REDOX EQUILIBRIUM IN THE ACCEPTOR QUINONE COMPLEX OF ISOLATED REACTION CENTERS AND THE MODE OF ACTION OF *O*-PHENANTHROLINE

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1. Introduction

The electron acceptor reactions of isolated reaction centers from *Rhodopseudomonas sphaeroides* involve two specialized ubiquinone molecules, Q_I and Q_{II} [1–3]. Both Q_I and Q_{II} interact with an iron atom [1,4,5], forming what may be termed the acceptor quinone complex. In the presence of electron donors to re-reduce the primary donor (P) of the reaction center, and electron acceptors to serve as an electron acceptor pool for the acceptor quinone complex, a series of flashes elicits binary oscillations in the formation and disappearance of an anionic ubisemiquinone [2,3]. These oscillations are out-of-phase with oscillations in the formation of reduced quinol [2] and proton uptake [6]. This behavior can be accounted for by the following scheme:

$$\text{First flash:}\quad Q_IQ_{II} \xrightarrow{h\nu} Q_I^{\cdot-}Q_{II} \longrightarrow Q_IQ_{II}^{\cdot-}$$

$$\text{Second flash:}\quad Q_IQ_{II}^{\cdot-} \xrightarrow{h\nu} Q_I^{\cdot-}Q_{II}^{\cdot-} \xrightarrow{2H^+} Q_IQ_{II}H_2$$

Scheme 1

Details of the reaction steps, particularly those involving H^+-uptake, have been presented [7].

In the absence of added electron donors, the electron in the acceptor quinone complex is able to back react with oxidized P (P^+). When electron transfer from $Q_I^{\cdot-}$ to Q_{II} is blocked (Q_{II} extracted or inhibitor present) the back reaction from $Q_I^{\cdot-}$ to P^+ occurs in ~60 ms [8,9]. If electron transfer to Q_{II} does occur, the charge recombination from $Q_{II}^{\cdot-}$ to P^+ is considerably slower ($t_{1/2}$ ~1 s) [8,9]. By assuming that this slow process is a true back reaction from $Q_{II}^{\cdot-}$ and proceeds via Q_I, at near equilibrium, we have estimated the redox midpoint potential span (ΔE_m) between Q_I and Q_{II} for 1 e^- oxidation–reduction to be ~80 mV at pH <8 [7].

Blankenship and Parson independently arrived at a similar conclusion but found that *o*-phenanthroline (*o*-phen), a well known inhibitor of forward electron transfer from $Q_I^{\cdot-}$, did not affect the slow recombination kinetics from $Q_{II}^{\cdot-}$ when added immediately after the flash [10]. Thus, the recombination from $Q_{II}^{\cdot-}$ would appear not to be a true back reaction via Q_I but a separate, essentially shortcircuit, pathway. They therefore concluded that the estimated ΔE_m, which assumes a back reaction mechanism, was only a minimum value. In response to this, however, we have suggested that *o*-phen may fail to inhibit electron transfer when added after a flash, when the acceptor complex is in the state $Q_IQ_{II}^{\cdot-}$, and have briefly described evidence in support of this [11]. Through studies on the light-induced redox changes of the quinones in isolated reaction centers and the oxidation of cytochrome *c* in a series of flashes, we show here that *o*-phen interacts very weakly and slowly with the acceptor quinone complex in the $Q_IQ_{II}^{\cdot-}$ state and is, indeed, a poor inhibitor of electron transfer when added to reaction centers in this state. Thus, our original estimate of the redox midpoint potential span between Q_I and Q_{II} (ΔE_m ~80 mV [7]) may well be correct.

2. Materials and methods

Reaction centers were isolated and purified from

Rhodopseudomonas sphaeroides, strain R26, as in [7]. Kinetic measurements of the light-induced semiquinone ΔA (at 433 or 450 nm) and of cytochrome *c* oxidation (at 550 nm) were performed on a single beam spectrophotometer, with xenon flash excitation, as in [7]. For experiments where *o*-phen was added after a variable number of preconditioning flashes, the addition was made by hand and the total time between the preconditioning flashes and the measuring flash sequence was 5 s.

3. Results

Fig.1 shows the inhibition of electron transfer from Q_I to Q_{II} by 4 mM *o*-phen at various flash repetition rates. Clearly, the full effect of *o*-phen on the oscillatory activity of the acceptor quinone complex is only felt at $\lesssim$100 ms after a flash. The loss of inhibition had $t_{1/2}$ ~0.6 s (fig.1e). In this time the semiquinone signal does not decay at all and the loss of inhibition is not due to reoxidation of the acceptor complex as a whole. It is readily accounted for, however, by supposing that the electron leaks past the *o*-phen block, allowing the formation of $Q_{II}^{\cdot -}$ in an essentially normal, but slowed, reaction. This can be seen directly at 433 nm, a shoulder on the semiquinone absorbance spectrum where $Q_I^{\cdot -}$ absorbs less strongly than $Q_{II}^{\cdot -}$ [7,12]. In the presence of *o*-phen, the flash-induced semiquinone signal exhibits a small, slow rise phase ($t_{1/2}$ ~0.5 s) as electron transfer through the *o*-phen block occurs (fig.1d).

However, the slow electron transfer is not a simple leak leaving the *o*-phen block intact. Fig.2 shows that at pH 7.2 the disappearance of $Q_{II}^{\cdot -}$ following a second flash, given at sufficiently long time after the first, is fast regardless of the presence or absence of 2 mM *o*-phen. Thus, *o*-phen does not inhibit the $Q_I^{\cdot -}Q_{II}^{\cdot -} \longrightarrow Q_IQ_{II}H_2$ electron transfer.

Turnover of the reaction centers was also assessed by monitoring the oxidation of cytochrome *c* in a series of flashes (fig.3). The relative amount of cytochrome oxidized on the second flash indicates the extent to which $Q_I^{\cdot -}$ has been reoxidized between flashes. At high repetition rates, inhibition of the second flash cytochrome oxidation was half maximal, at pH 8, at ~0.4 mM *o*-phen. At low repetition rates, the effectiveness of inhibition diminished markedly and *o*-phen >10 mM was needed for half maximal inhibition when the time between flashes was >2–3 s. In the absence of *o*-phen, turnover on the second flash was diminished by ~6% due to the intrinsic equilibrium between $Q_I^{\cdot -}Q_{II}$ and $Q_IQ_{II}^{\cdot -}$.

Cytochrome *c* oxidation was also monitored in samples exposed to a variable number of preconditioning flashes, followed by the addition of 4 mM *o*-phen. A measuring flash sequence was then given at a high repetition rate (30 Hz). The relative extent of cyto-

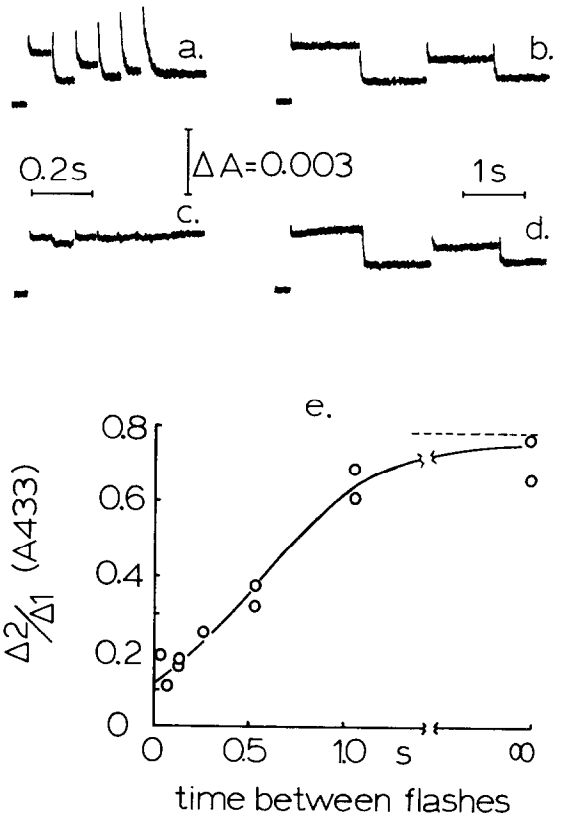


Fig.1. Oscillations in semiquinone formation and disappearance, measured at the isosbestic wavelength for cytochrome *c* oxidation, near 433 nm: 2 μM reaction centers; 15 μM cytochrome *c*; 20 μM ubiquinone-10; 10 μM 1,4-naphthoquinone; 20 μM diaminodurene; 0.06% Triton X-100 in 0.1 M NaCl, 10 mM Tris–HCl (pH 8.0). *o*-Phenanthroline (4 mM) was present in traces c,d. A series of 6 flashes at 15 Hz (a,c) or flashes at 0.94 Hz (b,d) were given. The flashes were 89% saturating. From experiments at various flash repetition rates, the relative amount of turnover on the second flash in the presence of 4 mM *o*-phen was obtained. This is plotted (e) as a function of dark time between the flashes. The 'end-point' (at ∞ s) was taken in the absence of inhibitor, corrected for the difference in absorption coefficients of $Q_I^{\cdot -}$ and $Q_{II}^{\cdot -}$ (see text). The dashed line is the expected limit for 89% flash saturation.

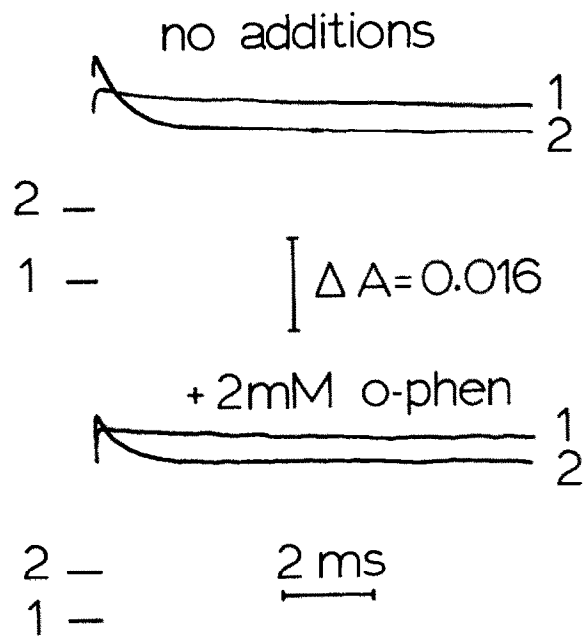
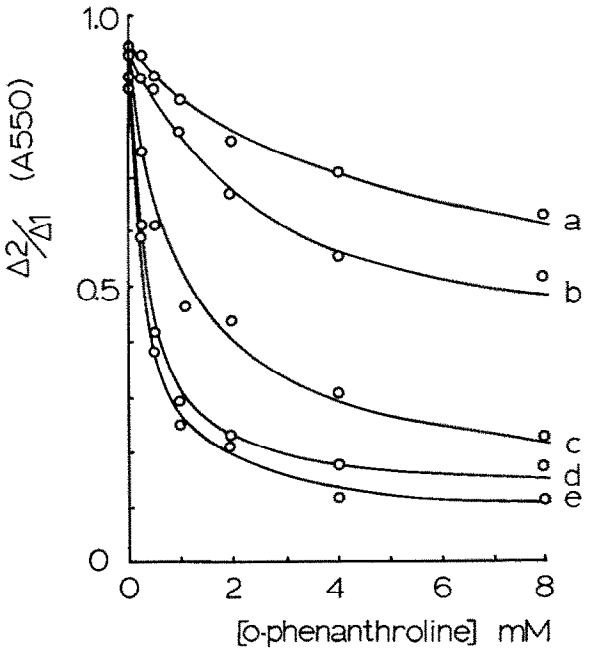


Fig.2. ΔA_{450} due to P^+ and the semiquinones: 5.1 μM reaction centers; 100 μM diaminodurene; 40 μM ubiquinone-10; 10 μM 1,4-naphthoquinone; 0.12% Triton X-100 in 0.1 M KCl; 10 mM MOPS, pH 7.2. First and second flash traces are shown, indicated by 1 and 2. P^+ decays very slowly on this time scale and the rapid decay phase following the second flash indicates the electron transfer: $Q_I^- Q_{II}^- \longrightarrow \longrightarrow Q_I Q_{II} H_2$. Top traces: no further additions. Bottom traces: plus 2 mM *o*-phen.



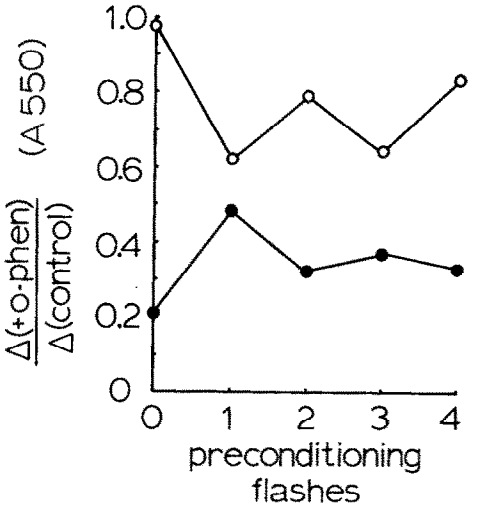


Fig.4. Oscillations in cytochrome *c* oxidation, measured at 550 nm. After a variable number of preconditioning flashes, *o*-phen (4 mM) or an equivalent volume of ethanol was added and cytochrome oxidation was measured on first (○) and second (●) subsequent flashes; 2 μM reaction centers; 20 μM cytochrome *c*; 20 μM ubiquinone-10; 10 μM, 1,4-naphthoquinone; 0.06% Triton X-100 in 10 mM Tris–HCl (pH 8). Flash repetition rate, 30 Hz.

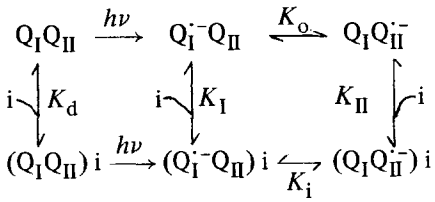
chrome oxidation by the first flash of the measuring flash series indicates the proportion of reaction centers with Q_I oxidized. This is determined by the 1 e^- equilibrium between Q_I and Q_{II} [7]. The second flash indicates the degree of inhibition by *o*-phen of the electron transfer following the first flash. Fig.4 (closed circles) shows that the extent of cytochrome oxidation on the second flash oscillated with the number of preconditioning flashes and was maximal after an odd number. This is consistent with the conclusion that *o*-phen does not inhibit the reaction centers in the $Q_I Q_{II}^-$ state. The extent of cytochrome

Fig.3. Inhibition of cytochrome *c* oxidation, measured at 550 nm: 2 μM reaction centers; 15 μM cytochrome *c*; 20 μM ubiquinone-10; 10 μM 1,4-naphthoquinone; 0.06% Triton X-100 in 10 mM Tris–HCl (pH 8.0). Xenon flashes were given at variable repetition rates and the inhibition of second flash turnover, relative to the first flash, was determined as a function of [*o*-phen]. The curves are all drawn according to scheme 2, with $K_Q = 15$, $K_I/K_{II} = 100$; (a) 0.47 Hz, K_I = 1200 M^{-1}; (b) 0.94 Hz, K_I = 7200 M^{-1}; (c) 1.88 Hz, K_I = 13 000 M^{-1}; (d) 7.5 Hz, K_I = 30 000 M^{-1}; (e) 30 Hz, K_I = 60 000 M^{-1}.

oxidation on the first flash of the series also oscillated (fig.4, open circles), but was maximal after an even number of flashes. Since the extents are expressed relative to those of a similar flash series given in the absence of *o*-phen, this demonstrates that the 1 e^- equilibrium between Q_I and Q_{II} was displaced by *o*-phen in favor of $Q_I^{\cdot -}$, causing subsequent blockage of photochemical turnover.

4. Conclusions

These observations can be accounted for by the following simple scheme, in which the inhibitory action of *o*-phen is equated with binding to a specific site in the acceptor quinone complex. i represents *o*-phenanthroline:



Scheme 2

The inhibitory activity of *o*-phen added before illumination is associated with a relatively high affinity of *o*-phen for the fully oxidised quinone complex (Q_IQ_{II}); half maximal inhibition at pH 8 required 0.4 mM *o*-phen. The poor inhibition by *o*-phen added after one flash (i.e., 1 e^- in the acceptor quinone complex) is readily accounted for by weak binding, but the decrease in the extent of cytochrome oxidation on the first flash after an odd number of preconditioning flashes indicates that *o*-phen binds more strongly to $Q_I^{\cdot -}Q_{II}$ than to $Q_IQ_{II}^{\cdot -}$. The net effect is to displace the equilibrium in favor of $Q_I^{\cdot -}$. In terms of the total concentrations of $Q_I^{\cdot -}$ (I) and $Q_{II}^{\cdot -}$ (II) in the overall equilibrium:

$$\frac{I}{II} = \frac{1}{K_o} \cdot \frac{(1 + K_I\,(i))}{(1 + K_{II}\,(i))}$$

At high concentrations of *o*-phen the maximal failure in turnover, according to this scheme, gives K_i. From fig.3 we obtain $K_i \sim 0.15$ at high *o*-phen and $K_o \sim 15$ in the absence of *o*-phen. Thus, $(K_o/K_i) = (K_I/K_{II}) = 100$, and this value has been used to draw all the curves of fig.3. As the dark time between flashes was lengthened, the concentration of *o*-phen needed for inhibition increased. The absolute values of K_I and K_{II}, therefore, varied with the flash repetition rate but maintained the same ratio. The time dependence of the inhibition arises from slow equilibration between the different acceptor quinone states of scheme 2 and this is the principle source of the normal inhibitory action of *o*-phen. Clearly, the overall equilibrium constant does not account for the inhibition as the electron does eventually pass to Q_{II} at all levels of *o*-phen commonly in use (0.5–4 mM). Slow equilibration between states I and II requires that at least 2 of the 4 steps equilibrate slowly. The rate constants that constitute K_o are known to be fast [7] and it is likely that it is the two binding equilibria, K_I and K_{II}, that are established slowly.

At low repetition rates the inhibition of turnover reflects the net binding constant for *o*-phen to the semiquinone states of the acceptor complex:

$$K_{net} = K_I \frac{(1 + K_i)}{(1 + K_o)}$$

Below 1 Hz the concentration for half maximal inhibition of turnover approaches a value somewhat >10 mM ($K_{net} \leq 10^2\ M^{-1}$). Using the values of K_o and K_i given above, we obtain $K_I \leq 10^3\ M^{-1}$ and $K_{II} \leq 10\ M^{-1}$. Thus, although the equilibrium scheme predicts an enhanced level of $Q_I^{\cdot -}$ that should have accelerated the back reaction in [10], we can now see that such an effect requires very high concentrations of *o*-phen, perhaps unattainably high in view of its limited solubility in water. Here we saw an effect (10–30%) on first flash cytochrome oxidation with 4 mM *o*-phen. In [10] 2 mM *o*-phen was used, which would give a barely observable displacement of the equilibrium. Furthermore, the binding equilibrium for the semiquinone states is established slowly and occurs on the same time scale ($t_{1/2} \sim 0.5$–1 s) as the back reaction itself. It is also possible that the presence of P^+ after the flash affects the binding equilibrium and kinetics. Our experiments show clearly that the interaction of *o*-phen with the acceptor quinone complex is strongly modulated by the redox state of the $Q_{II}/Q_{II}^{\cdot -}$ couple such that the electron transfer

$Q_I^{\cdot-}Q_{II}^{\cdot-} \longrightarrow Q_IQ_{II}H_2$ is not significantly inhibited.

The lack of significant inhibition of electron transfer by *o*-phen added after the first flash means that the charge recombination between $Q_{II}^{\cdot-}$ and P^+, in isolated reaction centers, can be a true back reaction. Thus, the calculation [7] of the redox midpoint potential span between $Q_I/Q_I^{\cdot-}$ and $Q_{II}/Q_{II}^{\cdot-}$ (ΔE_m ~80 mV) may still be valid.

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