# THE HIGH POTENTIAL SEMIQUINONE-IRON SIGNAL IN *RHODOPSEUDOMONAS VIRIDIS* IS THE SPECIFIC QUINONE SECONDARY ELECTRON ACCEPTOR IN THE PHOTOSYNTHETIC REACTION CENTRE

A. W. RUTHERFORD and M. C. W. EVANS

*Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, England*

Received 12 June 1979

## 1. Introduction

On illumination of the purple photosynthetic bacterial reaction centre an electron moves from a special pair of bacteriochlorophyll molecules [1] to a bacteriopheophytin molecule in less than 10 psec and then to the primary quinone acceptor, $Q_1$, in approximately 200 psec [2,3]. $Q_1$ is closely associated with a ferrous iron atom [4] which seems to be responsible, at least in part, for the quinone's unusual ability to form the stable unprotonated semiquinone [5] and may play a role in transport to the secondary acceptor [6]. It is this $Q_1^-$-Fe interaction that is responsible for the distorted e.p.r. signal observed at $g$=1.82 [7,8].

It has been recognised for some years that the secondary electron carrier is also a quinone molecule [9] and recently it has been shown in the reaction centres [10,11] and chromatophores [12] of *Rhodopseudomonas sphaeroides* R-26 that this is a special quinone which accepts two consecutive electrons before donating to the ubiquinone pool. It was also shown that in the reaction centres of this bacterium the secondary quinone is associated with a ferrous iron atom, $Q_2^-$-Fe, to give a similar but not identical e.p.r. signal to that of $Q_1^-$-Fe [13].

Recently we reported a $g$=1.82 signal in *Rps. viridis* chromatophores which was observed at higher potentials ($E_{m\,8.0} \simeq +70$ mV) than $Q_1^-$-Fe ($E_{m\,8.0} \simeq -165$ mV) in this bacterium [14]. Despite an interaction effect when both semiquinones were present at the same time, we were unable to determine the role of this species without direct evidence of its function and the possibility remained that the signal was due to another special semiquinone in the secondary electron transport chain, e.g. Z, which is thought to function between cytochrome *b* and *c* in other photosynthetic bacteria and has a midpoint potential in this range also [15–17]. We have now identified the high potential semiquinone-iron signal as being due to $Q_2^-$-Fe.

## 2. Materials and methods

*Rps. viridis* was grown to late log phase in a modified Hutner's medium and chromatophores were prepared as previously described [18]. Reaction centres were prepared using L.D.A.O. detergent treatment and adsorption chromatography on hydroxyapatite essentially as described in [19], but collected from the wash and concentrated by ultrafiltration rather than by ammonium sulphate precipitation.

Samples in 3 mm quartz e.p.r. tubes were illuminated by 10 μsec flashes of light from a 20 joule Xenon flash lamp and frozen quickly to 120°C in an isopentane/cyclohexane (5 : 1) freezing bath and then to liquid nitrogen temperature with a total freezing time of about 10 sec. When chromatophores were used for these flash experiments their redox potential was adjusted using small additions of freshly made potassium ferricyanide solution. EPR spectroscopy was carried out at liquid helium temperatures using a JEOL FE1X spectrometer with a cylindrical cavity as described earlier [18]. Orthophenanthroline and antimycin *A* were supplied by Sigma (London) Chemical Co.

## 3. Results

Dark adapted chromatophores poised at around +150 mV were found to have no e.p.r. signal in the $g$=1.82 region (fig.1b) but after one flash at room temperature the signal shown in fig.1a was observed. This signal has the characteristic double peak at $g$=1.82 and associated $g$=1.75 signal of the high potential $Q^-$-Fe obtained by equilibrium redox titration [14,18]. In order to establish the position of operation in the electron transport chain of this flash induced signal an experiment was carried out using the inhibitors *o*-phenanthroline, which inhibits between $Q_1$ and $Q_2$ [21], and antimycin *A*, which inhibits between cytochrome *b* and Z [22]. Antimycin *A* (fig.1g and h) has no effect on the size of the flash induced signal which is induced to almost the same extent as in the ethanol control (fig.1c and d). With *o*-phenanthroline however, no signal can be flash induced (fig.1e and f). These results indicate that the flash induced signal is $Q_2^{\cdot}$. However the possibility cannot be ruled out that this signal is due to a component operating between $Q_2$ and the antimycin *A* block. In order to substantiate the identity of the signal as $Q_2$, purified reaction centres were prepared and flash experiments were carried out on these. Figure 2a and b show that after one flash the characteristic high potential $Q^-$-Fe signal was produced. Figure 2c shows the $Q_1^-$-Fe signal which is irreversibly photoinduced at low temperatures in reaction centres which have the cytochrome $c_{553}$ reduced by small additions of sodium dithionite.

When samples of chromatophores and reaction centres which had the flash induced signal were illuminated at 7K shape and size changes occured which we attribute to interaction between $Q_1^-$ and $Q_2^-$. These changes were not as large as those observed in titrations of chromatophores at pH 10 [14]. The decrease in this effect is probably due to the redox state of the preparations which have only a small amount of the cytochrome $c_{553}$ in the reduced form ($E_m \simeq +45$ mV) [18]. At the same time as the changes at $g$=1.82 we observed small changes in the $g$=2 region, these may be the result of the interaction between $Q_1^-$ and $Q_2^-$ but we were unable to obtain sufficiently reproducible results to confirm this.

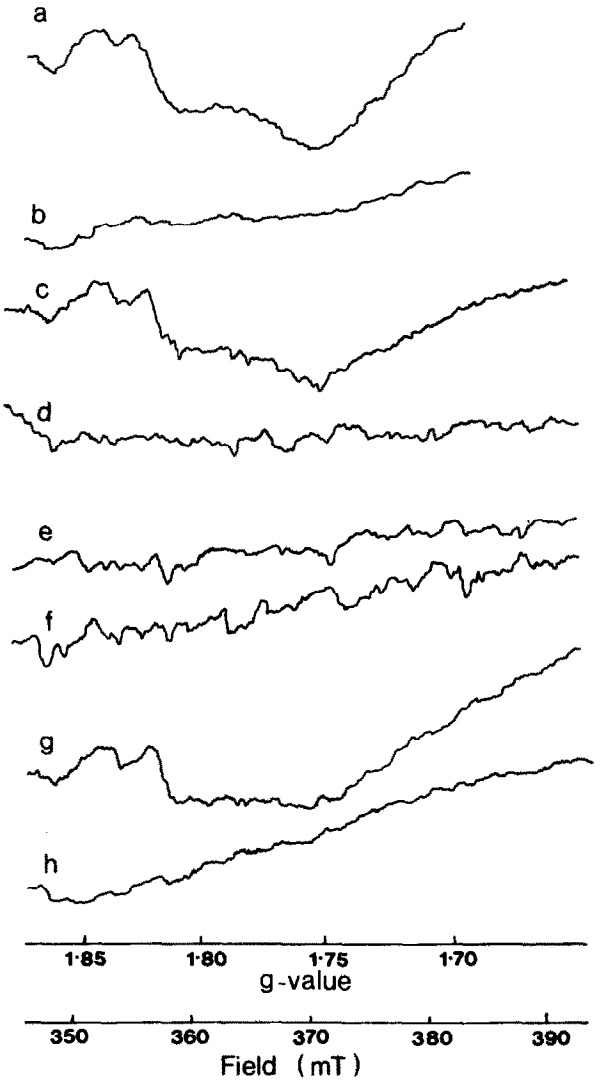


Fig.1. Flash-induced $g$=1.82 signals in *Rps. viridis* chromatophores. The chromatophores (≃ 3 mM BChl b) were in the presence of 2 mM potassium ferricyanide. Samples a and b were dark adapted at room temperature for 5 min, (a) given 1 flash and rapidly frozen, (b) frozen dark. Samples c and d were dark adapted for 10 min in the presence of 10% ethanol, (c) 1 flash and rapidly frozen, (d) frozen dark. Samples e and f were dark adapted for 10 min in the presence of ≃ 10 mM *o*-phenanthroline in ethanol making the sample 10% ethanol, (e) 1 flash and rapidly frozen, (f) frozen dark. Samples g and h were dark adapted for 10 min in the presence of antimycin *A* (≃ 10 mM) in ethanol making the sample 10% ethanol, (g) 1 flash and rapidly frozen (h) frozen dark. Spectra were recorded at 6K with the following e.p.r. conditions: microwave power, 20 mW; frequency, 9.09 GHz; modulation amplitude, 1.0 mT; gain 1000.

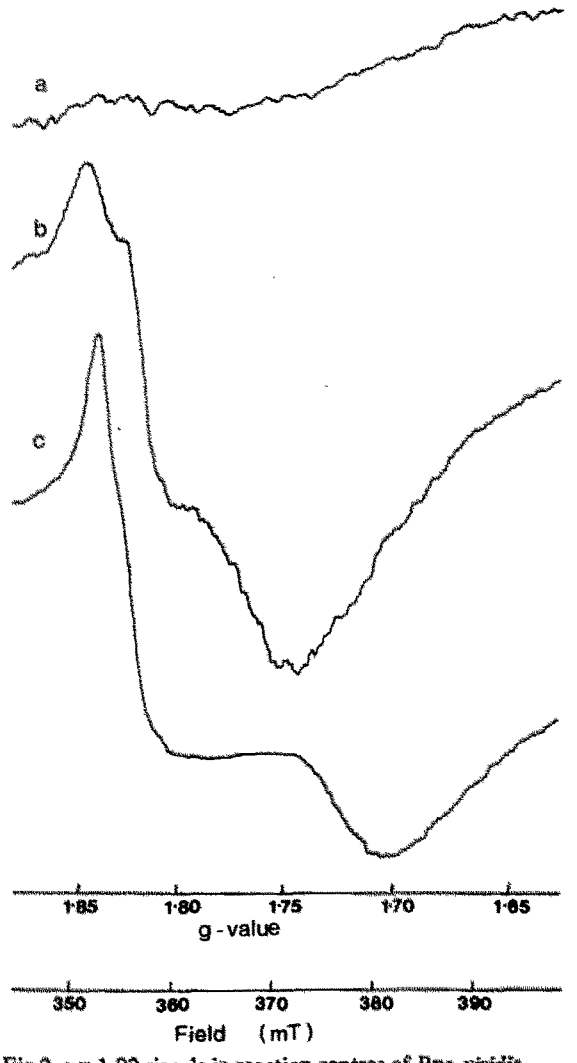


Fig.2. $g = 1.82$ signals in reaction centres of *Rps. viridis*. Samples were dark adapted for 5 min, (a) frozen dark, (b) 1 flash and rapidly frozen, (c) the signal observed in a sample in the presence of 0.2% sodium dithionite after illumination for 30 sec at 7K. e.p.r. conditions were as in fig.1, except (c) was recorded at a gain of 500.

## 4. Discussion

The identification of the high potential $Q^-$-Fe signal of *Rps. viridis* chromatophores as $Q_2$ is important as the first demonstration that the specialized secondary acceptor operating in the native membrane is a quinone–iron complex. A quinone–iron signal attributed to $Q_2$ has been shown in *Rps. sphaeroides* reaction centres in the presence of added quinone [6,10,13]. Our experiments show that this is a native component and that it is probably a common component to all purple photosynthetic bacteria. Since the position and function of this component in the electron transport chain is now known the redox data obtained earlier can be regarded as the first directly measured midpoint potentials of $Q_2$. However until the effects of pH and proton binding are investigated in this species we are unable to assign an operating potential to the $Q_2/Q_2^-$ couple. If a proton is involved then the operating potential will be that of the $E_{m\,7.0}$ ($\simeq +130$ mV) if not then it will be the $E_m$ at the p$K$ [21] ($E_{m\,10.0} \simeq -15$ mV) [14].

The presence of the double interaction between $Q_1^-$ and $Q_2^-$ via the iron atom [14] supports the hypothesis that the iron atom plays a role in the electron transfer from $Q_1^-$ to $Q_2$ [6].

## Acknowledgements

This work was supported in part by grants from the U.K. Science Research Council. A.W.R. is the recipient of an S.R.C. studentship.

## References

[1] Norris, J. R. and Katz, J. J. (1978) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) Plenum Press Co., New York, 397–416.
[2] Rockley, M. G., Windsor, M. W., Cogdell, R. J. and Parson, W. W. (1975) Proc. Natl. Acad. Sci. USA 72, 2251–2252.
[3] Kaufman, K. J., Dutton, P. L., Netzel, T. L., Leigh, J. S. and Rentzepis, P. M. (1975) Science 188, 1301–1304.
[4] Feher, G. and Okamura, M. Y. (1976) Brookhaven Symp. Biol. 28, 183–194.
[5] Tiede, D. M., Prince, E. C. and Dutton, P. L. (1978) Biophys. J. 21, 196a.
[6] Okamura, M. Y., Isaacson, R. A. and Feher, G. (1978) Biophys. J. 21, 196a.
[7] McElroy, J. D., Feher, G. and Mauzerall, D. (1970) Biophys. Soc. Abstract 10, 204.
[8] Leigh, J. S. and Dutton, P. L. (1972) Biochem. Biophys. Res. Commun. 46, 414–421.
[9] Halsey, Y. D. and Parson, W. W. (1974) Biochim. Biophys. Acta 347, 404–416.
[10] Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516–524.
[11] Wraight, C. A. (1977) Biochim. Biophys. Acta 459, 525–531.

[12] Barouch, Y. and Clayton, R. K. (1977) Biochim. Biophys. Acta 462, 785–788.
[13] Wraight, C. A. (1977) FEBS Lett. 93, 283–288.
[14] Rutherford, A. W. and Evans, M. C. W. (1979) FEBS Lett. 100, 305–308.
[15] Cogdell, R. J., Jackson, J. B. and Crofts, A. R. (1973) J. Bioenerg. 4, 211–227.
[16] Evans, E. H. and Crofts, A. R. (1974) Biochim. Biophys. Acta 357, 89–102.
[17] Prince, R. C. and Dutton, P. L. (1977) Biochim. Biophys. Acta 462, 731–747.
[18] Rutherford, A. W., Heathcote, P. and Evans, M. C. W. (1979) Biochem. J. in press.
[19] Clayton, R. K. and Clayton, B. J. (1978) 501, 478–487.
[20] Dutton, P. L. (1971) Biochim. Biophys. Acta 226, 63–80.
[21] Prince, R. C. and Dutton, P. L. (1978) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) Plenum Press Co., New York, 439–453.
[22] Dutton, P. L. and Prince, R. C. (1978) in: The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R. eds) Plenum Press Co., New York, 525–570.