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The iron-quinone acceptor complex in *Rhodospirillum rubrum* chromatophores studied by EPR

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A new EPR signal is reported in *Rhodospirillum rubrum* chromatophores. The signal is attributed to $Q_B^-Fe^{2+}$, the semiquinone-iron complex of the secondary quinone electron acceptor, on the basis of the following observations. (1) It is induced by a single laser flash given a room temperature and is stable. (2) It is present after odd-numbered flashes and absent after even-numbered flashes when a series of flashes is given. (3) When it is already present, low-temperature illumination results in the disappearance of the signal due to formation of the $Q_A^-Fe^{2+}Q_B^-$ state. (4) Its formation is inhibited by the presence of ortho-phenanthroline at normal values of pH. The $Q_B^-Fe^{2+}$ signal has two main features, one at $g = 1.93$ and the other at $g = 1.82$. The two features have different microwave power and temperature dependences, with the $g = 1.82$ signal being more difficult to saturate and requiring lower temperatures to be observable. Raising the pH leads to an increase in the $g = 1.82$ feature, while the $g = 1.93$ signal decreases in amplitude. It is suggested that the two parts of the signal may represent two EPR forms due to structural heterogeneity. The low-field feature of the $Q_B^-Fe^{2+}$ signal shifts to lower field as the pH is raised and a pK for this change seems to occur at pH 9.4. The $Q_A^-Fe^{2+}$ signal at $g = 1.88$ also shifts as the pH is increased; however, the shift is less marked than that seen for $Q_B^-Fe^{2+}$, the shift is to higher field and the range over which it occurs is wider and depends upon the temperature of $Q_A^-Fe^{2+}$ formation. This effect may be due to a pK on a protein group being shifted to higher pH by the presence of Q_A^-. *ortho*-Phenanthroline broadens and shifts the $Q_A^-Fe^{2+}$ signal. The inhibition of electron transfer between Q_A^- and Q_B by *ortho*-phenanthroline becomes less effective at high pH. The new $Q_B^-Fe^{2+}$ signal is unlike other semiquinone-iron signals reported in the literature in bacteria; however, it is remarkably similar to the $Q_B^-Fe^{2+}$ signal reported in Photosystem II.

Introduction

In photosynthesis, energy conversion occurs via charge separation which takes place in the pigment-protein complex called the reaction centre (RC). Upon the absorption of a photon a (bacterio)chlorophyll molecular complex, known as the primary electron donor or P, becomes highly reducing and an electron moves from P to a series of electron accceptors. It is now well established that, in bacterial RCs, the electron passes from P

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Abbreviations: BPheo, bacteriopheophytin; Hepes, 4-(2-hyroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; P, the primary electron donor; PS II, Photosystem II; Q_A, the first quinone electron acceptor; Q_B, the second quinone electron acceptor; RC, reaction centre; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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via an intermediary electron acceptor, a bacteriopheophytin molecule (BPheo), to a primary quinone, Q_A, and from Q_A to a secondary quinone, Q_B. Q_A functions as a one-electron acceptor under normal conditions, while Q_B accumulates two consecutive electrons before donating to the ubiquinone pool (for reviews, see Refs. 1 and 2). Both the Q_A^- and Q_B^- semiquinone molecules are magnetically coupled to a high-spin ferrous-ion, Fe^{2+}, resulting in characteristic EPR signals. The strong anti-ferromagnetic coupling causes the very broadened form, the temperature sensitivity and saturation properties of the semiquinone-iron EPR signals [3]. The EPR signals from $Q_B^-Fe^{2+}$ in *Rhodobacter sphaeroides* [4] and *Rhodopseudomonas viridis* [5] resemble those of $Q_A^-Fe^{2+}$ in both signal shape and the *g*-value of the major peak (i.e., centered at $g = 1.82$).

In plant photosynthesis, the primary electron-transfer steps in Photosystem II (PS II) are similar to those occurring in purple bacteria. In particular, the electron acceptor side shows remarkable structural and functional analogies (for recent reviews, see Refs. 2 and 6). In PS II, Q_A is also associated with a ferrous ion and a $Q_A^-Fe^{2+}$ EPR signal is observed which resembles that found in most purple bacterial species, having also a *g*-value of 1.82 [7,8]. However, in most PS II preparations a broader signal at $g = 1.90$ is present as well as, or instead of, the signal at $g = 1.82$ [9]. The two signals seem to represent two interconvertible EPR forms and the interconversion is affected by pH [9], the binding of inhibitors [10] and the binding of some anions [11]. An EPR signal has also been preliminary attributed to $Q_B^-Fe^{2+}$ in PS II [12]. However, the signal was at a higher *g*-value than the $Q_A^-Fe^{2+}$ signal and did not resemble EPR signals reported from $Q_B^-Fe^{2+}$ in purple bacteria.

In the purple bacterium *Rhodospirillum rubrum* the $Q_A^-Fe^{2+}$ does not give rise to the normal bacterial semiquinone-iron signal. Instead a signal at $g = 1.87$ was observed [13]. The similarity between this signal and that found in PS II is striking. In this work, we have looked more closely at the semiquinone-iron complex in *R. rubrum*. We report a new signal from $Q_B^-Fe^{2+}$ which resembles that of PS II. In addition, we have studied the effects of pH and inhibitor binding on the semiquinone-iron EPR signals.

Materials and Methods

R. rubrum strain G9 cells were washed in a 50 mM Tris/HCl (pH = 8) buffer and stored in the same buffer at −30°C. After thawing the cells were spun out of this buffer. The pellet was divided and each part was suspended in a buffer with a different pH. The suspensions were French pressed twice at 96 MPa. After a centrifugation to remove unbroken cell debris, the chromatophores were pelleted by centrifugation for 1 h at approx. $200\,000 \times g$. The pellets were resuspended in buffer at the appropriate pH. The chromatophore concentration of each suspension was measured and adjusted to an absorbance at 865 nm of 90. The buffers used were: 0.1 Mes (pH = 6.0), 0.1 M Hepes (pH = 7.0, pH = 7.5 and pH = 8.0), 0.05 M Tricine (pH = 8.5), 0.1 M glycine (pH = 9.0, pH = 9.4, pH = 9.7, pH = 10.0 and pH = 10.5) and 0.2 M glycine (pH = 11.5). In most experiments the chromatophore suspension was used immediately to prepare EPR samples. In some experiments chromatophores were used which had been stored at −80°C. No significant differences were observed between the freshly prepared and the stored chromatophores.

Each EPR sample, unless otherwise stated, was made by putting 200 μl of the chromatophore suspension in a calibrated quartz EPR-tube, adding 1 μl of 0.1 M EDTA and letting it become dark adapted for 10 min on ice. 4 μl of 0.1 M diaminodurene in dimethylsulphoxide and 2 μl 0.5 M sodium ascorbate were then added, followed by freezing to 77 K via freezing to 200 K using an ethanol-solid CO_2 mixture. The EPR spectra were taken with a Bruker ER 200 t X-band spectrometer, fitted with an Oxford Instruments cryostat and temperature control system, and equipped with a Bruker ER 41 VR water-cooled cavity. Illuminations were done with a 800 W projector. For the flash experiments a Quantel Nd-YAG laser was used giving a 15 ns, 100 mJ flash at 530 nm. This flash was saturating at the chromatophore concentration used. When several flashes were given, the flash frequency was 1 Hz. The samples were rapidly (less than 2 s) frozen to 200 K after flash excitation and stored at 77 K. For some experiments 3 μl 0.5 M *o*-phenanthroline in dimethylsulphoxide was also added.

Results and Discussion

Continuous illumination of dark adapted *R. rubrum* chromatophores at 5 K results in a reversible charge separation:

$$\mathrm{P\ BPh\ Q_AFe^{2+}\ Q_B \xrightarrow{h\nu} P^+\ BPh\ Q_A^-\ Fe^{2+}\ Q_B}$$

Fig. 1a shows the P^+ radical signal (inset), centered at $g = 2.0026$ and the $Q_A^-Fe^{2+}$ signal at $g \approx 1.88$. The $Q_A^-Fe^{2+}$ signal is similar to that reported by Prince and Thornber [13]. However, a small signal at $g = 1.82$ is also present, which is very temperature sensitive and not observable at temperatures higher than approx. 10 K.

Illumination at room temperature followed by freezing under illumination fills up the quinone pool at the expense of the exogeneous electron donors and results in the trapping of Q_A^-. The $Q_A^-Fe^{2+}$ signal formed in this way (Fig. 1b) closely resembles that formed under illumination at 5 K.

Illumination at 5 K of a sample in which Q_A^- is already present, results in the formation of the P^+BPh^- state which recombines rapidly to form the ^3P triplet state. This triplet which decays to the ground state in approx. 110 μs is detectable by conventional EPR (see reviews Refs. 1 and 2). The triplet state is only photo-induced in reaction centers in which Q_A^- is already present. Conversely, the P^+ signal is only photoinduced in centers in which Q_A is oxidized. In Fig. 1, the absence of P^+ and the presence of a large ^3P signal show that Q_A is completely reduced (Fig. 1b inset).

There are a number of reports [14–17], which indicate that in *R. rubrum* Q_B functions as two-electron gate in a manner comparable to that in *Rb. sphaeroides*. The formation of semiquinone on odd-numbered flashes followed by its disappearance on even-numbered flashes has been observed in *Rb. sphaeroides* with optical techniques [18,19] and by EPR [4,21]. We have looked for EPR signals arising from $Q_B^-Fe^{2+}$ in *R. rubrum* after flash excitation.

Fig. 1c shows the EPR spectrum obtained in a sample after a single flash was given at room temperature. The spectrum is different from that arising from $Q_A^-Fe^{2+}$ (Fig. 1a and b) having a low field line at $g = 1.93$ and a signal at $g = 1.82$. We have characterized the new signal by two g-values: by measuring the vertical amplitudes of the two parts of the spectrum and taking the g-value of

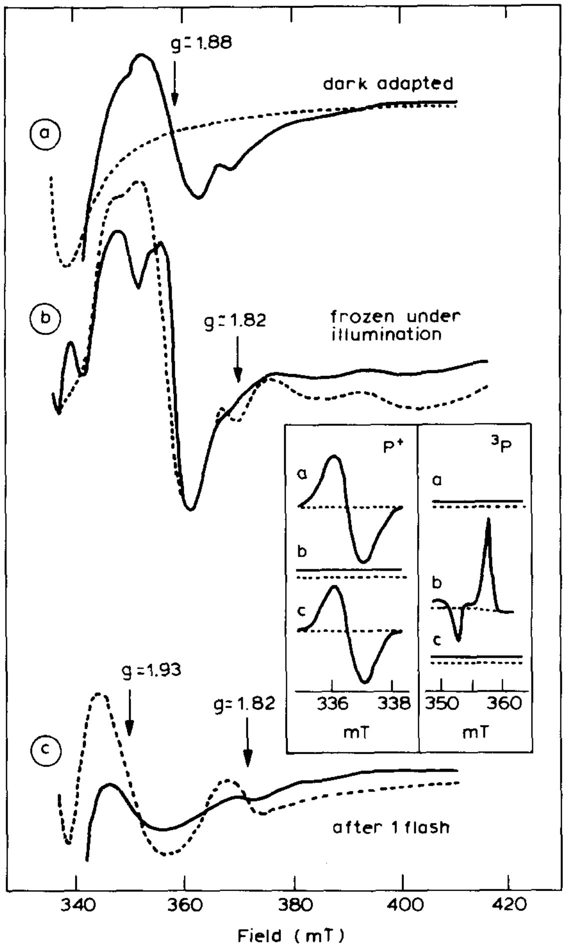

Fig. 1. EPR signals photoinduced in *R. rubrum* chromatophores. Broken lines are spectra recorded in darkness and solid lines were recorded during continuous illumination in the EPR cavity. Samples were frozen after dark adaptation (a), or under continuous illumination (b), or after being given a single flash at room temperature (c). All samples contained sodium ascorbate (5 mM) and diaminodurene (2 mM) as electron donors and were in buffer at pH 8.0 (see Materials and Methods). Instrument settings were as follows: temperature, 4.8 K; microwave power, 8 dB down from 200 mW (35 mW); modulation amplitude, 32 G. The inset shows the P^+ radical and the ^3P, triplet state photoinduced in the same samples described above. For P^+ the instrument settings were as follows: microwave power, 65 dB down from 200 mW; modulation amplitude, 2 G. For ^3P the instrument settings were as follows: microwave power, 20 dB down from 200 mW; modulation amplitude, 12.5 G.

the signal at half the height. This gives the approximate g-value of the absorption maximum in a X'' vs. H plot. From the extent of P^+ generated by low temperature illumination and the absence of ^3P, it is clear that Q_A is completely oxidized under these conditions (Fig. 1c, inset). It seems likely that the new signal arises from $Q_B^- Fe^{2+}$.

In *Rps. viridis*, when $Q_B^- Fe^{2+}$ was formed at room temperature, illumination at low temperature resulted in the disappearance of the semiquinone-iron signal. This was interpreted as being due to formation of the $Q_A^- Fe^{2+} Q_B^-$ state [5,22]. In *Rb. sphaeroides*, the existence of a similar state was discussed [4]. Illumination of *R. rubrum* samples at low temperature in which the flash-induced signal is present, results in a marked decrease in signal amplitude (Fig. 1c). This is attributed to the formation of the $Q_A^- Fe^{2+} Q_B^-$ state. The residual EPR signal is attributed to centers in which the $Q_A^- Fe^{2+} Q_B^-$ is not present and so $Q_B^- Fe^{2+}$ is still observed. This partial effect is due to the illumination at low temperature being less than saturating. When the samples were diluted so that the continuous illumination at low temperature was saturating, the complete disappearance of the signal was observed (see Fig. 2d). The presence of this effect supports the identification of the flash-induced signal as $Q_B^- Fe^{2+}$. The effect itself is a striking demonstration of the magnetic interactions between Q_A^-, Fe^{2+} and Q_B^-.

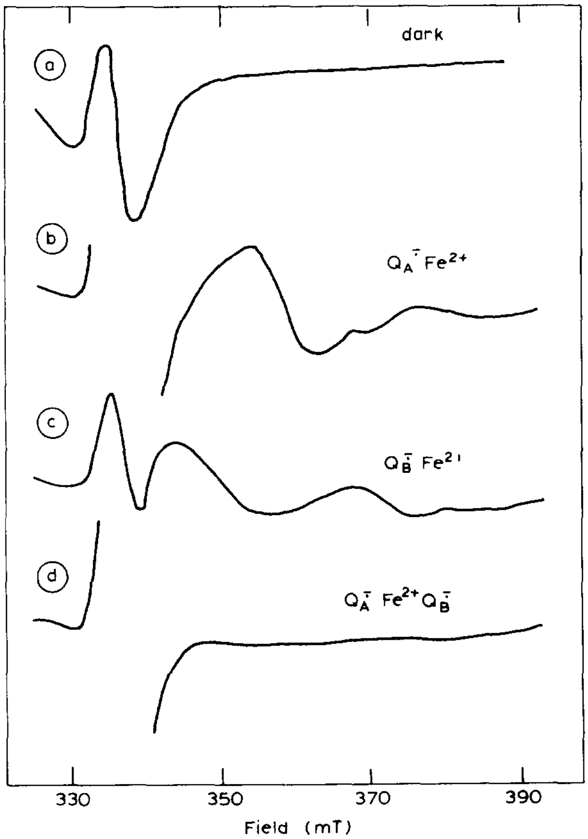

Fig. 2. Semiquinone-iron interactions in *R. rubrum* chromatophores. Samples were treated as in Fig. 1, except that the chromatophore suspension (at pH 7.5) was diluted (absorbance$_{865\ nm}$ = 25) until the continuous illumination at low temperature was saturating. (a) Dark adapted and recorded in the dark. (b) Dark adapted and recorded under continuous illumination. (c) given a single flash at room temperature and recorded in the dark. (d) Given a single flash at room temperature and recorded under continuous illumination. EPR conditions were as described in Fig. 1.

When a series of flashes was given to *R. rubrum* chromatophores, it was found that the flash-induced signal was present on odd numbered flashed and disappeared on even numbered flashes (Fig. 3). These binary oscillations are expected from $Q_B^- Fe^{2+}$. Upon even numbered flashes a second electron reduces Q_B^- to give the fully reduced form which is thought to be replaced by a ubiquinone molecule from the pool [23,24]. Fig. 3 shows data from an experiment in which six separate samples were given 0–5 flashes. The same results were obtained using a single sample from which EPR spectra were recorded after five separate thaw/one flash/freeze cycles. This illustrates the stability of Q_B^- at room temperature and also the insensitivity of the chromatophores to freezing damage.

From the data in Figs. 1–3 it is clear that the flash-induced signal arises from $Q_B^- Fe^{2+}$. The signal is remarkable similar to that preliminary attributed to $Q_B^- Fe^{2+}$ in PS II [12] and this spectral similarity adds weight to the assignment in PS II.

In Fig. 4, the microwave power saturation characteristics of the $Q_B^- Fe^{2+}$ signals at $g = 1.93$ and $g = 1.82$ are compared. Like the $Q_A^- Fe^{2+}$ signal (open circles) both parts of the $Q_B^- Fe^{2+}$ signal are rather difficult to saturate even at very low temperature. However, the $Q_B^- Fe^{2+}$ signal at $g = 1.93$ is clearly more easily saturated than that at 1.82. The $Q_A^- Fe^{2+}$ signal seems to saturate even more easily but a direct comparison is not possible,

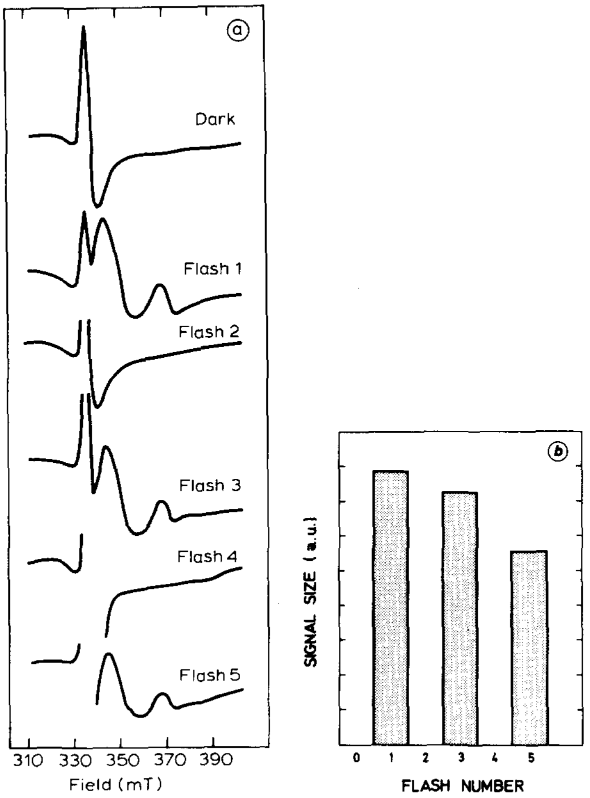

Fig. 3. (A) EPR signals in *R. rubrum* chromatophores recorded after a series of flashes given at room temperature. Each EPR spectrum is from a separate sample. EPR spectra were recorded using the instrument settings described in Fig. 1. (B) A plot of the signal amplitude at $g = 1.93$ against flash number shows period of two oscillations.

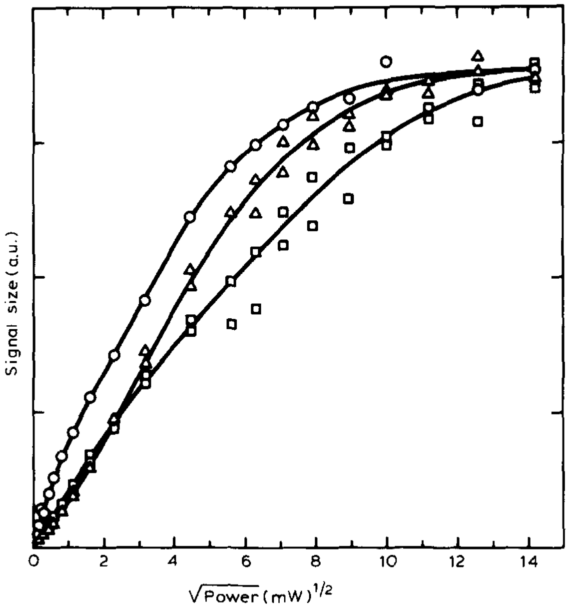

Fig. 4. The effect of microwave power on the EPR signals from the semiquinone-iron complex in *R. rubrum*. Open circles, the $Q_A^- Fe^{2+}$ at $g \approx 1.87$ formed by continuous illumination of a dark adapted sample. Open triangles, the feature of the $Q_B^- Fe^{2+}$ signal at $g \approx 1.93$. Open squares, the feature of the $Q_B^- Fe^{2+}$ signal at $g = 1.82$. $Q_B^- Fe^{2+}$ was formed by a single flash at room temperature. EPR instrument settings were as follows: temperature, 5 K; modulation amplitude, 22 G; the microwave power was varied.

since $Q_A^- Fe^{2+}$ is formed under continuous illumination which inevitably results in a higher sample temperature. The different saturation behaviour of the two parts of the $Q_B^- Fe^{2+}$ signals may indicate the presence of two EPR forms arising from two populations of centers in which the semiquinone-iron interaction is different.

The two parts of the $Q_B^- Fe^{2+}$ signal also behave differently with temperature. The $g = 1.82$ part is observable only at temperatures below approx. 10 K, while the $g = 1.93$ signal is still easily observable at 20 K. These differential effects of temperature may also support the idea of the existence of different population of RCs, although in the most recent model of the electronic interactions responsible for the semiquinone iron signal in *Rb. sphaeroides*, differential effects of temperature on different parts of the signal are inherent in the model [3] without involving structural heterogeneity.

In PS II it has been shown that there is an effect of pH on the relative amplitudes of the two EPR signals attributed to $Q_A^- Fe^{2+}$ [9]. We have investigated the effect of pH on the semiquinone-iron EPR signals in *R. rubrum*. For the $Q_A^- Fe^{2+}$ signal no interconversion of the $g = 1.87$ form to the $g = 1.82$ form could be observed. However, for the $Q_B^- Fe^{2+}$ signal the amplitude of the signal in the $g = 1.93$ region decreases and that of the signal at $g = 1.82$ increases as the pH is raised. This could reflect an interconversion of the two EPR forms, similar to that for $Q_A^- Fe^{2+}$ in PS II. However, a quantitative interconversion is not clear, due to the differential temperature sensitivity of the two parts of the signal and to changes in the position and shape of the low field form. Upon raising the pH, the $g = 1.82$ signal remains at the same position, while the signal in the region $g = 1.93$ shifts markedly to lower field (see Fig. 5). A plot of the position of the low field signal with pH is shown in Fig. 6. It is clear that the shift takes place over a narrow range of pH values between pH = 9.0 and pH = 9.7.

Since the optical absorption spectrum of Q_B^-, measured in *Rb. sphaeroides* is characteristic of the anionic form throughout a wide range of pH values, it is supposed that the proton binding which influences Q_B^- formation is to the protein rather than to the semiquinone itself [25]. In this case, since $Q_B^- Fe^{2+}$ is formed at room temperature and since it is frozen after 1–2 s, we are unable to determine whether the protonation occurs as a result of semiquinone formation or if the proton-induced change which influences the EPR signal is already present prior to Q_B^- formation.

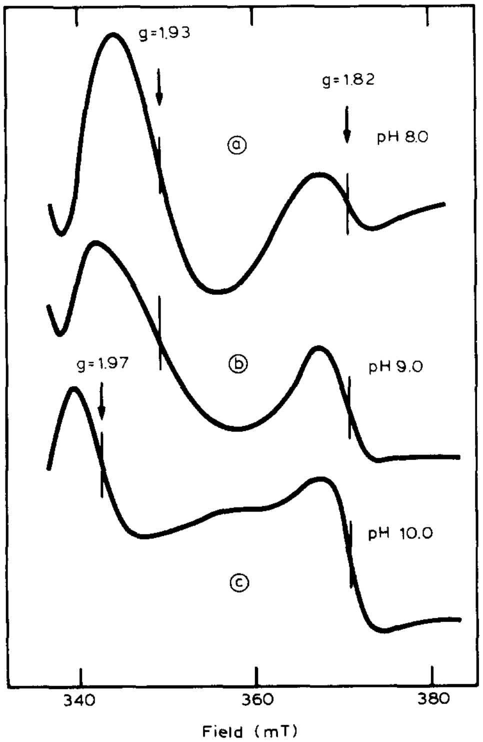

Fig. 5. The effect of pH on the EPR signal of $Q_B^- Fe^{2+}$ induced by a single flash at room temperature. Samples were prepared as described in the materials and methods. EPR conditions were as in Figure 1. (a) pH 8.0; (b) pH 9.0; (c) pH 10.0.

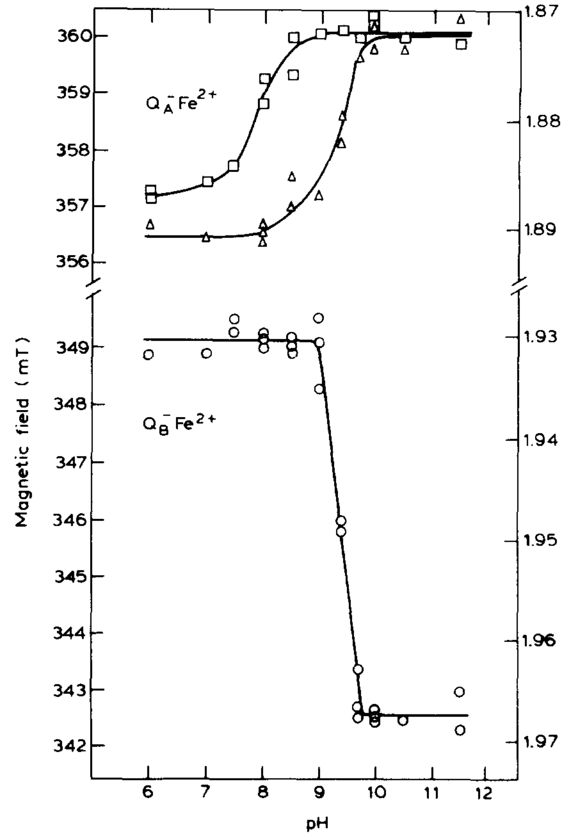

Fig. 6. The effect of pH on the semiquinone-iron signals in *R. rubrum*. The plot shows the field position of the centre of the EPR signal (see text) as it changes with pH. Open circles, the low-field feature of the $Q_B^- Fe^{2+}$ signal generated by a single flash at room temperature. Open squares, the $g = 1.88$ feature of the $Q_A^- Fe^{2+}$ signal generated under illumination of 5 K in a dark adapted sample. Open triangles is the same $Q_A^- Fe^{2+}$ feature but generated by giving continuous illumination while freezing. EPR conditions were as described in Fig. 1.

However, Verméglio [16] has shown that in *R. rubrum*, unlike *Rb. sphaeroides*, the pH does not affect the rate of Q_B^- formation. Thus, it seems likely that the protonation event, reflected by the $Q_B^- Fe^{2+}$ EPR signal occurs prior to Q_B^- formation.

An increased damping of oscillations and progressive electron transfer blockage between Q_A^- and Q_B were observed in *R. sphaeroides* as the pH was raised above pH = 9.0 [25]. A similar but less marked phenomenon is evident here in *R. rubrum*. This is observed as the presence of some $Q_A^- Fe^{2+}$ signal formed after a single flash at pH values of pH = 10.0 and higher and this is visible as a minor peak between the two $Q_B^-Fe^{2+}$ signals (see Fig. 5c). This corresponds to the formation of a small amount of ^3P triplet signal when illumination is given at low temperatures. At the same time the $Q_A^-Fe^{2+}$ signal becomes more evident due to $P^+Q_A^-Fe^{2+}$ formation in centers in which neither Q_A^- nor Q_B^- were trapped after the flash. However, in most of the centers $Q_B^-Fe^{2+}$ was formed by the flash (even at pH = 11.5 more than 80%) and low temperature illumination resulted in $Q_A^-Fe^{2+}Q_B^-$ formation.

Also shown in Fig. 6 is a plot of a pH-induced shift at the field position of the $Q_A^-Fe^{2+}$ signal. The shift is smaller (it shifts by 35 G) than that observed for the low-field part of $Q_B^-Fe^{2+}$ signal (which shifts by 74 G) and is in the opposite direction. The pH range over which the shift takes place is different depending upon whether $Q_A^-Fe^{2+}$ is generated by illumination at 5 K, or by illumination at room temperature. For the signal photoinduced at 5 K, the pH range over which the shift takes place is from pH = 7.0 to pH = 9.0, while that for the signal formed at room temperature is from pH = 8.0 to pH = 10.0. In both cases this rather broad range may indicate the presence of more than one p*K*. In *Rb. sphaeroides* rapid proton binding to the reaction centre protein is thought to occur upon Q_A^- formation and the p*K* for this protonation corresponds to that obtained by redox titrations of Q_A/Q_A^- [26]. In *R. rubrum*, the pH range associated with the shift of $Q_A^-Fe^{2+}$ signal when formed at room temperature corresponds with the p*K* at pH 8.9 measured by redox titrations of Q_A/Q_A^- in this species [27]. The different pH dependence for the $Q_A^-Fe^{2+}$ signal shift when formed at low temperature might be explained by the protonation being blocked at cryogenic temperatures. The pH range over which the shift occurs in the $Q_A^-Fe^{2+}$ signal when formed at cryogenic temperature, would then represent a p*K* on the protonatable group in the absence of Q_A^-. This p*K* would shift from pH 8 to pH 9 when Q_A^- was formed resulting in a proton uptake (at room temperature) in the pH range between pH 8 and pH 9. It should be pointed out, however, that another important difference between the $Q_A^-Fe^{2+}$ formed at low temperature compared with that generated at room temperature is the presence of the fully reduced secondary quinone acceptor in the latter case. The presence of one or both protons on Q_BH_2 could also influence the $Q_A^-Fe^{2+}$ signal position.

ortho-Phenanthroline inhibits electron transfer from Q_A^- to Q_B by competing with Q_B for the Q_B-binding site [23,24,28]. The $Q_A^-Fe^{2+}$ EPR signal is modified by *o*-phenanthroline addition in *Rb. sphaeroides* [3] and in PS II [10]. Fig. 7 shows that the $Q_A^-Fe^{2+}$ signal in *R. rubrum* is also influenced by *o*-phenanthroline binding. The signal becomes broader and is shifted to higher field. In these experiments *o*-phenanthroline was added in dimethylsulphoxide instead of ethanol since ethanol itself had a broadening effect on both the $Q_A^-Fe^{2+}$ and the $Q_B^-Fe^{2+}$ signals (not shown).

A single flash given to samples in the presence of *o*-phenanthroline resulted in the formation of the modified $Q_A^-Fe^{2+}$ signal and no $Q_B^-Fe^{2+}$ was

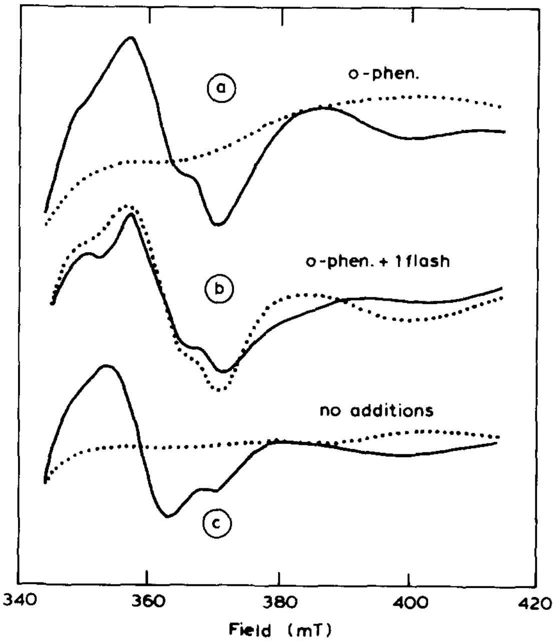

Fig. 7. The effect of *ortho*-phenanthroline on the semiquinone-iron complex in *R. rubrum*. (a) A dark adapted sample in the presence of 7.5 mM *o*-phenanthroline . (b) A sample in the presence of *o*-phenanthroline which was given a single flash at room temperature. (c) A sample comparable to (a), but in the absence of *o*-phenanthroline as a control. Broken lines were recorded in the dark and solid lines were recorded during illumination at 5 K. EPR conditions were as in Fig. 1 and the samples were suspended in buffer at pH 6.0.

formed. When illuminated at 5 K the flash-induced signal was virtually unchanged (Fig. 7b). This effect, along with the appearance of ^3P and the corresponding decrease in P^+ formation upon illumination at low temperature (not shown), verified the identity of the signal as being due to flash-induced $Q_A^-Fe^{2+}$.

We have investigated the effect of pH on *R. rubrum* chromatophores in the presence of *o*-phenanthroline. The $Q_A^-Fe^{2+}$ signal photo-induced at low temperature does not show further obvious field shifts as the pH is raised, but the detection of small shifts would be difficult due to the greater signal width. At high pH values some $Q_B^-Fe^{2+}$ signal was induced upon a single flash in the *o*-phenanthroline-treated chromatophores (Fig. 8). Clearly the inhibitory action of *o*-phenanthroline decreases at high pH.

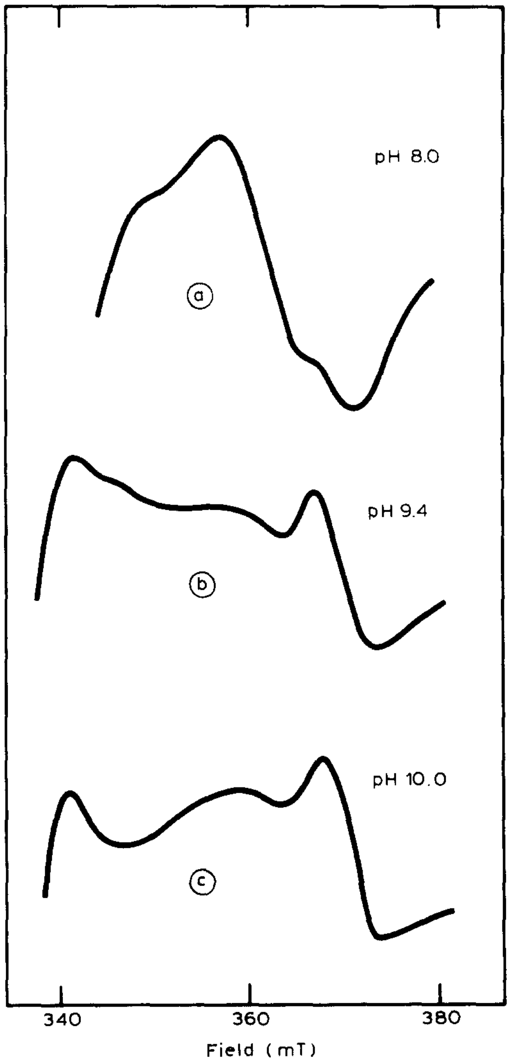

Fig. 8. The effect of pH on *o*-phenanthroline inhibition in *R. rubrum* chromatophores. *o*-Phenanthroline at 7.5 mM was present in all samples. A single flash was given to each sample before freezing. Spectra were recorded in darkness using the EPR conditions as described in Fig. 1. (a) pH 8.0; (b) pH 9.4; and (c) pH 10.0.

Because of the similarities between the EPR signals arising from the semiquinone iron complexes of *R. rubrum* and PS II [9,12] we looked at the effects of treatments on *R. rubrum* which are known to have marked effects on PS II. In PS II, sodium formate removes the $g = 1.9$ form of $Q_A^-Fe^{2+}$ and increases the amplitude of the $g = 1.82$ form by more than a factor 10 [11]. Formate (200 mM, pH 6.0) has no effect in *R. rubrum*. In PS II the iron can be oxidized by a number of high-potential semiquinones generated by flash illumination [29]. No iron oxidation could be observed upon flash excitation in *R. rubrum* in the presence of 1 mM phenyl-*p*-benzoquinone.

Conclusions

The semiquinone-iron complex of *R. rubrum* is functionally very similar to that of *Rb. sphaeroides* having two quinones, Q_A working as a one-electron carrier and Q_B working as a two-electron gate. Both quinones are associated with a ferrous ion giving rise to semiquinone-iron EPR signals which are similar but not identical. As with *Rb. sphaeroides* and *Rps. viridis*, the semiquinone-iron interaction, and hence the semiquinone–iron distance, is similar for $Q_A^-Fe^{2+}$ and $Q_B^-Fe^{2+}$ and a structure compatible with that obtained for the *Rps. viridis* reaction centre [30] can be envisioned for *R. rubrum*. At a more precise level, however, the semiquinone-iron interactions in *R. rubrum* are different from those seen in the other species of bacteria studied. Interestingly, both the $Q_A^-Fe^{2+}$ and the $Q_B^-Fe^{2+}$ signals are almost identical to those reported in PS II [9,12]. Both in PS II and in *R. rubrum*, the semiquinone-iron complexes give rise to two signals, one at $g = 1.82$, the same as that seen in other species of purple bacteria, and another signal at higher *g*-value ($g = 1.88$ for $Q_A^-Fe^{2+}$ and $g = 1.93$ for $Q_B^-Fe^{2+}$). The proportions of the two signals vary and are considered to represent structural heterogeneity between two specific conformations. In *R. rubrum*, the higher *g*-value forms sense protonation events in the reaction centre in that the EPR signals shift their field position. *ortho*-Phenanthroline binding to the Q_B site is also sensed by the Q_A^-Fe signal, shifting and broadening the signal. The effect of this inhibitor is greatly diminished at high pH, indicating that a deprotonation takes place which lowers the affinity of the binding site for *o*-phenanthroline relative to that for Q_B. A study of the amino acid sequence of the reaction centre subunits of *R. rubrum* in the light of the crystal structure of *Rps. viridis* [28,30] may show specific amino acids which could be candidates for these deprotonation phenomena.

Although the structural relationships between the semiquinones and the iron must be remarkably similar in the reaction centres of purple bacteria (particularly *R. rubrum*) and PS II, the semiquinone-iron complex of PS II differs from that of bacteria in a number of respects. These include a more labile iron [31], an iron atom that is redox active [29,32], sensitivity to a wider range of herbicides (see Ref. 33 for a review) and a marked effect of formate binding [11]. Some or all of these phenomena might be explained by the iron atom, in the PS II reaction centre, being more easily accessible from the outside. It is of note in this regard that the glutamic acid residue on the M subunit in *Rps. viridis* reaction centres, which forms a bidentate ligand to the iron [28], is not conserved in the corresponding subunit, D_2, in PS II [34]. A free or replaceable ligand to the iron in the PS II RC could be the origin of the differences between the semiquinone-iron complex of PS II and that of purple bacteria.

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