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DAMPING OF OSCILLATIONS IN THE SEMIQUINONE ABSORPTION IN REACTION CENTERS AFTER SUCCESSIVE FLASHES

DETERMINATION OF THE EQUILIBRIUM BETWEEN $Q_A^-Q_B$ AND $Q_AQ_B^-$

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A quantitative model for the damping of oscillations of the semiquinone absorption after successive light flashes is presented. It is based on the equilibrium between the states $Q_A^-Q_B$ and $Q_AQ_B^-$. A fit of the model to the experimental results obtained for reaction centers from *Rhodopseudomonas sphaeroides* gave a value of $\alpha = [Q_A^-Q_B]/([Q_A^-Q_B] + [Q_AQ_B^-]) = 0.065 \pm 0.005$ ($T = 21°C$, pH 8).

The optical absorption due to semiquinone formation in bacterial reaction centers exhibits damped oscillations when reaction centers are excited with a series of laser flashes in the presence of exogenous electron donors and acceptors. These oscillations, first observed by Vermeglio [1] and by Wraight [2] in bacterial reaction centers and by Bouges-Bocquet [3] and Velthuys and Amesz [4] in Photosystem II of green plants, arise from a serial transfer of electrons from the donor (D) to the primary (Q_A) and secondary (Q_B) quinone acceptors (for a review see Ref. 5). While Q_A is only a single-electron acceptor, Q_B accepts two electrons and functions as a 'gate' between the single-electron processes following photon absorption and the two-electron transfer step to the exogenous quinone pool. The stable semiquinone state $DQ_AQ_B^-$ is formed on the first and subsequent odd flashes; the unstable state $DQ_AQ_B^{2-}$ is formed on even flashes. Thus, the semiquinone signal is expected to oscillate, undamped, with a period of two flashes.

Damping of the oscillations will result from any mechanism that prevents the complete conversion of DQ_AQ_B to $DQ_AQ_B^-$ after odd flashes and the complete regeneration of DQ_AQ_B after even flashes. While this includes non-ideal external conditions (e.g., multiple turnover or nonsaturating flashes), the oscillations must dampen because of the inherent equilibrium between the semiquinone states $DQ_A^-Q_B$ and $DQ_AQ_B^-$, the former being photochemically inactive. This mechanism was suggested in green plants by Diner (Ref. 6; see also Refs. 7–9 and the review in Ref. 10) and bears resemblances to the proposals of Joliot [11,12] and Kok [13] used to explain oxygen evolution by chloroplasts (for a review see Ref. 14).

In this work we present a quantitative model for the amplitude of semiquinone absorption after successive flashes in terms of the equilibrium between the states $Q_A^-Q_B$ and $Q_AQ_B^-$. Thus, the model serves as the basis for an assay of the

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** See footnote on p. 407.

Abbreviation: DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; UQ-0, 2,3-dimethoxy-6-methyl-1,4-benzoquinone.

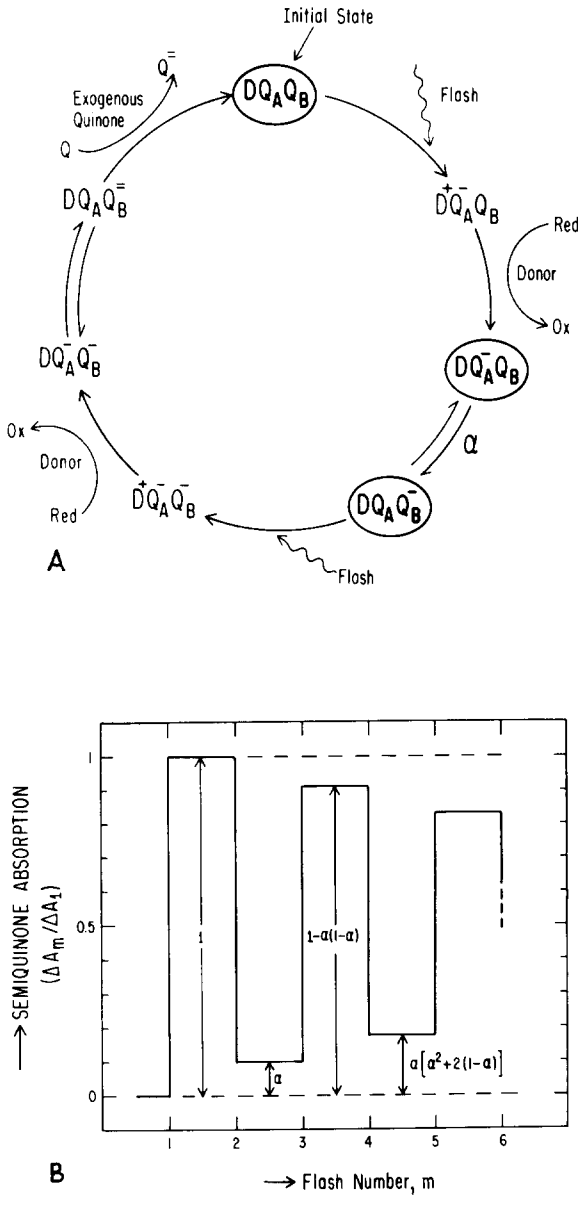

Fig. 1. (A) The light-driven electron-transfer steps for reaction centers in the presence of exogenous donors and (quinone) acceptors. The stable states are circled; $DQ_A^-Q_B$ is photochemically inactive. The scheme assumes that the reaction between D^+ and exogenous donors occurs rapidly compared to charge recombination within the reaction center and that all intermediate reactions shown occur to completion between flashes. (B) The relative amplitude ($\Delta A_m/\Delta A_1$) of semiquinone absorption predicted from the previous scheme, as a function of flash number (m). The amplitudes are given by Eqn. 6, with α chosen as 0.1 for purpose of illustration only.

partition coefficient α**, given by:

$$\alpha = \frac{[Q_A^- Q_B]}{[Q_A^- Q_B] + [Q_A Q_B^-]} \tag{1}$$

The model was tested using bacterial reaction centers for which the value of α has been obtained by different methods [15,16]. A preliminary account of this work has been presented [17].

The overall electron-transfer process is modeled by the scheme illustrated in Fig. 1A, with the predicted semiquinone absorption after each flash illustrated in Fig. 1B. On the first flash, the full semiquinone signal is formed. On the second flash, a fraction α of the reaction centers are in the photochemically inactive state $DQ_A^-Q_B$; the remainder loose two electrons to regenerate the initial state DQ_AQ_B. With succeeding flashes the semiquinone absorption oscillates in a damped manner as the concentrations of DQ_AQ_B, $DQ_A^-Q_B$ and $DQ_AQ_B^-$ approach their respective steady state values.

The difference equations governing the concentrations of the various reaction center states after m flashes are given by:

$$[Q_AQ_B]_m = [Q_AQ_B^-]_{m-1} \tag{2a}$$

$$[Q_A^-Q_B]_m = \alpha([Q_AQ_B]_{m-1} + [Q_A^-Q_B]_{m-1}) \tag{2b}$$

$$[Q_AQ_B^-]_m = (1-\alpha)([Q_AQ_B]_{m-1} + [Q_A^-Q_B]_{m-1}) \tag{2c}$$

The initial conditions are:

$$[Q_AQ_B]_0 = N_0, \quad [Q_A^-Q_B]_0 = 0, \quad [Q_AQ_B^-]_0 = 0 \tag{3}$$

where N_0 is the initial reaction center concentration. The solutions of these equations are:

$$[Q_AQ_B]_m = N_0 \frac{1-\alpha}{2-\alpha}\{1-(-1)^{m-1}(1-\alpha)^{m-1}\} \tag{4a}$$

$$[Q_A^-Q_B]_m = N_0 \frac{\alpha}{2-\alpha}\{1-(-1)^{m}(1-\alpha)^{m}\} \tag{4b}$$

$$[Q_AQ_B^-]_m = N_0 \frac{1-\alpha}{2-\alpha}\{1-(-1)^{m}(1-\alpha)^{m}\} \tag{4c}$$

** The partition coefficient α is related to the equilibrium constant K and the free-energy difference ΔG by:

$$1/\alpha = 1 + K = 1 + e^{-\Delta G/kT}$$

where k is Boltzmann's constant and T is the temperature.

The measured semiquinone absorption contains contributions from both Q_A^- and Q_B^- and is given by:

$$\Delta A_m = \varepsilon_{A^-}[Q_A^- Q_B]_m + \varepsilon_{B^-}[Q_A Q_B^-]_m \quad (5)$$

where ε_{A^-} and ε_{B^-} are the extinction coefficients for the states $DQ_A^-Q_B$ and $DQ_AQ_B^-$, respectively. Substituting Eqn. 4b and c into Eqn. 5 gives:

$$\Delta A_m = \frac{\Delta A_1}{2-\alpha}\{1-(-1)^m(1-\alpha)^m\} \quad (6)$$

The oscillations result from the changing sign of the $(-1)^m$ term on alternate flashes; the damping results from the $(1-\alpha)^m$ term. As $m \to \infty$, ΔA_m assumes the limiting value of $\Delta A_1/(2-\alpha)$. For $\alpha \ll 1$, $(1-\alpha)^m = e^{-\alpha m}$ and the oscillations decay with an apparent rate constant α. The relative change in semiquinone absorption between two sequential pairs of flashes, obtained from Eqn. 6, is:

$$\frac{\Delta A_{m+1} - \Delta A_m}{\Delta A_m - \Delta A_{m-1}} = 1-\alpha \quad (7)$$

To determine the equilibrium partitioning using this model, the semiquinone absorption was measured optically at 450 nm using reaction centers isolated from *Rhodopseudomonas sphaeroides* R-26

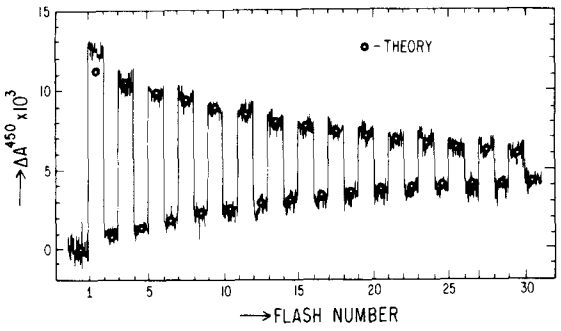

Fig. 2. The semiquinone absorption change of reaction centers, monitored at 450 nm, after successive laser flashes. 2.8 μM reaction centers, 1.0 mM UQ-0 and 0.5 mM DAD in 10 mM Tris-HCl, pH 8.0, 0.025% lauryldimethylamine N-oxide, at $T = 21.5\,^\circ$C. Dye laser flashes ($\lambda_0 = 584$ nm, approx. 0.15 J per pulse, 0.4 μs duration) occurred at 0.5 s intervals. A small long-term drift in the absorption, caused by instrumental instabilities and photoproducts of the exogenous reactants, was subtracted out. Theoretical absorption values (O) were calculated using Eqn. 6 with $\Delta A_1 = 11.2 \cdot 10^{-3}$ and $\alpha = 0.065$.

and purified on a cytochrome *c* (cyt *c*; horse heart type III, Sigma) affinity column [18]. Optical measurements were performed with a kinetic spectrophotometer of local design [16].

The exogenous electron donor was diaminodurene (DAD; 2,3,5,6-tetramethyl-*p*-phenylenediamine, Aldrich), chosen for its lack of absorption at 450 nm. The photooxidized donor, D^+, must be reduced by DAD before charge recombination with Q_A^- or Q_B^- occurs. The DAD to D^+ electron transfer was found to follow second order kinetics with $k_{DAD} = 3 \cdot 10^5\ M^{-1} \cdot s^{-1}$ (pH 8.0, $I = 10$ mM, 0.025% lauryldimethylamine *N*-oxide, $T = 21.5\,^\circ$C; $k_{DAD} \propto [H^+]^{0.04}$ over the pH range 7.0–11.0).The donor recovery rate for either $D^+Q_AQ_B^-$ [19] or $D^+Q_AQ_B^{2-}$ was determined to be approx. 1 s^{-1} at pH 8. This is approx. 100 times slower than the DAD reduction rate of D^+ ([DAD] = 0.5 mM) and satisfies, therefore, the condition outlined above.

The exogenous quinone acceptor was ubiquinone-0 (UQ-0; 2,3-dimethoxy-6-methyl-1,4-benzoquinone, World Chemicals) selected for its relatively high solubility in water. UQ-0 must accept the two electrons from $Q_AQ_B^{2-}$ in a time fast compared to the time interval between the laser flashes (0.5 s). By using cyt c^{2+} as the exogenous donor and optically ($\lambda = 550$ nm) monitoring the cyt c^{2+} oxidation rate under conditions in which electron transfer from Q_B^{2-} to UQ-0 was the limiting step, we determined that the transfer between Q_B^{2-} and UQ-0 followed second order kinetics with $k_{UQ\text{-}0} = 1 \cdot 10^7\ M^{-1} \cdot s^{-1}$ ($7.0 \leqslant pH \leqslant 9.0$, $I = 10$ mM, 0.025% lauryldiethylamine *N*-oxide, $T = 21.5\,^\circ$C). We used [UQ-0] = 1.0 mM, making the transfer time approx. 10^{-4} s. The electron transfer time between $Q_A^-Q_B^-$ and $Q_AQ_B^{2-}$ had been determined to be less than or equal to $5 \cdot 10^{-2}$ s ($pH \leqslant 9.0$) [8,20], which is at least 100 times faster than the time interval between laser flashes. Thus, the conditions for the model to hold were satisfied for the reactions at both the donor and the acceptor side of the reaction center.

The damped oscillations observed for the semiquinone absorption after successive flashes at pH 8.0 are shown in Fig. 2. The best fit to the model (i.e., Eqn. 6) was found with (see O in Fig. 2):

$\alpha = 0.065 \pm 0.005$

The discrepancy after the first flash is due in part to reaction centers with only a single quinone (approx. 10%, determined as in Ref. 21). The value for α found in this work agrees, within experimental error, with the value found by other, independent, techniques [15,16]. Similar agreements were obtained at pH 7.0 and 9.0. The advantage of obtaining α with the present method is that, by averaging the absorption changes over many flashes, a high degree of accuracy is achieved.

Oscillations observed with reaction centers purified by ammonium sulfate fractionation [22] (rather than using a cyt *c* affinity column) fit the model well beginning only after the first three flashes. This suggest that a fraction (approx. 20%) of these reaction centers have lost the ability of Q_B^{2-} to react with exogenous quinone and are consequently left in the state $DQ_A^-Q_B^{2-}$. Difficulties in observing oscillations with reaction centers purified in this manner have been reported [1,2].

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