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ELECTRON ACCEPTORS OF PHOTOSYNTHETIC BACTERIAL REACTION CENTERS

DIRECT OBSERVATION OF OSCILLATORY BEHAVIOUR SUGGESTING TWO CLOSELY EQUIVALENT UBIQUINONES

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SUMMARY

When reaction centers are illuminated by a series of single turnover flashes ubisemiquinone is formed and destroyed on alternate flashes. This oscillatory behaviour can be observed with both optical and electron spin resonance techniques. The oscillations are dependent upon the presence of excess ubiquinone in a manner which suggests that two molecules may act almost equivalently as metastable primary acceptors forming a two-electron gate between the one-electron primary photoact and a two-electron secondary acceptor pool.

INTRODUCTION

After many years of controversy over the chemical nature of the metastable primary electron acceptor in photochemical reaction centers of photosynthetic bacteria, this function has recently been rather convincingly bestowed upon quinones [1–3]. Okamura and coworkers [2, 3] have shown *Rhodopseudomonas sphaeroides* to utilize ubiquinone in this capacity whereas *Chromatium vinosum* utilizes menaquinone. Illumination or chemical reduction produces the semiquinone but the free electron interacts magnetically with an iron atom to cause a marked distortion of the ESR spectrum resulting in a highly asymmetrical signal centered at g 1.82, originally ascribed to the iron itself [4, 5]. Certain treatments, such as with the detergent SDS, interrupt this magnetic interaction, permitting the appearance of a normal semiquinone free-radical signal which has been identified as the anionic form [6–8]. The anionic semiquinone has also been observed in isolated reaction centers from *Rps. sphaeroides* and *Rhodospirillum rubrum* by optical spectroscopy [9, 10]. The anionic nature of the semiquinone is consistent with the lack of H^+-binding following a single flash [11].

Studies on the kinetic behaviour of the optically observed semiquinone, however, have revealed discrepancies with a simple view of a single ubiquinone

acting as a one-electron intermediate between the photochemistry and the secondary components of the cyclic electron transport chain. The semiquinone, termed A450 after its absorption maximum, was observed to be formed following a single flash but even in the presence of excess secondary acceptors was rather stable and decayed only in minutes. More surprising was the observation that photochemical competence, the ability to oxidise cytochrome *c* as an electron donor on subsequent flashes, was not impeded by the retention of the first electron on the semiquinone.

This report describes further observations on the semiquinone signal by optical and electron spin resonance spectroscopy both of which provide direct evidence of a two-electron gating process in the primary acceptor region giving rise to strong oscillations with a period of two.

MATERIALS AND METHODS

Reaction centers were prepared from *Rps. sphaeroides*, R26, by detergent fractionation using lauryldimethylamine *N*-oxide (LDAO or Ammonyx LO, Onyx Corporation, Division of Millimaster, Jersey City, N.J. 07023) as described by Clayton and Wang [13]. However, the detergent extract was purified by ion exchange chromatography on DEAE-cellulose (Whatman DE 52) in 0.1 % LDAO, 10 mM Tris-HCl, pH 8.0 rather than by ammonium sulfate fractionation. In most cases ammonium sulfate precipitation was avoided altogether and concentration of the reaction centers was performed by ultrafiltration.

Optical spectroscopy was performed on an unchopped double-beam spectrophotometer in single-beam mode and signals were recorded on a storage oscilloscope (Tektronix D15). Flash excitation was provided by a liquid dye laser (Phase-R, DL 1100) with rhodamine 6G as lasing solution. Saturation was routinely checked. Each sample contained roughly 2 μM reaction centers in 5 mls of 10 mM Tris · HCl, pH 8.0, in an anaerobic cuvette. Ubiquinone was added as a sonicated suspension in 10 % LDAO so that for a final concentration of 10–20 μM UQ the LDAO concentration was about 0.1 %. This additional LDAO had no noticeable effect on the activity of the reaction centers. As secondary donor both reduced cytochrome *c* ($\approx$ 25 μM) and diaminodurene (DAD, 80–100 μM) were used. The latter is useful for optical measurements of the semiquinone signal at about 450 nm while cytochrome *c* has the advantage of reacting very rapidly at low ionic strengths [12] and was used primarily for the ESR measurements.

ESR spectroscopy was performed on 3 mm sample tubes containing approximately 50 μM reaction centers and, when present, a 5–10 fold excess of ubiquinone and reduced cytochrome *c*. Samples were illuminated at room temperature with short saturating flashes from a ruby laser. Up to five flashes were given at 10 s intervals and at the end of each flash series the sample was frozen in isopentane/cyclohexane (5 : 1) freezing mixture at liquid nitrogen temperature. Freezing was complete in 3–5 s. Spectra were run at liquid helium or liquid nitrogen temperature on a Varian E4 spectrometer. All ESR sample tubes were calibrated with 0.5 mM $CuSO_4$, 5 mM EDTA at liquid nitrogen temperature.

RESULTS

The generation of the anionic semiquinone signal at room temperature by

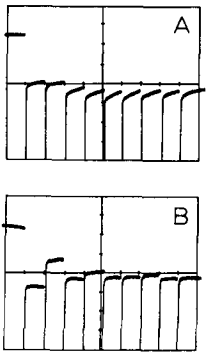


Fig. 1. Flash production of ubisemiquinone measured at 450 nm. Samples were in an anaerobic cuvette and contained 1.9 μM reaction centers and 80 μM diaminodurene; $E_h \approx +175$ mV. (A) No further additions. (B) Plus 12 μM ubiquinone. Flash excitation was given at 5 s intervals as indicated by the sharp spikes of P^+ oxidation and rereduction. Vertical scale = 0.0022 A per division.

single turnover excitation is shown in Fig. 1. The onset, which is too rapid to register on the oscilloscope, includes an absorption increase due to P^+. This rapidly decays due to reduction by the donor (DAD) giving the appearance of spikes. The stable absorbance remaining is due to the anionic semiquinone. In the absence of added ubiquinone as secondary acceptor the signal generated after the first flash is more or less stable with respect to subsequent flashes (Fig. 1a). In the presence of excess ubiquinone, however, the absorbance change is largely reversed following the second flash and is regenerated on the third (Fig. 1b). The spectra of the absorbance changes following the first and second flashes are similar in the limited wavelength region examined (400–480 nm) (Fig. 2).

The precise form of the oscillatory behaviour depends on what might be called the degree of coupling to the added quinones and varies somewhat from one batch of reaction centers to another and on their mode of preparation. The nature of this variability is currently under investigation; it is dependent, in part, on the amount of endogenous ubiquinone. Fig. 3a shows the oscillatory behaviour observed when the coupling is strong. Oscillations can be observed for 15–20 flashes in such samples. The damping process may be fairly well approximated by assuming a 10 % miss factor

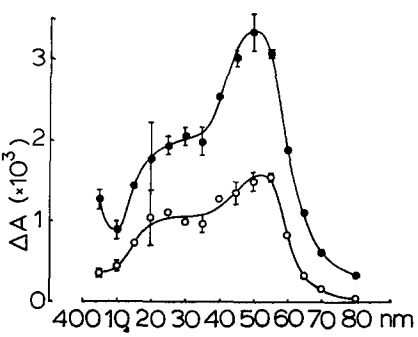


Fig. 2. Flash induced spectra for first (●) and second (○) flashes. Aerobic sample, with 1.3 μM reaction centers, 100 μM diaminodurene and 25 μM ubiquinone.

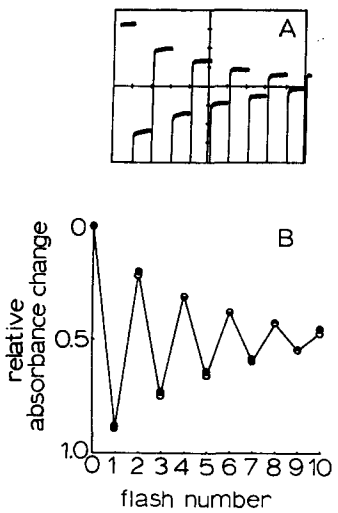


Fig. 3. Distinct oscillations of the ubisemiquinone at 450 nm. Aerobic sample with 2.2 μM reaction center, 100 μM diaminodurene and 35 μM ubiquinone. (A) Oscilloscope recording; scales as for Fig. 1. (B) Comparison of data from A (○) with expectations assuming 11 % misses (●).

for each flash as shown in Fig. 3b. Misses could arise from imperfect coupling of the secondary donor system (DAD) to the rereduction of P^+, allowing partial back reaction of the primary donor-acceptor pair (P^+X^-). Such a mechanism is in agreement with the observation that rereduction of P^+ is accelerated roughly ten-fold by the levels of DAD used compared to the back reaction rate in the absence of DAD; however, the correlation is not exact.

This novel behaviour of the optically detected semiquinone prompted a study of the ESR component attributed to this species, the $g = 1.82$ signal. Samples were flash illuminated at room temperature and frozen for examination at low temperature as described in Methods. Fig. 4 shows the amplitude of the g 1.82 signal observed in the dark as a function of flash number. Strong oscillations are apparent with the g 1.82 signal almost maximal after flashes 1 and 3 but much diminished after flashes 2 and 4. The fifth flash appears anomalous but examination of the g 3 region showed that this flash had produced no further oxidised cytochrome, indicating that the secondary donor pool was exhausted. In the absence of cytochrome *c* as donor almost no stable g 1.82 signal was formed by the flash treatment. In the absence of added UQ, the damping of the oscillations was much stronger.

The g 1.82 signal is extremely temperature sensitive and it is difficult to obtain meaningful quantitative data from the light-induced response of this component. Nevertheless it was apparent that continuous illumination at liquid helium temperature generated the g 1.82 signal in samples exposed to 0, 2 or 4 flashes but not 1 or 3. On the other hand, the light-induced g 2 signal was large in all cases so that when the g 1.82 component is formed at room temperature a second site of electron acceptance is still available at low temperature. A search for a new light-inducible ESR component revealed no distinct signals at liquid helium or nitrogen temperatures but the light-induced g 2 signal itself was subject to significant oscillations (Fig. 4). These amounted to about 25 % of the total signals and were of the correct phase

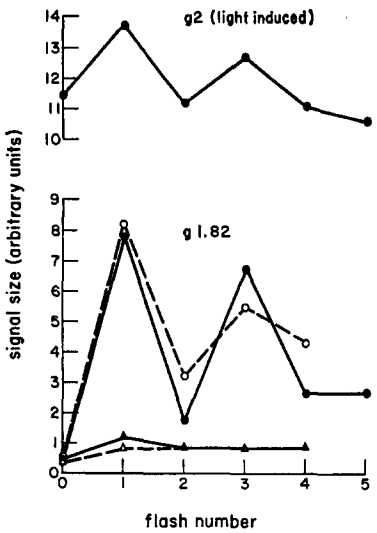


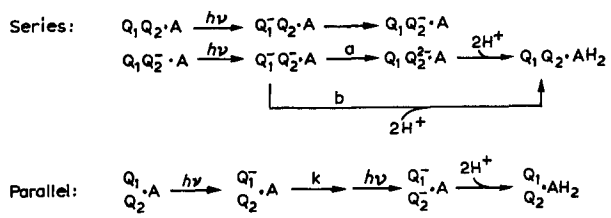
Fig. 4. Effect of room temperature flash excitation on low temperature ESR components. All samples contained approximately 50 μM reaction centers; ●, plus 250 μM reduced cytochrome *c* and 400 μM ubiquinone; ○, plus 250 μM reduced cytochrome *c* only; ▲, plus 400 μM ubiquinone only; △, no further additions. All ESR spectra were run at 6 °K with a modulation frequency of 100 KHz. Top: Light induced g 2 signal; modulation amplitude was 1 Gauss, microwave power was 0.5 mW. Bottom: Dark *g* 1.82 signal; modulation amplitude was 20 Gauss, microwave power 20 mW. The two vertical scales are not equivalent.

with respect to the flash sequence to account for an alternative electron acceptor (with $g \approx 2.0$) operative at low temperature when the *g* 1.82 component is reduced by single flashes at room temperature.

DISCUSSION

These new data on the behaviour of the primary acceptor of reaction centers suggest an intriguing dynamic relationship between two roughly equivalent ubiquinone molecules. Estimates of the ubiquinone content of the reaction centers prepared by LDAO fractionation have varied between 0 and 2 [1, 6, 14, 15]. Some variability is to be expected in view of the recent work of Okamura et al. [2] who have concluded from extraction and reconstitution studies that reaction centers under normal conditions contain two molecules of ubiquinone, one relatively easily extracted the other much more firmly bound, with the implication that one is the primary acceptor and the other a secondary acceptor. Such a distinction toward extraction, however, does not mean that the two molecules are different when both are present, since extraction of one could affect the binding of the other. Similarly, the fact that only one electron appears to contribute to the *g* 1.82 signal does not necessarily mean that the two alternative acceptors are different when both are oxidised. Indeed the ESR data show that the *g* 1.82 signal can herald the presence of either the first or second electron but not both and that when two electrons are present the first is manifested as the *g* 1.82 signal while the other may contribute a more normal free-radical component in the *g* 2 region.

Oscillations with a period of two have been indirectly observed for charge accumulation on the acceptor side of Photosystem II in green plants [16, 17] and plastosemiquinone has been identified as the reduced form of the primary acceptor of Photosystem II in deoxycholate subchloroplast fragments [18]. The similarities between the direct observations of oscillations in a chemical component reported here for the bacterial reaction center and the properties of the Photosystem II acceptor region are obvious and evolutionarily intriguing. It has been suggested that the oscillatory behaviour in Photosystem II is due to the operation of a two-electron gate acting in series with a one-electron primary acceptor [17]. The results presented here for the bacterial system indicate a rather close equivalence between two quinone molecules which, together, act as a two-electron buffer between the primary photoact and the secondary acceptor pool. Furthermore, simultaneous transfer of two electrons to the mobile secondary acceptor pool (A in scheme below) is supported by preliminary data on flash induced H^+-uptake in reaction center suspensions which show distinct binary oscillations with proton uptake occurring only on even flashes (unpublished observations). A two electron gate can thus be proposed for the bacterial reaction center, similar to that for Photosystem II [16, 17] although there would appear to be no compelling reason for excluding the operation of the two quinones in parallel. Series and parallel schemes may be represented as follows:



Parson has shown [19] that in *Chromatium vinosum* roughly 60 μs must elapse following a saturating flash before a second flash can elicit photochemistry and that the limitation is on the acceptor side of the reaction center. A parallel model for this would require that diffusion or rearrangement of Q_1 and Q_2 introduces a dark step (k) into the turnover time of the primary acceptor. There has been no reason to exclude such a possibility and the data presented here show that the two bound quinones of the reaction center are very similar in their optical and ESR spectral properties suggesting roughly equal interaction with the iron. However, this near equivalence is destroyed upon receipt of a single electron and it is difficult to distinguish between a series and parallel operation of the two potential acceptors on the basis of this work.

Recent studies of Photosystem II in green plants, however, have shown that when the two-electron transfer to the A pool is inhibited by bicarbonate depletion a total of three electrons can be held in the Q region [20]. This supports the series model with Q_2^{2-} functioning in the two-electron transfer (route a in the scheme above) thus allowing a third electron to be deposited on Q_1. Work is currently in progress to determine which scheme operates in bacterial reaction centers and chromatophores.

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