A HIGH POTENTIAL SEMIQUINONE–IRON TYPE EPR SIGNAL IN *RHODOPSEUDOMONAS VIRIDIS*

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1. Introduction

It is generally accepted that the classically defined primary acceptor of photosynthesis [1] in purple photosynthetic bacteria is a quinone molecule which is associated with a ferrous iron atom. When this quinone (Q_1) is reduced to the semiquinone form (Q_1^-) a characteristic electron paramagnetic resonance (EPR) signal at $g = 1.82$ is observable at liquid helium temperature [2,3]. The unique position and assymetry of this signal is a result of antiferronmagnetic coupling between the semiquinone and the ferrous iron atom (Q_1^-–Fe) [4]. By carrying out flash experiments with *Rhodopseudomonas sphaeroides* it has recently been shown that the secondary acceptor is also a specialised quinone molecule (Q_2) [5,6]. In its semiquinone form, (Q_2^-), it also exhibits an EPR signal which is similar to the signal attributed to Q_1^-–Fe [6,7].

In this work a new semiquinone–iron type signal is reported in *Rhodopseudomonas viridis* which is significantly different from the Q_1^-–Fe signal in this species and the Q_2^-–Fe as reported in *R. sphaeroides*, and has an E_m value that suggests a role in secondary electron transport.

2. Materials and methods

Chromatophores from *R. viridis* were prepared by French pressure cell treatment and centrifugation [8]. Oxidation–reduction potentiometry was carried out as in [9] by the method in [10]. Triton X-100 (0.2%, w/v) was added to the particles to allow better equilibration. Titrations at pH 8.0 were carried out in 0.05 M Tris/HCl buffer (pH 8.0) and titrations at pH 10 were carried out in 0.1 M glycine/KOH buffer (pH 10). EPR spectra were obtained with a Jeol FE1 X ESR spectrometer at liquid helium temperatures using an Oxford instrument cryostat and temperature monitoring system. Theoretical curves for a one-electron accepting centre were fitted using a Tektronix 4051 computer.

3. Results and discussion

Two EPR signals were observed in the $g = 1.82$ region (fig.1a,b). The first (fig.1a) is attributed to Q_1^-–Fe which is chemically reducible with a mid-point potential (E_m) of −170 mV and a pK at pH~7.6 [8,11] and is also irreversibly photoreducible at potentials where cytochrome c_{553} is reduced ($E_m \simeq 50$ mV) [8]. The second is a broader $g = 1.82$ signal (fig.1b) which has a prominent high field signal (g_x) at $g = 1.75$ (by comparison, the g_x of Q_1^-–Fe is at $g = 1.69$). In oxidation–reduction potential titration this component appears at a more oxidised potential than Q_1^-–Fe ($E_{m,8.0} = 67$ mV (fig.2) and $E_{m,10.0} = -15$ mV (fig.3). Both titrations show that the high potential semiquinone–iron signal starts to decrease in size soon after the maximum is reached, and, in the pH 10 titration (fig.3), a theoretical curve for a one-electron accepting centre has been fitted to the disappearance of this signal. The E_m of this curve is −155 mV. This transition presumably represents the double reduction of the quinone.

When samples with the high potential signal chemically induced were illuminated at 7 K significant

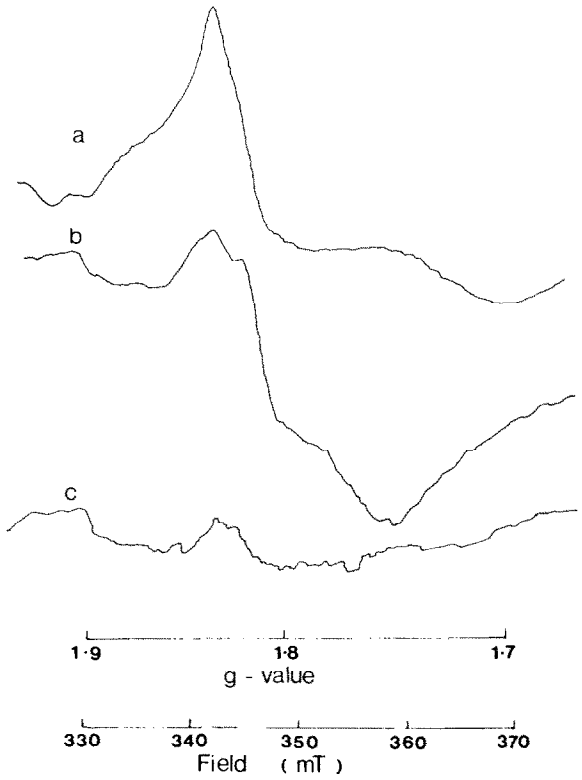

Fig.1. EPR spectra of semiquinone–iron type components in *R. viridis* chromatophores. EPR samples in 3 mm quartz tubes, contained 3 mM bacteriochlorophyll *b*. Spectra were recorded at 7 K with the following instrument settings: microwave power, 20 mW; frequency, 8.79 GHz; modulation amplitude, 1 mT. (a) shows the dark spectrum of a sample poised at −400 mV at pH 10. (b) shows the dark spectrum of a sample poised at −81 mV at pH 10. (c) shows the same sample as in (b) after illumination for 30 s with a 1000 W projector at 7 K.

size reduction and shape change was observed (fig.1c). This is presumably due to an interaction between the photoreduced Q_1^-–Fe and the high potential semiquinone–iron complex. Microwave power saturation investigations have been carried out on these $g = 1.82$ signals (fig.4). The unusual microwave power saturation characteristics of the Q_1^-–Fe signal (fig.4a) are also exhibited by the high potential semiquinone–iron signal (fig.4b). Also it can be seen that the small $g = 1.82$ signal remaining after illumination (fig.1c) has similar power saturation characteristics to the other semiquinone–iron signals (fig.4c). Therefore, it

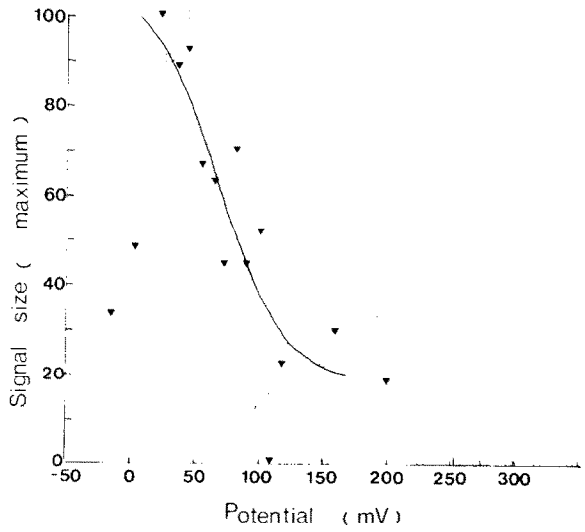

Fig.2. Oxidation–reduction potential titration at the high potential semiquinone–iron component in *R. viridis* chromatophores at pH 8.0. The chromatophores were equilibrated with the appropriate mediators for 40 min. Triton X-100 (0.2%, w/v) was added to allow better equilibration. The titration was performed in near darkness and samples were frozen in complete darkness. EPR spectra were recorded in the dark with EPR conditions as described for fig.1. Signal height was measured at $g = 1.82$ at 7 K. The results of 3 different titrations are presented (○ ▼ □). The curve drawn is a theoretical curve for one-electron acceptor with an E_m at 67 mV.

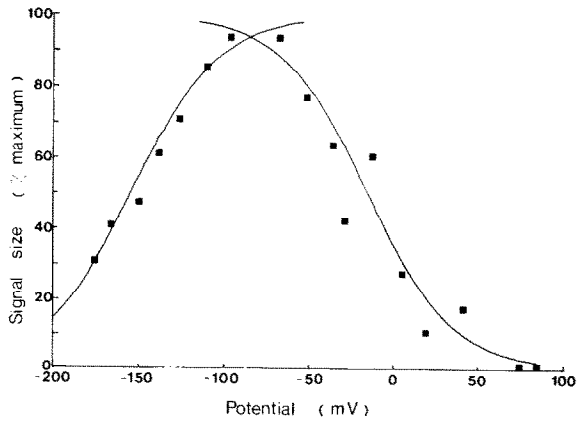

Fig.3. Oxidation–reduction potential titration of a high potential semiquinone–iron component in *R. viridis* at pH 10. The titration was carried out in 0.1 M glycine/KOH (pH 10) but otherwise as in fig.2. EPR conditions were as in fig.1. Signal size was measured as the height of the $g = 1.82$ signal at 7 K. Two theoretical curves for one-electron acceptors have been fitted with E_m at −15 mV and −155 mV.

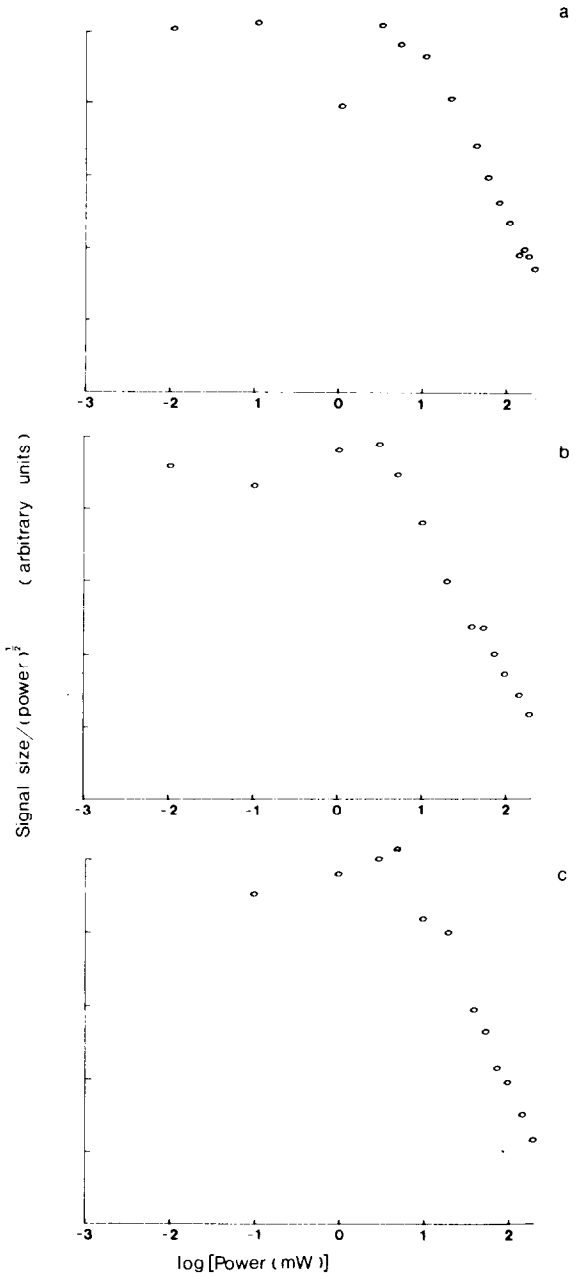

Fig.4. Power saturation characteristics of $g = 1.82$ EPR signals found in *R. viridis* chromatophores. The samples used were those whose spectra are shown in fig.1. Spectra were measured at 4.2 K with the following instrument settings: frequency, 8.79 GHz; modulation amplitude, 0.5 mT; with microwave power as indicated: a, $g = 1.82$ signal at −400 mV; b, $g = 1.82$ signal at −81 mV; c, $g = 1.82$ signal at −81 mV after illumination at 7 K.

is probable that this signal is due to residual unaffected semiquinone–iron complex and not to a modified form resulting from the double interaction.

From the position, shape and saturation characteristics of the EPR signal it is reasonable to suggest that the new signal is produced by a semiquinone molecule interacting with a ferrous iron atom in a similar, but not identical, fashion to Q_1^-–Fe. The component must also be close enough to Q_1^-–Fe to allow the strong interaction effect observed here. A size reduction was observed [12] in the flash-induced Q_1^-–Fe $g = 1.82$ signal when Q_1^-–Fe was reversibly photo-induced in *R. sphaeroides* reaction centres. To explain this result it was suggested that the two semiquinone species were interacting with the same iron atom causing a disappearance of the $g = 1.82$ signal. Such an explanation can be applied to the interaction observed here if it is assumed that the high potential quinone is Q_2.

However, such an assumption is not justified on the basis of the data available. In fact, the EPR spectrum of the high potential Q^-–Fe complex reported here is markedly different from the Q_2^-–Fe signal reported in *R. sphaeroides*, having its high field component at $g = 1.75$ instead of $g = 1.63$ [12]. Also the E_m value for the single reduction of this component is more compatible with a function in the secondary electron transport chain. Interestingly, the E_m value reported here is very similar to that found for the component Z, which has been reported in *R. sphaeroides* [13] and *Rhodopseudomonas capsulata* [14]. Z is thought to be a special quinone [15] which operates in some way between cytochrome *b* and a high potential cytochrome *c* in cyclic electron transport. It is tempting to assign the high potential Q^-–Fe signal reported here to Z, however, since it is thought that Z operates on the opposite side of the membrane to the acceptor of the reaction centre, a magnetic interaction between these components would not be expected. Further experimentation attempting to establish the identity of this high potential Q^-–Fe is underway.

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