Electron-Paramagnetic-Resonance Measurements of the Electron-Transfer Components of the Reaction Centre of *Rhodopseudomonas viridis*

OXIDATION-REDUCTION POTENTIALS AND INTERACTIONS OF THE ELECTRON ACCEPTORS

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Oxidation-reduction potentiometry was carried out on *Rhodopseudomonas viridis* chromatophores. Measurements of e.p.r. signals of the semiquinone-iron type at $g = 1.82$ have revealed a more complex situation than previously reported. The presence of three different components is indicated. The midpoint potential (E_m) of the primary acceptor quinone/semiquinone couple was found to be approx. -165 mV at pH 10, with a pK being reached at around pH 7.5. The primary acceptor also accepts a second electron with an E_m of -525 mV, but this redox transition exhibits a hysteresis effect. Interaction effects indicate the presence of another component with E_m values at pH 10 of approx. -165 mV (pK reached at around pH 7.5) for single reduction and -350 mV (pK at pH 10 or greater) for double reduction. It is suggested that this component is the secondary acceptor. Another semiquinone-iron-type component which gives a $g = 1.82$ signal is also present. This component is distinguishable from the primary acceptor by its e.p.r. spectrum, which shows a double peak at $g = 1.82$ and a g_x line at $g = 1.76$. This component has E_m values at pH 10 for single and double reduction of -15 mV and approx. -150 mV respectively. Both of these E_m values are pH-dependent. The presence of an interaction between this component and the photoreduced primary acceptor indicates the close proximity of these components. However, the midpoint potential of this component indicates a function as a secondary electron-transport component rather than an electron acceptor in the reaction centre. The dependence of the bacteriopheophytin intermediate (I) doublet e.p.r. signal on the presence of the semiquinone-iron form of the primary acceptor is demonstrated. The midpoint potential of the I/I⁻ couple is estimated to be lower than -600 mV.

In recent years much progress has been made towards the elucidation of the structure and function of the bacterial photosynthetic reaction centre (see review by Dutton *et al.*, 1976). In purple photosynthetic bacteria it is generally accepted that the primary donor is a specialized bacteriochlorophyll pair which, on illumination, donates an electron via a bacteriopheophytin intermediate (I) to a quinone primary acceptor (Q_1). The changes of oxidation state involved in these processes have been observed spectrophotometrically and by low-temperature e.p.r. spectrometry.

The reduced Q_1 has been shown to be anti-ferromagnetically coupled (Butler *et al.*, 1978) to a nearby ferrous iron atom (Q_1^- -Fe), giving a characteristically asymmetrical e.p.r. signal with g_y and g_x values at $g = 1.82$ and $g = 1.68$ respectively (Dutton *et al.*, 1973a). If this interaction with the iron is disturbed, some or all of this e.p.r. signal is replaced by another at $g \approx 1.9$ (Tiede *et al.*, 1978). If the interaction is absent, a normal semiquinone free-radical signal

is seen at $g = 2.0045$ with a linewidth of approx. 0.8 mT (Prince *et al.*, 1977; Tiede *et al.*, 1978). The loss of this interaction also affects the electron-accepting properties of the quinone (Tiede *et al.*, 1978).

Midpoint-potential (E_m) determinations of the Q_1/Q_1^- couple have been carried out on several different species of bacteria (Prince & Dutton, 1976). Results have varied, but generally it has been found that in chromatophores the E_m is pH-dependent. Since the primary reaction occurs much more rapidly than does protonation, the operating E_m of the Q_1/Q_1^- couple is thought to be one that is pH-independent, i.e. the E_m at the pK.

In isolated reaction centres of *Rhodopseudomonas sphaeroides* R-26 mutant the E_m of the Q_1/Q_1^- couple was found to have a more positive value and to be independent of pH (Dutton *et al.*, 1973b).

Only rarely have E_m determinations been carried out that have measured the $g = 1.82$ signal itself (Dutton *et al.*, 1973b; Evans *et al.*, 1974); the majority have measured indirect changes. In

Rhodospseudomonas viridis, the organism studied in the present work, Thorner & Olson (1971), Cogdell & Crofts (1972) and Prince *et al.* (1976) measured the E_m of the Q_1/Q_1^- couple by following the light-induced cytochrome changes spectrophotometrically. Prince *et al.* (1976) reported an E_m value of -150 mV at pH 8, with a pK being reached at pH 7.8.

It has been shown that the secondary acceptor (Q_2) is also a specialized quinone closely associated with an iron atom (Wraight, 1977; Okamura *et al.*, 1978). This acts as a two-electron gate, becoming doubly reduced and protonated before donating both electrons to the quinone pool (Vermeglio, 1977; Wraight, 1978a).

Most e.p.r. studies of intermediate I have been carried out with *Chromatium vinosum* and *R. viridis* because they possess *c*-type cytochromes which are closely associated with the reaction centre (Dutton *et al.*, 1976). If Q_1 is reduced and material is illuminated under special conditions (Evans *et al.*, 1974; Tiede *et al.*, 1976), the cytochrome *c* can donate an electron to the oxidized bacteriochlorophyll dimer and consequently trap intermediate I in its reduced form. It is also possible to trap form I^- in *R. sphaeroides* reaction centres by using an external donor and illumination at room temperature (Okamura *et al.*, 1977).

The trapped form I^- has been found to be exchange- and dipolar-coupled (Prince *et al.*, 1977) to the Q_1^- -Fe species, giving rise to a characteristic doublet signal centred at $g = 2.003$. Without this interaction an unsplit I^- free-radical signal appears; this was demonstrated in *R. viridis* reaction centres which were prepared by digestion with the detergent sodium dodecyl sulphate, in which the Q^- -Fe interaction is absent (Prince *et al.*, 1977). From measurements *in vitro* Fajer *et al.* (1975, 1976) have estimated E_m values for the reduction of bacteriopheophytin *a* and bacteriopheophytin *b* to be -550 mV and -500 mV respectively. Determinations of the E_m of the I/I^- couple *in vivo* have been reported in *R. viridis*. This bacterium was used because, as well as its cytochromes functioning at low temperature as described above, it is a bacteriochlorophyll *b*-containing species which absorbs less-energetic longer-wavelength light, and therefore is expected to have a smaller redox span and consequently an intermediate with a more positive E_m .

By using several different methods, including the observation of the attenuation of a light-induced triplet and of the 1301 nm absorbance attributed to the oxidized bacteriochlorophyll dimer, values for the E_m of the I/I^- couple at around -400 mV were obtained (Prince *et al.*, 1976; Netzel *et al.*, 1977). In contrast with this, Klimov *et al.* (1977) found a value of -620 mV by monitoring optically an absorbance change at 760 nm attributed to the reduction of the bacteriopheophytin.

The present work was undertaken to obtain redox titrations of species Q^- -Fe and I^- by direct measurement of e.p.r. signals attributed to these species in an attempt to resolve the E_m discrepancy of the I/I^- couple *in vivo* and to clarify further the function of the reaction-centre components.

Materials and Methods

R. viridis was grown in a modified Hutner's medium as described by Cohen-Bazire *et al.* (1957) with the addition of 0.003 M-acetate, 0.0017 M-glutamate and 0.1 % (w/v) yeast extract, and harvested in a continuous-flow centrifuge after 3 days' growth. The cells were washed in 0.05 M-Tris/HCl, pH 8.0, and stored in liquid nitrogen until required.

Chromatophores were prepared by suspending the cells 1:2 (w/v) in the same buffer and passing them through an Aminco French pressure cell twice at 138 MPa (20000 lb/in²) with the addition of deoxyribonuclease (EC 3.1.4.5) after the first pressing. Cell debris was sedimented by centrifugation at 38000g for 20 min. The chromatophores themselves were sedimented by centrifugation at 200000g for 2 h, washed once and resuspended in the same buffer to a concentration of approx. 0.003 M-bacteriochlorophyll *b*. Bacteriochlorophyll *b* concentration was determined by measuring the long-wavelength absorbance maximum at 1010 nm on a Cary 14 spectrophotometer and by using the approximate ϵ of 10^5 (Olson & Nadler, 1965). The chromatophores were stored at 4°C in the dark for up to 4 days.

Oxidation-reduction potentiometry was carried out as described earlier (Evans *et al.*, 1974) by the method of Dutton (1971). Titrations were carried out with the addition of Triton X-100 (0.2%, w/v) into the titration vessel to allow better equilibration. The following compounds were used as appropriate as mediators between the electrode and the electron carriers in the chromatophores: triquat ($E'_0 = -540\text{ mV}$), 100 μM ; Methyl Viologen ($E'_0 = -436\text{ mV}$), 100 μM ; diquat ($E'_0 = -350\text{ mV}$), 100 μM ; Benzyl Viologen ($E'_0 = -311\text{ mV}$), 100 μM ; Safranin T ($E'_0 = -289\text{ mV}$), 100 μM ; phenosafranin ($E'_0 = -255\text{ mV}$), 100 μM ; 2-hydroxy-1,4-naphthoquinone ($E'_0 = -145\text{ mV}$), 100 μM ; indigodisulphonate ($E'_0 = -125\text{ mV}$), 100 μM ; Methylene Blue ($E'_0 = 11\text{ mV}$), 100 μM ; 5-hydroxy-1,4-naphthoquinone ($E'_0 = 30\text{ mV}$), 100 μM ; thionine ($E'_0 = 60\text{ mV}$), 100 μM ; 1,2-naphthoquinone ($E'_0 = 145\text{ mV}$), 100 μM . The potential of the reaction mixture was adjusted with 0.1 M-potassium ferricyanide or 1 % (w/v) sodium dithionite in 0.1 M-Tris/HCl buffer, pH 9.0. Values of E_m obtained in repeated titrations normally agreed within $\pm 10\text{ mV}$. Unless mentioned in the text, values obtained by oxidative or reductive titrations were also within this range.

Titration at pH 6.5 were carried out with 0.1 M-Mes (4-morpholine-ethanesulphonic acid)/KOH, pH 6.5, and titrations at pH 10 were done in the presence of 0.1 M-glycine/KOH buffer, pH 10. The pH was monitored and maintained throughout the course of the titrations.

E.p.r. spectra were obtained with a Jeol J.E.S. FEIX spectrometer. Samples were cooled to liquid-helium temperature by using an Oxford Instruments cryostat and monitoring system. Illumination at 200 K was carried out in a solid CO₂/ethanol bath made up in an unsilvered dewar. Illumination in the cavity and at 200 K was done with a 1000 W projector.

Light-dark spectra were obtained by using a Nicolet 1020 A signal averager. Theoretical curves for a one-electron acceptor were fitted, by using a Tektronix 4051 computer.

Triquat and diquat were gifts provided by Imperial Chemical Industries (Bracknell, Berks, U.K.). The naphthquinone derivatives were obtained from Koch-Light Ltd. (Colnbrook, Bucks., U.K.). Mes, Tris, Triton X-100 and deoxyribonuclease were obtained from Sigma (London) Chemical Co. (Kingston-on-Thames, Surrey, U.K.). Other chemicals and mediators were obtained from British Drug Houses (Poole, Dorset, U.K.).

Results

Fig. 1 shows the results of a redox titration carried out at pH 6.5. Fig. 1(a) shows the extent of the $g = 1.82$ signal measured in dark samples. A high-potential component is present at the most oxidizing potentials; this signal disappears with an E_m at 110 mV. A second, larger, component titrates in at lower potentials and, despite overlap with the high-potential component, a one-electron curve with an E_m at -91 mV can be fitted to the points.

Fig. 1(b) shows the $g = 1.82$ signal after illumination at liquid-helium temperature, and Fig. 1(c) shows the extent of the light-induced signal (i.e. the light-dark signal size). The ability to photoreduce this signal titrates in with an E_m of +58 mV. This value reflects the redox state of cytochrome *c*₅₅₃, which donates to the reaction centre at low temperature and is slightly more oxidized than the reported E_m for this component of -12 mV (Cogdell & Crofts, 1972; Prince *et al.*, 1976). As expected, the ability to photoinduce any increase in $g = 1.82$ signal is lost as Q₁ is chemically reduced. However, the titration shows a marked decrease in signal size before the maximum chemically induced value is reached (Fig. 1b).

After illumination at 200 K the $g = 1.82$ signal disappears (Fig. 1d) and a one-electron curve with an E_m at -117 mV can be fitted to these points. This disappearance of the $g = 1.82$ signal matches exactly the appearance of the split signal attributed to species I⁻ after illumination at 200 K (Fig. 1e).

The same measurements when carried out on redox titrations done at pH 10 showed a more complex situation (Fig. 2). Fig. 2(a) shows the extent of the $g = 1.82$ signal in the dark. Again a high-potential component is present; however, the appearance of this signal is moved to a more reducing potential and is within the titration range used. A one-electron curve with an E_m at -15 mV can be fitted to the appearance of this component. The e.p.r. spectrum of this component is different from previously reported signals, which are attributed to Q₁⁻-Fe (Fig. 3a). It is narrower, has two peaks at $g = 1.82$ and has a g_x at a lower field value. The extent of this signal decreases at potentials lower than -100 mV. Between -130 mV and -180 mV a gradual change in shape takes place from the double-peak type to the more usual single-peak signal shown in Fig. 3(b). The latter rises to a plateau at -230 mV. This change-over of species giving rise to the $g = 1.82$ signal is responsible for the increase in the scatter of the points between -150 mV and -200 mV. Between -300 mV and -400 mV there is a dramatic rise in this signal, and a one-electron curve with an E_m of -350 mV has been fitted. The signal at its maximum extent (Fig. 3c) is typical of Q₁⁻-Fe signals previously reported (Dutton *et al.*, 1973b).

This Q₁⁻-Fe signal disappears (Fig. 3d) as the potential is lowered further. A one-electron curve with an E_m at -525 mV has been fitted to this disappearance, but this value is only given tentatively, since, although the effect is completely reversible in extent, a marked hysteresis effect is present. It was found that when titrating in an oxidizing direction over the range -650 mV to -250 mV a plateau at 40% maximum extent is reached, without going through a region of maximum height. However, when the material was re-reduced to -420 mV the usual rise to the maximum value with an E_m at -350 mV is seen.

The potential dependency of the $g = 1.82$ signal after illumination at liquid-helium temperature is shown in Fig. 2(b) and the light-induced signal size (light-dark) is plotted in Fig. 2(c). As shown in the pH 6.5 titration (Fig. 1), the ability to photo-induce Q₁⁻-Fe is dependent on the redox state of cytochrome *c*₅₅₃. As expected, the E_m of the cytochrome is essentially pH-independent (Cogdell & Crofts, 1972; Prince *et al.*, 1976) and a value of approx. +50 mV is found. As the Q₁ is chemically reduced, the ability to obtain a light-induced increase of the $g = 1.82$ signal is lost. This does not, however, occur at the maximum size of the $g = 1.82$ signal, but at the plateau of 40% maximum height. At potentials lower than this, illumination at this temperature has no effect and the points are identical with those measured in the dark. Also shown in Figs. 2(b) and 2(c) is the marked trough at -100 mV. This coincides closely with the presence of the high-potential $g = 1.82$ signal seen in the dark.

Fig. 1. Oxidation-reduction-potential dependence of the semiquinone-iron-type components and the trapped I^- doublet signal in *R. viridis* chromatophores at pH 6.5

R. viridis chromatophores (3 mM-bacteriochlorophyll *b*) in 0.1 M-Mes/KOH buffer, pH 6.5, were equilibrated with the appropriate mediators (see the Materials and Methods section) 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. E.p.r. spectra were recorded in the dark and after illumination for 30 s at liquid-helium temperature. Samples were then illuminated at 200 K for 10 min, then e.p.r. spectra re-recorded. Signal height at $g = 1.82$ (difference between $g = 1.84$ and $g = 1.81$) and the high-field part of the trapped I^- doublet signal at $g \approx 1.97$ were measured. E.p.r. spectra were recorded at 7 K with the following instrument settings: microwave power, 20 mW; modulation amplitude, 1 mT; frequency, 8.98 GHz; scan rate, 50 mT/min; instrument gain, 1000 (for $g = 1.82$ signals) and 50 (for $g \approx 1.97$ signals). The curves drawn are the theoretical curves for a one-electron-accepting centre and have been drawn to facilitate interpretation of the results. (a), Extent of the $g = 1.82$ signal in the dark; the E_m values of the curves are 110 mV and -91 mV. (b), Extent of the $g = 1.82$ signal after illumination at 7 K; the E_m values of the curves are 110 mV, 58 mV, -50 mV and -130 mV. (c), Light-dark $g = 1.82$ signal size after illumination at 7 K; the E_m values of the curves are 33 mV and -50 mV. (d), Extent of the $g = 1.82$ signal after illumination at 200 K; the E_m value of the curve is -117 mV. (e) Extent of the $g \approx 1.97$ signal after illumination at 200 K; the E_m value of the curve is -117 mV.

Fig. 2(d) shows the $g = 1.82$ signal after illumination at 200 K. At potentials more oxidizing than -100 mV the points are similar to those seen after liquid-

helium-temperature illumination. However, at potentials below -100 mV the signal size does not increase to a maximum and disappears at approx.

Fig. 2. Oxidation-reduction-potential dependence of the semiquinone-iron type components and trapped I^- doublet signal in *R. viridis* chromatophores at pH 10

R. viridis chromatophores (3 mM-bacteriochlorophyll *b*) in 0.1 M-glycine/KOH buffer, pH 10, were used. The titration conditions, treatment of samples and e.p.r. conditions were as described in the legend to Fig. 1. The points presented are the results of three different titrations (■, ○, ●) carried out over different potential ranges. The curves drawn are the theoretical curves for a one-electron-accepting centre and have been drawn to facilitate interpretation of the results. (a), Extent of the $g = 1.82$ signal difference between $g = 1.84$ and $g = 1.81$ in dark samples; the E_m values of the curves are -15 mV, -150 mV, -350 mV and -525 mV. (b), Extent of the $g = 1.82$ signal after illumination at 7 K; the E_m values of the curves are $+50$ mV, -55 mV, -150 mV, -225 mV, -350 mV and -525 mV. (c), The $g = 1.82$ light-dark signal size after illumination at 7 K; the E_m values of the curves are 45 mV, -25 mV, -160 mV and -200 mV. (d) Extent of the $g = 1.82$ signal after illumination at 200 K; the E_m value of the curve is -200 mV. (e), Extent of the $g \approx 1.97$ signal after illumination at 200 K; the E_m values of the curves are -165 mV, -330 mV and -522 mV.

-300 mV. A one-electron curve with an E_m of -225 mV has been fitted to this disappearance.

A marked two-step increase is seen in the titration of species I^- after 200 K illumination (Fig. 2e). Two one-electron curves have been fitted, with E_m values of -165 mV and -330 mV (Figs. 4a and 4b). The induced I^- species disappears at very reduced potentials

paralleling the disappearance of the $g = 1.82$ signal, with an E_m of -522 mV. Here again the same hysteresis effect is present, although the effect is again almost totally reversible in extent.

In very reduced samples where the $g = 1.82$ signal has disappeared and the ability to induce the I^- doublet has been lost, illumination at 200 K induces a

Fig. 3. E.p.r. signals in the $g = 1.82$ region in dark samples of *R. viridis* chromatophores titrated at pH 10

The e.p.r. spectra shown are of samples from the titrations presented in Fig. 2(a). E.p.r. conditions are as described in the legend of Fig. 1, except that a frequency of 9.09 GHz was used. Spectrum (a) is of a sample poised at -85 mV and has a g_x value of $g \approx 1.76$. Spectrum (b) is of a sample poised at -290 mV. Spectrum (c) is of a sample poised at -470 mV and has a g_x value of $g \approx 1.67$. Spectrum (d) is of a sample poised at -620 mV.

broad free-radical signal with a peak-to-peak width of $14.5g$ (Fig. 5). From its width it was thought that this could be the unsplit radical signal from species I^- ; two experiments were carried out which substantiated this identification. Firstly, the light-induced triplet signal that is present during continuous illumination at liquid-helium temperature was studied. This signal arises from a bacteriochlorophyll dimer triplet that is formed as an intermediate when electrons return from species I^- to the oxidized bacteriochlorophyll. The attenuation of this triplet has been used as an indirect measurement of the reduction of species I (Prince *et al.*, 1976). In samples poised at -600 mV the triplet was still present, but after the broad radical signal was induced by illumination at 200 K the triplet had disappeared. Secondly, it is known that, if a sample with species I^- trapped by illumination at 200 K is thawed out under nitrogen in the

Fig. 4. E.p.r. doublet signal attributed to the photoreduced intermediate (I^-) after illumination at 200 K of samples of *R. viridis* chromatophores titrated at pH 10

The e.p.r. spectra are of samples from the titrations shown in Fig. 2. E.p.r. conditions are as described in Fig. 1, except that a frequency of 9.09 GHz was used. Spectrum (a) is of a sample poised at -290 mV and spectrum (b) is of a sample poised at -470 mV.

Fig. 5. Free-radical signal that is induced by illumination at 200 K of *R. viridis* chromatophores poised at -590 mV (pH 10)

The e.p.r. spectrum is a light-dark spectrum recorded at 7 K with the following instrument settings: microwave power, 1 mW; modulation amplitude, 0.32 mT; frequency, 8.79 GHz; scan rate, 10 mT/min; instrument gain, 125.

dark and refrozen, the I^- doublet is lost and the original dark e.p.r. spectrum is obtained; when this is re-illuminated at 200 K the I^- doublet is regenerated. The broad radical signal trapped by illumination at 200 K also exhibits this unusual behaviour.

At the lowest potentials that could be obtained at this pH (−650 mV) despite the presence of a large mediator radical, some I^- radical was still inducible by illumination at 200 K, indicating that the E_m of the I/I^- couple is lower than −600 mV.

Discussion

The most striking feature of the titration results is the presence of a two-stage rise of the Q^- -Fe signal (and consequently the I^- signal) at pH 10, whereas only a single curve for this rise is seen at pH 6.5. The difference between the titrations is easily explained by assuming that there are two single-electron redox changes which have similar E_m values, but reach pK values at different values of pH. Since both redox changes have E_m values around −100 mV at pH 6.5, and the E_m is expected to shift by −59 mV per pH unit as the pH is increased, then it can be seen that at pH 10 the E_m values of −165 mV and −350 mV correspond to a situation where one of these redox changes reaches a pK at around pH 7.5, whereas the other remains pH-dependent up to pH 10.

Redox titrations carried out at pH 8.0 (results not shown) support this explanation.

What these two redox changes represent in terms of the reductions of reaction-centre components is much more difficult to decide. It is possible to fit several different schemes to the results; however, a systematic consideration of each change allows most of these schemes to be ruled out and a tentative solution to be offered.

In the titrations at both pH values a $g = 1.82$ signal is present at relatively high potentials. Because of its shape and position it is almost certainly attributable to a semiquinone-iron complex. It is, however, easily distinguishable from the Q_1^- -Fe signal by its width, fine structure and g_x value. When species Q_1^- is photoinduced at liquid-helium temperature in samples where the high-potential Q^- -Fe is present, no increase in $g = 1.82$ signal occurs. In fact, where the high-potential Q^- -Fe signal is at its maximum size, a light-induced decrease of the $g = 1.82$ signal is seen. This phenomenon is attributed to the presence of an interaction between the two Q^- -Fe species. This interaction is lost at potentials lower than −100 mV, allowing the light-induced Q_1^- -Fe signal to attain its maximum size again. The loss of the interaction is almost certainly due to the decrease and subsequent disappearance of the high-potential Q^- -Fe signal itself, presumably by double reduction. The curves fitted for this double reduction are affected by overlap and interaction with other species also

undergoing redox changes, and therefore the E_m values at pH 10 of −150 mV (Figs. 2a and 2b) and −160 mV (Fig. 2c) can only be considered an approximation.

It is tempting to assume that the high-potential Q^- -Fe signal is due to the secondary acceptor, Q_2 , which is expected to be present in chromatophore preparations. However, the measured E_m value is more positive than would be expected for Q_2 .

Wraight (1978b) has published an e.p.r. spectrum of Q_2^- -Fe in *R. sphaeroides*. This spectrum is different from that of the Q_1^- -Fe, but is also different from the high-potential Q^- -Fe spectrum reported here.

It is possible that the high-potential Q^- -Fe is a component operating in the secondary electron-transport chain. Such a component, however, would have to be close enough to the Q_1^- -Fe species to allow the interaction to take place.

The Q_1^- -Fe signal is more easily identified. Its shape, whether photochemically reduced or chemically reduced (E_m approx. −115 mV at pH 6.5, and approx. −165 mV and −350 mV at pH 10) is typical of previously reported Q_1^- -Fe signals (Dutton *et al.*, 1973a). Also, the presence of the Q_1^- -Fe is accompanied by an ability to induce species I^- to proportional extent.

At pH 10, even though there are two clearly distinguishable one-electron steps before the Q_1^- -Fe (and consequently the I^-) signal reaches its maximum extent, there are several pieces of evidence which show that the reduction of Q_1 to Q_1^- is totally accounted for by the reduction at −165 mV and pK at pH 7.5. Firstly, there is the attenuation of the increase in liquid-helium-temperature light-induced Q_1^- -Fe signal at around −225 mV. This would only be expected to occur when Q_1^- is fully chemically induced. Secondly, preliminary results (not described here) show that light-induced changes in the $g = 3$ region, which are attributed to cytochrome oxidation, attenuate at this potential also. Thirdly, after illumination at 200 K the Q_1^- -Fe signal disappears totally before the second step is begun. The more reduced value for the attenuation of the light-induced Q_1^- -Fe signal of −225 mV (Figs. 2b and 2c) is probably a result of overlap with the antagonistic effects of the double reduction of the high-potential quinone. Thus we regard the value of −165 mV, as reflected by the curve for induction of species I^- (Fig. 2e), to be a closer estimation of the E_m of the Q_1/Q_1^- couple.

The E_m and pK value for Q_1 are in good agreement with those reported by Prince *et al.* (1976) of an E_m of −150 mV and a pK at pH 7.8 obtained by measuring spectrophotometrically the attenuation of light-induced cytochrome oxidation.

The anomalously small size of the e.p.r. signal of the fully reduced Q_1 and its subsequent increase in

size at -350mV in a one-electron event which does not involve redox changes of Q_1 itself can be explained by invoking the presence of another component. This component has the following properties: firstly, it becomes reduced with an E_m value close to that of $Q_1\text{-Fe}$; secondly, in this redox state, it interacts with $Q_1\text{-Fe}$ in such a way that the $g = 1.82$ signal only reaches 40% of its maximum size; and thirdly, it is further reduced at -350mV to a form that no longer interacts with $Q_1\text{-Fe}$. Such properties would be expected from the secondary acceptor, Q_2 . The redox changes occurring at pH 10 in the acceptors of the reaction centre can therefore be presented as follows:

Although at pH 6.5 all three one-electron events overlap (see Fig. 1a), evidence of their presence is found in Fig. 1(b), which shows the extent of the $g = 1.82$ signal after illumination at liquid-helium temperature. The decrease in signal size with an E_m at around -50mV and subsequent rise to a maximum (E_m at -130mV) indicate the presence of $Q_1\cdots\cdots\text{Fe}\cdots\cdots Q_2^-$ and $Q_1\text{-Fe } Q_2^{2-}$ respectively.

The concomitant changes in inducibility of species I^- in titrations at both pH values are simply a consequence of the doublet's dependency on the presence of $Q_1\text{-Fe}$.

At pH 10, where more reducing conditions are possible, the signal for $Q_2\text{-Fe}$ and consequently that for species I^- disappear with E_m values of -525mV . This disappearance is attributed to the double reduction of Q_1 to give the e.p.r.-silent, fully reduced form:

The hysteresis effect observed in this redox change is probably due to accessibility and equilibration effects; however, conformational changes, perhaps involving a proton or protons, may well be involved. Further investigation of this redox change is required.

Double reduction of Q_1 was first suggested by Okamura *et al.* (1977) to explain the disappearance of the $g = 1.8$ signal during illumination of *R. sphaeroides* reaction centres at 300K in the presence of an external donor. This disappearance occurred in the 'lag' before species I^- was photoreduced which was observed as an unsplit free-radical signal. Similarly, in the redox titrations carried out here, an unsplit I^-

radical signal is induced after the $Q_1\text{-Fe}$ signal has disappeared. Thus the disappearance of the I^- doublet at -525mV is caused by the loss of the interaction with $Q_1\text{-Fe}$ and not by any change in the redox state of species I itself.

Since this I^- radical could not be totally chemically reduced at the lowest potentials available (-650mV), it is concluded that the I/I^- couple has an E_m which is significantly lower than that reported by Prince *et al.* (1976) and Netzel *et al.* (1977) of -400mV , but may be in the same region as that reported by Klimov *et al.* (1977) of -620mV .

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References

- Butler, W. F., Johnston, D. C., Okamura, M. Y., Shore, H. B. & Feher, G. (1978) *Biophys. J.* **21**, 8a
- Cogdell, R. J. & Crofts, A. R. (1972) *FEBS Lett.* **27**, 176-178
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957) *J. Cell. Comp. Physiol.* **49**, 25-68
- Dutton, P. L. (1971) *Biochim. Biophys. Acta* **226**, 63-80
- Dutton, P. L., Leigh, J. S. & Reed, D. W. (1973a) *Biochim. Biophys. Acta* **292**, 654-664
- Dutton, P. L., Leigh, J. S. & Wraight, C. A. (1973b) *FEBS Lett.* **36**, 169-173
- Dutton, P. L., Prince, R. C., Tiede, D. M., Petty, K. M., Kaufman, K. J., Netzel, T. L. & Rentzepis, P. M. (1976) *Brookhaven Symp. Biol.* **28**, 213-237
- Evans, M. C. W., Lord, A. V. & Reeves, S. G. (1974) *Biochem. J.* **138**, 177-183
- Fajer, J., Brune, D. C., Davis, M. S., Forman, A. & Spaulding, L. D. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4956-4960
- Fajer, J., Davis, M. S., Brune, D. C., Spaulding, L. D., Borg, D. C. & Forman, A. (1976) *Brookhaven Symp. Biol.* **28**, 74-104
- Klimov, V. V., Shuvalov, V. A., Krakhaleva, I. N., Klevanik, A. V. & Krasnovskii, A. A. (1977) *Biokhimiya* **42**, 519-530
- Netzel, T. L., Rentzepis, P. M., Tiede, D. M., Prince, R. C. & Dutton, P. L. (1977) *Biochim. Biophys. Acta* **460**, 467-479
- Okamura, M. Y., Isaacson, R. A. & Feher, G. (1977) *Biophys. J.* **17**, 149a

- Okamura, M. Y., Isaacson, R. A. & Feher, G. (1978) *Biophys. J.* **21**, 8a
- Olson, J. M. & Nadler, K. D. (1965) *Photochem. Photobiol.* **4**, 783-791
- Prince, R. C. & Dutton, P. L. (1976) *Arch. Biochem. Biophys.* **172**, 329-334
- Prince, R. C., Leigh, J. S. & Dutton, P. L. (1976) *Biochim. Biophys. Acta* **440**, 622-636
- Prince, R. C., Tiede, D. M., Thornber, J. P. & Dutton, P. L. (1977) *Biochim. Biophys. Acta* **462**, 467-490
- Thornber, J. R. & Olson, J. M. (1971) *Photochem. Photobiol.* **14**, 329-342
- Tiede, D. M., Prince, R. C., Reed, G. H. & Dutton, P. L. (1976) *FEBS Lett.* **65**, 301-304
- Tiede, D. M., Prince, R. C. & Dutton, P. L. (1978) *Biophys. J.* **21**, 196a
- Vermeglio, A. (1977) *Biochim. Biophys. Acta* **459**, 516-524
- Wraight, C. A. (1977) *Biochim. Biophys. Acta* **459**, 525-531
- Wraight, C. A. (1978a) *Biophys. J.* **21**, 8a
- Wraight, C. A. (1978b) *FEBS Lett.* **92**, 283-288