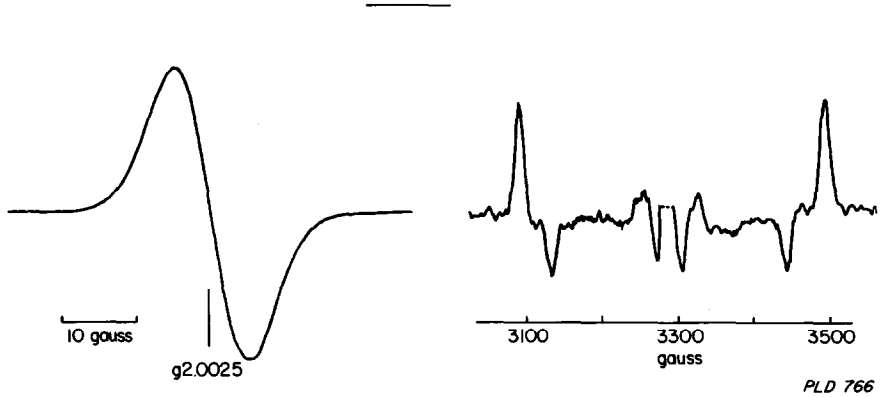


Fig.3. EPR spectra of the $(BChl)_2$. Reaction centers (~ 50 μM) were suspended in 50 mM Tris–HCl, pH 8.0. Both the spectra shown are light minus dark difference spectra; on the left that due to $(BChl)_2^+$, measured in a sample where the 'primary acceptor' was oxidized prior to illumination; on the right that due to the triplet state of the $(BChl)_2$, measured when the 'primary acceptor' was reduced prior to illumination. Both signals decayed completely when the illumination ceased. Spectrometer settings; microwave power, 1 mW; temperature, 7.5°K; modulation amplitude, 5 G $(BChl)_2^+$ and 20 G for the triplet.

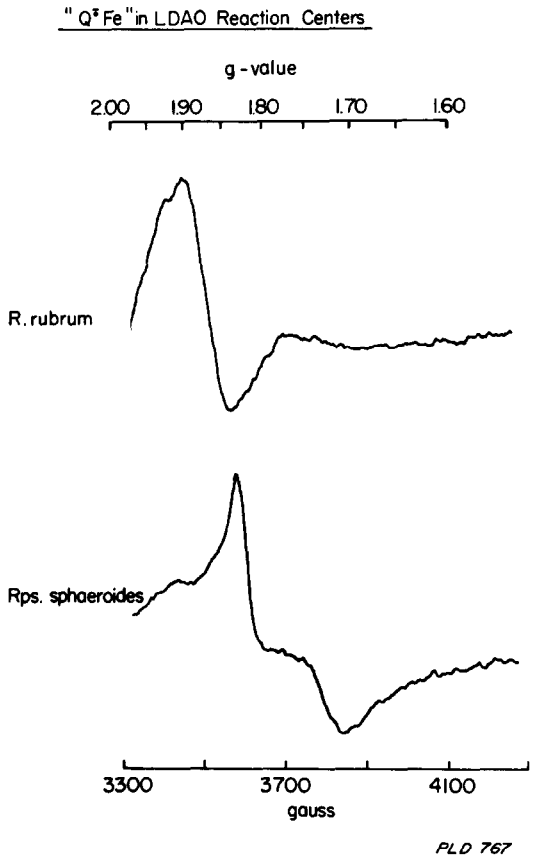


Fig.4. EPR spectrum of the primary acceptor. Reaction centers (~ 50 μM) were suspended in 50 mM Tris–HCl, pH 8.0, together with enough sodium dithionite to completely eliminate any loss of absorbance at 870 nm (due to photooxidation of $(BChl)_2$) when the sample was exposed to light in the IR2 mode of a Cary 14R spectrophotometer. Spectrometer settings; microwave power, 20 mW; temperature, 6°K; modulation amplitude, 25 G. The spectrum of the 'primary acceptor' in LDAO reaction centers isolated from *Rps. sphaeroides* R-26 [27] which was reduced under identical conditions, is shown for comparison.

have similar magnetic properties; thus, both signals are difficult to observe at temperatures above 15°K, and both are difficult to saturate with microwave power (cf. [7]).

## 4. Discussion

We have described a simple and rapid procedure for the isolation of photochemically active reaction centers from *R. rubrum*. The final chromatographic step on hydroxylapatite probably removes all excess LDAO from the reaction center preparation, and this apparently results in a preparation that is more stable than others described for this organism (cf. [10]). The spectroscopic properties of the primary electron donor $(BChl)_2$ are within experimental error of the values measured in whole cells and are very similar to those of other bacteriochlorophyll *a*-containing species (Rhodspirillaceae and Chromataceae). However the EPR spectrum of the 'primary acceptor', QFe, with its prominent band at $g$ 1.87, is quite distinct from that of other bacteriochlorophyll *a*- or *b*-containing purple bacteria examined, which have their major band at $g_y$ 1.82 [3–8].

The first question to be asked is whether this $g$ 1.87 signal could be an artefact of the reaction center purification procedure. This seems unlikely because the $g$ 1.87 signal can be observed in whole cells under certain conditions: The presence in whole cells of a Rieske-type $g$ 1.90 iron–sulfur center which has very similar properties to that of the $g$ 1.90 center in *C. vinosum* [17,18], *Rps. sphaeroides* [19] and *Rps. capsulata* [19], obscures the $g$ 1.87 signal except at very low temperatures and very high powers when the Rieske iron–sulfur centers are saturated. Furthermore, if the 'primary acceptor' in *R. rubrum* were identical to those in the other purple bacteria studied to date, a $g$ 1.82 signal would have been clearly seen in the whole cells.

The next question to be considered is the chemical nature of the species giving rise to the $g$ 1.87 signal, and this is perhaps best approached by considering the $g$ 1.82 signal of the other species. This latter signal is thought to be that of an unpaired electron residing chiefly on a quinone [4] in close association with an iron atom (see Introduction); in *Rps. sphaeroides* the quinone is ubiquinone [20], while in *C. vinosum* [21] and *Rps. viridis* [22] it is menaquinone or menaquinone-like, respectively. Evidence from Mossbauer [23] and EPR [24] spectroscopy, and from magnetic susceptibility [23] suggest that the iron moiety of QFe is a high-spin ferrous ion, and it is the very rapid relaxation rate of this species which gives the $g$ 1.82 signal its unusual temperature and power saturation properties [7,24]. If the iron is removed or disturbed in some way, the reduced quinone can be detected as a semiquinone radical at $g$ 2.0045 ([25], see also [3]). This 'primary

acceptor' signal was in fact first seen in iron-free subchromatophore fragments of *R. rubrum* [26], and it has provided the strongest evidence that a quinone functions as the 'primary acceptor' in this organism. The unusual temperature and power dependencies of the *g* 1.87 signal reported here, which are similar to those of the *g* 1.82 signal discussed above, suggest that under more physiological conditions the quinone of the *R. rubrum* 'primary acceptor' might be magnetically coupled to a high-spin ferrous iron. If this interpretation is correct, the differences between the *g* 1.82 and *g* 1.87 QFe complexes might be due to subtle differences in the relative arrangement of the magnetic partners.

To date we have been unable to trap the intermediary carrier, I, in its reduced state, but in other species I seems to involve one of the bacteriopheophytin molecules in the reaction center [1,2]. The reaction center of *R. rubrum* clearly contains bacteriopheophytin, and we have shown that it can also be resolved in the spectrum of unfractionated whole cells (fig.2) so that it is unlikely to be an artefact of the preparative procedure. Experiments are underway in an attempt to chemically reduce I to see whether bacteriopheophytin is involved in the primary photochemistry of *R. rubrum.*

## Acknowledgements

This work was supported by National Science Foundation grants PCM 76 14209 and BMS 75-20252. JPT gratefully acknowledges receipt of a Guggenheim Memorial Fellowship. The authors thank Dr P. Leslie Dutton for his help and interest in the research reported here.

## References

[1] Dutton, P. L., Prince, R. C., Tiede, D. M., Petty, K. M., Kaufmann, K. J., Netzel, T. L. and Rentzepis, P. M. (1976) Brookhaven Symp. Biol. 28, 213–237.
[2] Fajer, J., Davis, M. S., Brune, D. C., Spaulding, L. D., Borg, D. C. and Forman, A. (1976) Brookhaven Symp. Biol. 28, 74–104.
[3] Prince, R. C., Tiede, D. M., Thornber, J. P. and Dutton, P. L. (1977) Biochim. Biophys. Acta, in press.
[4] Bolton, J. R. and Cost, K. (1973) Photochem. Photobiol. 18, 417–421.
[5] Leigh, J. S. and Dutton, P. L. (1972) Biochem. Biophys. Res. Commun. 46, 414–421.
[6] McElroy, J. D., Feher, G. and Mauzerall, D. (1970) Biophys. J. 10, 204a.
[7] Dutton, P. L., Leigh, J. S. and Reed, D. W. (1973) Biochim. Biophys. Acta 292, 654–664.
[8] Prince, R. C., Leigh, J. S. and Dutton, P. L. (1976) Biochim. Biophys. Acta 440, 622–636.
[9] Noel, H., Van der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta 275, 219–230.
[10] Gingras, G. (1977) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) Plenum Press in press.
[11] Clayton, R. K. (1973) Ann. Rev. Biophys. Bioenerg. 2, 131–156.
[12] McElroy, J. D., Feher, G. and Mauzerall, D. C. (1972) Biochim. Biophys. Acta 267, 363–374.
[13] Norris, J. R., Uphaus, R. A., Crespi, H. L. and Katz, J. J. (1971) Proc. Natl. Acad. Sci. USA 68, 625–628.
[14] Dutton, P. L., Leigh, J. S. and Seibert, M. (1972) Biochem. Biophys. Res. Commun. 46, 405–413.
[15] Uphaus, R. A., Norris, J. R. and Katz, J. J. (1974) Biochem. Biophys. Res. Commun. 61, 1057–1063.
[16] Hoff, A. J. (1976) Biochim. Biophys. Acta 440, 765–771.
[17] Dutton, P. L. and Leigh, J. S. (1973) Biochim. Biophys. Acta 314, 178–190.
[18] Evans, M. C. W., Lord, A. V. and Reeves, S. G. (1974) Biochem. J. 138, 177–183.
[19] Prince, R. C., Lindsay, J. G. and Dutton, P. L. (1975) FEBS Lett. 51, 108–111.
[20] Okamura, M. Y., Isaacson, R. A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.
[21] Okamura, M. Y., Ackerson, L. C., Isaacson, R. A., Parson, W. W. and Feher, G. (1976) Biophys. J. 16, 233a.
[22] Pucheu, N. L., Kerber, N. L. and Garcia, A. F. (1976) Arch. Microbiol. 109, 301–305.
[23] Feher, G. and Okamura, M. Y. (1976) Brookhaven Symp. Biol. 28, 183–194.
[24] Tiede, D. M., Prince, R. C. and Dutton, P. L. (1977) Biophys. J. 17, 198a.
[25] Feher, G., Okamura, M. Y. and McElroy, J. D. (1972) Biochim. Biophys. Acta 267, 222–226.
[26] Loach, P. A. and Hall, R. L. (1973) Proc. Natl. Acad. Sci. USA 60, 786–790.
[27] Clayton, R. K. and Wang, R. T. (1971) Meth. Enzymol. 23A, 696–704.