IRON–QUINONE INTERACTIONS IN THE ELECTRON ACCEPTOR REGION OF BACTERIAL PHOTOSYNTHETIC REACTION CENTERS

C. A. WRAIGHT

Department of Physiology and Biophysics and *Department of Botany, University of Illinois, Urbana, IL 61801, USA*

Received 30 June 1978

1. Introduction

Binary oscillations in electron transfer reactions of quinones on the acceptor side of the photoact have been widely observed in photosynthesis [1–5]. In reaction centers from *Rhodopseudomonas sphaeroides* this behaviour has been seen by both optical and ESR spectroscopy and can be explained by the concerted activity of two relatively specialised quinones in an acceptor region of the reaction center complex [3,4]. It is well established in bacteria that the primary quinone (Q_I) is associated with an iron atom [6,7] giving rise to a characteristic and highly distorted ESR signal of the semiquinone [6,8]. A similar ESR spectrum is seen for the semiquinone of the secondary quinone (Q_{II}) showing that this, too, interacts with the iron atom [3]. The function of the iron atom is currently unknown but its removal inhibits electron transfer from Q_I–Q_{II} [18]. It is shown here that the interaction with the iron is distinctly different for the two quinones. It is also suggested that magnetic coupling may occur between the two semiquinones when both are present which may be significant to the mechanism of electron transfer between them*.

2. Materials and methods

Reaction centers from *Rp. sphaeroides*, strain R 26, were prepared as in [3]. ESR samples, in 3 mm quartz tubes, contained 50–80 μM reaction centers supplemented with a 3-fold excess of ubiquinone-10, added as a suspension in Triton X-100, and a 5-fold excess of reduced cytochrome *c*. Samples prepared by flash illumination at room temperature prior to freezing ([3], see fig.1 legend) are described as pre-flashed samples. All experiments were run at liquid helium temperatures on a Varian E-9 ESR spectrometer using an Oxford helium cryostat and transfer system.

3. Results

Figure 1 shows the low temperature ESR signals of the iron–quinone complex. The dark-adapted sample (0-preflash) has no ESR signals in the regions of 3400–4200 G (340–420 mT). Illumination at low temperature caused a reversible charge separation in the reaction centers, generating oxidised bacteriochlorophyll (P^+, observable at g 2.0026 [9]) and the well-known reduced primary acceptor quinone signal at g 1.82 and g 1.68 [8] ascribed to an iron–semiquinone complex ($\dot{Q}_I^-$) [7]. After a single flash at room temperature, there is a well-developed g 1.82 signal seen in the dark at low temperature [3]. Since secondary electron transfer to Q_{II} occurs in less than 1 ms at room temperature [10,11] while freezing takes about 3 s, this signal is due to the stable semiquinone of Q_{II} ($\dot{Q}_{II}^-$). The high field component of this species is quite distinct from that of $\dot{Q}_I^-$ (g 1.62 compared to g 1.68). After two flashes at room temperature the pairwise transfer of electrons from the acceptor quinone complex to the pool of tertiary

* This work was presented in part at the 4th Int. Congr. Photosynthesis, Reading, England, September 1977

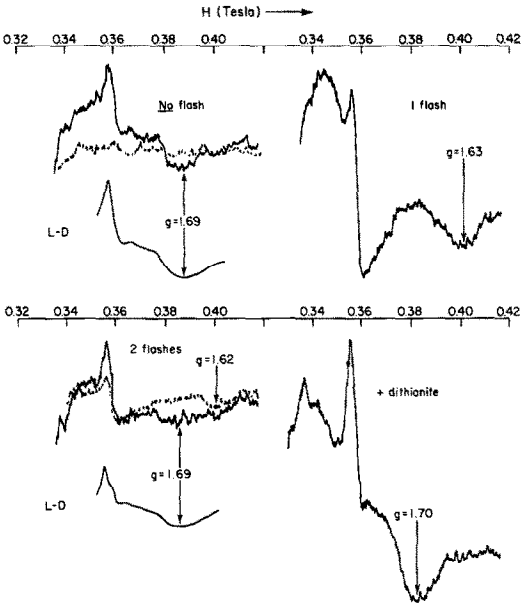


Fig.1. ESR spectra of primary and secondary iron-semiquinone complexes. Saturating flashes at 585 nm were administered at room temperature just prior to rapid freezing. Reduced cytochrome is present as donor to rapidly rereduce P^+ leaving the photoelectron trapped in the acceptor quinone complex:

$$PQ_IQ_{II} \xrightarrow{\text{1st flash}} P^+\dot{Q}_I^-Q_{II} \xrightarrow{D \rightarrow D^+} PQ_I\dot{Q}_{II}^- \text{ (stable)}$$

$$PQ_I\dot{Q}_{II}^- \xrightarrow{\text{2nd flash}} P^+\dot{Q}_I^-\dot{Q}_{II}^- \xrightarrow[A \rightarrow AH_2]{D \rightarrow D^+} PQ_IQ_{II} + 2\,H^+$$

Spectra were recorded at 6 K, 9.17 GHz, 20 mW microwave power, 2 mT field modulation. 1 flash and plus dithionite spectra are in the dark. For 0 and 2 flash samples, dashed lines are dark spectra, solid lines are with continuous illumination from a projector lamp filtered through water. Light–dark (L–D) difference spectra were obtained by hand subtraction.

Table 1
Characteristics of the $\dot{Q}_I^-$ and $\dot{Q}_{II}^-$ low temperature ESR signals

Samples type[a]		ΔH[b] (G) preparation 1[c]		ΔH[b] (G) preparation 2[c]	$P_{1/2}$ (mW)[d]	$T_{1/2}$ (K)[e]
		Buffer 0.5 M pH 8.5, $n = 2$	Buffer 0.07 M pH 8, $n = 4$	Buffer 0.07 M pH 8, $n = 5$		
$\dot{Q}_I^-$	$P^+\dot{Q}_I^-Q_{II}$	320	315 ± 17	270 ± 10	–	–[f]
	$PQ_I^-Q_{II}H_2$	325	275 ± 7	260 ± 12	95	3.4
$\dot{Q}_{II}^-$	$P^+Q_I\dot{Q}_{II}^-$	435	–	430 ± 11	125	1.9
	$PQ_I\dot{Q}_{II}^-$	430	440 ± 15	420 ± 9	110	1.8

[a] Sample preparation methods were: $P^+\dot{Q}_I^-$QII, illumination at low temperature of dark-adapted (0-preflash sample); $PQ_I^-Q_{II}H_2$, chemical reduction by dithionite; $P^+Q_I\dot{Q}_{II}^-$, frozen while illuminating with no donor present; $PQ_I\dot{Q}_{II}^-$, 1-preflash in the presence of donor prior to freezing

[b] ΔH is measured between the peak at g 1.82 (~3600 G) and the high-field trough at g 1.70–1.62 (3850–4050 G)

[c] Preparation 1 was freshly isolated reaction centers; preparation 2 had been stored refrigerated, but unfrozen, for several weeks. n refers to the number of samples of each type

[d] $P_{1/2}$, the microwave power for half-saturation was determined from plots of log (signal/$\sqrt{P}$) versus log P at 3.7 K [21]

[e] See text

[f] The temperature readout was not trustworthy under continuous illumination. However, $\dot{Q}_I^-$ generated in the light showed an almost identical temperature dependence to a chemically reduced sample under equivalent illumination

acceptors leaves only a residual signal due to damping of the binary oscillations [3]. Again this dark stable signal can be recognised as $\dot{Q}_{II}^{-}$ by the high field line at g 1.62–1.63. Illumination of this sample at low temperature caused an increase in the g 1.82 signal and the appearance of a component at g 1.68–1.69 which is clearly seen in the light–dark difference spectrum.

Table 1 summarizes the data available on the two semiquinone signals generated in different ways. $\dot{Q}_{I}^{-}$, generated either in the light or by chemical reduction, has a distinctly narrower signal (ΔH ~320 G (32 mT)) than $\dot{Q}_{II}^{-}$ (ΔH ~440 G (44 mT)). $\dot{Q}_{I}^{-}$ exhibits considerable variability from one reaction center preparation to another (ΔH 250–330 G (25–33 mT)) and, as noted [12], the narrower signals appear to be correlated with ageing of the preparation suggesting a gradual loss of the integrity of the Fe–Q_I interaction. In addition, however, we have found the widths of both $\dot{Q}_{I}^{-}$ and $\dot{Q}_{II}^{-}$ to be pH dependent, increasing with pH in the region of pH 9. This accounts, at least in part, for the narrower $\dot{Q}_{I}^{-}$ signals frequently seen on chemical reduction by dithionite, which causes a significant acidification unless heavily buffered (see table 1). This pH dependence correlates with protonation events in the two-electron reduction of Q_{II} and is discussed in detail in [11]. In addition to narrowing of the $\dot{Q}_{I}^{-}$ signal, a component at about g 1.88 is also seen in older preparations [12] and is evident in fig.1. A component in this region is seen in the $\dot{Q}_{II}^{-}$ spectrum even in fresh preparations. The variability in the semiquinone ESR signals suggests the probability of heterogeneity in the iron–quinone population, visible by ESR even though no clear functional variability is apparent [12]. Indeed, the possibility arises that a degree of heterogeneity is the norm.

Both semiquinones can be prepared in the presence or absence of P^+ (see table 1 and fig.1 legend). Taking into account the probable pH-origin of the narrower dithionite-reduced $\dot{Q}_{I}^{-}$ signal in columns 2 and 3, no obvious effect of P^+ on the line shapes could be discerned. Conversely, the P^+ signal (g 2.00) is not significantly broadened in the presence of either semiquinone (ΔH (g 2.00) 9.9 ± 0.2 G at 4.2 K), arguing against a close proximity of P^+ and either semiquinone radical. A similar conclusion for the primary quinone and P^+ in reaction centers from *Chromatium vinosum* has recently been drawn [13].

The two semiquinone signals are also distinguishable by their temperature dependences and microwave power saturation. The g 1.82 signals are anomalously temperature sensitive and do not follow the Curie-Weiss Law. The temperature curves conform closely to exponentials of the form $\exp(-T/T_{1/2})$ and the characteristic $T_{1/2}$ values are shown in table 1. At this time no physical significance can be associated with this particular behaviour, but the greater temperature sensitivity and less ready power saturation of $\dot{Q}_{II}^{-}$ are consistent with its origin in relaxation processes.

Illumination, at low temperature, of the 0- or 2-preflash samples produced a recognisable $\dot{Q}_{I}^{-}$ signal (g 1.82, g 1.68) (fig.1). Illumination of the 1-preflash sample, however, did not produce more g 1.82 even though charge separation is fully active as seen by the light-inducible g 2.00 signal of P^+ [3]. There was, instead, a large (20–50%) shrinkage of the g 1.82 signal. Dithionite reduction abolishes conventional photochemical activity but illumination still caused a significant (10–20%) shrinkage of the $\dot{Q}_{I}^{-}$ g 1.82 signal. The semiquinone signals are extremely temperature dependent (fig.2) and the shrinkage in this case was certainly due to warming of the sample by the actinic light. The temperature rise can be seen by direct measurement in the sample tube with a calibrated carbon resistor and at these low temperatures amounts to about 2 K.

The light-inducible g 2.00 signal at low temperature also oscillates with flash number and it was suggested that this could be due to contribution at g 2.00 by $\dot{Q}_{I}^{-}$ when $\dot{Q}_{II}^{-}$ was already present as the g 1.82 species [3]. This would imply that all the light-induced shrinkage of the $\dot{Q}_{II}^{-}$ was due to heating as seen for $\dot{Q}_{I}^{-}$ in the dithionite-reduced sample but, although the relatively larger shrinkage in $\dot{Q}_{II}^{-}$ is readily accommodated by the steeper temperature dependence of this signal (fig.2), the possibility remains that the light-generated $\dot{Q}_{I}^{-}$ radical couples with the $\dot{Q}_{II}^{-}$, perhaps via the iron atom as an exchange intermediary, to give rise to a diamagnetic state contributing to shrinkage. However, since the continuous illumination was only about 25% saturating for photochemistry, while the temperature rise of 1.6–2.0 K was sufficient to quench the $\dot{Q}_{II}^{-}$ signal by 50% or more, no temperature correction process could be devised that was reliable enough to reveal a distinct quenching

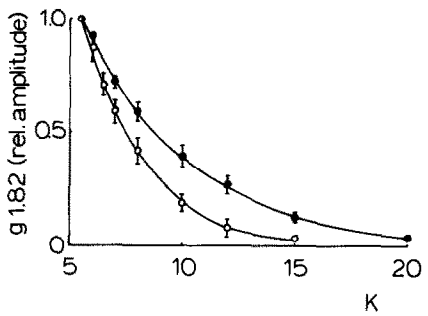


Fig.2. Temperature dependence of the $\dot{Q}_I^-$ and $\dot{Q}_{II}^-$ ESR signals. $\dot{Q}_I^-$ (•) was generated by chemical reduction; $\dot{Q}_{II}^-$ (○) by a single flash prior to freezing. Signals were recorded at 9.17 GHz, 50 mW microwave power, 4 mT field modulation. Signals are normalized to 1.0 at 5.5 K. Each curve is the average for 3 different samples.

of $\dot{Q}_{II}^-$ due to the presence of $\dot{Q}_I^-$. The question of possible interaction between $\dot{Q}_I^-$ and $\dot{Q}_{II}^-$ was therefore studied via the kinetics of the g 1.82 signal following a laser flash in the hope that part of the quenching of $\dot{Q}_{II}^-$ might recover with kinetics matching those of the back reaction (decay of $\dot{Q}_I^-$; $T_{½} \approx 20$ ms [14,15]). Again, however, the heating effect – even of the monochromatic laser flash (585 nm) – completely obscured any photochemical processes. With a dithionite-reduced sample, temperature quenching of the $\dot{Q}_I^-$ signal recovered in the 60 ms time range. Comparison of the flash-induced quenching with the temperature dependence of $\dot{Q}_I^-$ indicated a temperature jump of 12–13 K, sufficient to completely quench $\dot{Q}_{II}^-$ and most of $\dot{Q}_I^-$. In an untreated sample (0-preflash) flash-induced generation of the $\dot{Q}_I^-$ signal could be seen, as originally reported [16], but the decay kinetics and extent were highly distorted.

Since the direct approach to light-induced g 1.82 phenomena proved intractable, attention was turned to the oscillations in the light-induced g 2.00 signal. As originally reported [3], no g 2.00 signal distinct from P^+ could be detected in $\dot{Q}_{II}^-$-containing samples and further efforts have not been any more successful. The g 2.00 oscillations were therefore further scrutinized for other possible sources of this behaviour. The oscillations are somewhat variable but can comprise upto 25% of the basic P^+ signal ([3], see table 2). Since the continuous illumination available was far

Table 2
Light-induced g 2.00 signal amplitudes and light saturation characteristics

Flash number	0	1	2	3	4
g 2.00 in continuous light[a]	95	115	93	108	108
max. g 2.00 in saturating light[a,d]	465[c]	513	521	515	518
$I_{½}$[b,d]	407	355	476	395	394
Flash-induced g 2.00[a,e]	186	193	184	–	–

[a] Arbitrary units at 10×10^2 spectrometer gain, 0.1 mW microwave power, 2.5 G field modulation, 3.7 K
[b] Half-saturation intensity in arbitrary units relative to 100 for the maximum intensity of the continuous light
[c] This apparently anomalous value could not be checked as the sample tube shattered before calibration
[d] Correlation values for the least squares fits were >0.999 for all samples
[e] The lower values of the flash-induced signal relative to the saturating continuous light are due to the smaller area of illumination by the laser (~1 cm diam.); the ESR cavity was 2.2 cm long

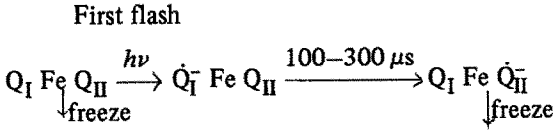
from saturating, light-saturation curves were obtained for all samples to give the maximum g 2.00 signal by extrapolation. The values obtained by linear regression are given in table 2 and it is clear that the oscillations observed at non-saturating intensities are absent at saturation. This behaviour, which accounts for a variable extent of the oscillations encountered in different experiments, indicates a flash-dependent light-saturability of the g 2.00 signal rather than of the intrinsic amplitude. This is supported by the observed variations in half-saturation intensities (table 2). An obvious source of this behaviour would be a dependence of the back reaction on the presence of $\dot{Q}_{II}^-$. An alternative reason, especially relevant at the low temperatures used here, could be the contribution of both the back reaction and spin-lattice relaxation to the thermal equilibration of the g 2.00 spin population. The back reaction decay constant (k_d), which is temperature independent [14,15], contributes significantly to the overall spin relaxation process at temperatures $\lesssim$15 K because the spin-lattice relaxation constant (k_1) decreases dramatically with temperature. This was first suggested in [15] where a very marked distortion of the g 2.00 decay kinetics

following illumination at 1.5 K was shown.

In preliminary experiments on the decay kinetics of the g 2.00 signal following a laser flash at low temperature we have observed a slower decay in the presence of $\dot{Q}_{II}^-$, consistent with the larger signal (greater degree of saturation) seen in such samples under continuous illumination. The lack of true g 2.00 oscillations was also confirmed by these measurements with saturating laser pulses (table 2). The decay kinetics, however, which were measured with near-saturating microwave power for high signal amplitude, were considerably complicated by the temperature jump caused by the laser pulse, even though the g 2.00 signal is much less temperature sensitive than the g 1.82 signals. It is thus not clear yet which of the two suggested origins of g 2.00 variability is operating (difference in k_1 or k_d), if either, and further work is in progress to clarify this question. It is relevant to note that an influence of $\dot{Q}_{II}^-$ on the forward reactions of the reaction center has been shown [17] at the level of the subnano-

leaves uncertain the state of the photoelectron on $\dot{Q}_I^-$ in the presence of $\dot{Q}_{II}^-$ but it is apparently not a normal semiquinone as previously suggested. Since both $\dot{Q}_I^-$ and $\dot{Q}_{II}^-$, alone, interact with the iron atom it seems probable that they do so also as $\dot{Q}_I^-$ Fe $\dot{Q}_{II}^-$. The considerable light-induced shrinkage of the $\dot{Q}_{II}^-$ signal could, therefore, include a diamagnetic coupling of the two spins mediated via the iron atom. The events leading to the ESR signals, described here, can be summarized by scheme 1.

The function of the iron is still unclear although it is certainly necessary for normal electron transport since its removal prevents the reoxidation of $\dot{Q}_I^-$ by secondary acceptors [18]. Since removal of the iron results in loss of stability of the $\dot{Q}_I^-$ anion radical and allows chemical reduction to the diamagnetic quinol, it is evident that the iron modulates the physical and chemical properties of Q_I [12]. An additional function in transferring the electron from Q_I to Q_{II} would be consistent with the suggested magnetic coupling between the two semiquinone radicals, a



Low temperature ESR signals		
in dark:	none	g 1.82, g 1.63 (Fe–$\dot{Q}_{II}^-$)
in light:	g 2(P^+); g 1.82, g 1.69 ($\dot{Q}_I^-$–Fe)	g 2(P^+); and ? ($\dot{Q}_I^-$ Fe $\dot{Q}_{II}^-$)

Scheme 1

second electron transfer from $\dot{I}^-$ to Q_I. This step was slowed in the presence of $\dot{Q}_{II}^-$ and either an electrostatic or structural effect might be responsible. Should the back reaction (k_d) also be slowed in the presence of $\dot{Q}_{II}^-$, a structural change would be implicated since any electrostatic contribution is likely to accelerate the back reaction.

4. Discussion

The lack of true oscillations in the g 2.00 signal

mechanism referred to as the 'iron wire' hypothesis [19].

The g 1.82 signals are not interpretable by simple g-tensor analysis but an additional exchange coupling term has recently been used to model the spectra with some success [20] and the similar positions of the major component at g 1.82 indicate that the magnetic interaction with the iron is similar for both $\dot{Q}_I^-$ and $\dot{Q}_{II}^-$. However, the broader, more temperature-dependent and less readily power-saturated character of $\dot{Q}_{II}^-$ (table 1) suggests that the environments are quite distinct. Since both quinones can be extracted

and reconstituted without loss of the iron [22], binding of the iron is not dependent on the quinones. The converse, however, is not true since functional Q_{II} cannot be restored in iron-depleted reaction centers [18]. In view of the complex protonation events leading to the normal, two-step reduction of Q_{II} to $Q_{II}H_2$ [11] and the abnormal stability of the anionic semiquinones responsible for the oscillatory phenomena, a role for the iron in charge stabilisation and Q_{II}-binding may also be indicated.

Acknowledgements

I am very grateful to Peter Debrunner and James Norris for helpful discussions throughout this work. Funding by NSF Grant PCM 77-25725 is also gratefully acknowledged.

References

[1] Bouges-Bouquet, B. (1973) Biochim. Biophys. Acta 314, 250–256.
[2] Velthuys, B. R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94.
[3] Wraight, C. A. (1977) Biochim. Biophys. Acta 459, 525–531.
[4] Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516–524.
[5] Barouch, Y. and Clayton, R. K. (1977) Biochim. Biophys. Acta 462, 785–788.
[6] Feher, G., Okamura, M. Y. and McElroy, J. D. (1972) Biochim. Biophys. Acta 267, 222–226.
[7] Feher, G., Isaacson, R. A., McElroy, J. D., Ackerson, L. C. and Okamura, M. Y. (1974) Biochim. Biophys. Acta 368, 135–139.
[8] Leigh, J. S. and Dutton, P. L. (1972) Biochem. Biophys. Res. Commun. 46, 414–421.
[9] McElroy, J. D., Feher, G. and Mauzerall, D. C: (1972) Biochim. Biophys. Acta 267, 363–374.
[10] Vermeglio, A. and Clayton, R. K. (1977) Biochim. Biophys. Acta 461, 159–165.
[11] Wraight, C. A. (1978) Biophys. J. 21, 8a; full ms in preparation.
[12] Dutton, P. L., Prince, R. C. and Tiede, D. M. (1978) Photochem. Photobiol. in press.
[13] Tiede, D. M., Leigh, J. S. and Dutton, P. L. (1978) Biochim. Biophys. Acta in press.
[14] Clayton, R. K. and Yau, H. F. (1972) Biophys. J. 12, 867–881.
[15] McElroy, J. D., Mauzerall, D. C. and Feher, G. (1974) Biochim. Biophys. Acta 333, 261–277.
[16] Leigh, J. S. and Dutton, P. L. (1973) Ann. NY Acad. Sci. 222, 838–845.
[17] Pellin, M. J., Wraight, C. A. and Kaufmann, K. J. (1978) Biophys. J. in press.
[18] Blankenship, R. E. and Parson, W. W. (1977) Abst. 4th Int. Congr. Photosynthesis, Reading, England, p. 37.
[19] Okamura, M. Y., Isaacson, R. A. and Feher, G. (1978) Biophys. J. 21, 8a.
[20] Butler, W. F., Johnston, D. C., Okamura, M. Y., Shore, H. B. and Feher, G. (1978) Biophys. J. 21, 8a.
[21] Beinert, H. and Orme-Johnson, W. H. (1966) in: Magnetic Resonance in Biological Systems (Ehrenberg, A., Malmström, B. G. and Vänngård, T. eds) pp. 221–247, Pergamon Press.
[22] Okamura, M. Y., Isaacson, R. A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.