CHARGE RECOMBINATION KINETICS AS A PROBE OF PROTONATION OF THE PRIMARY ACCEPTOR IN PHOTOSYNTHETIC REACTION CENTERS

D. KLIEFELD, M. Y. OKAMURA, AND G. FEHER
Department of Physics, University of California, San Diego La Jolla, California 92037

ABSTRACT The kinetics of the charge recombination \( \text{D}^+ Q_a^- \rightarrow \text{DQ}_a \) was used to probe the protonation of the primary acceptor in reaction centers from \textit{Rhodopseudomonas sphaeroides}, in which the native ubiquinone was replaced by anthraquinone. We found that \( Q_a^- \) is stabilized by the rapid \((t < 10^{-2} \text{ s})\) binding of a proton, with a \( pK \) of 9.8. The distance between \( Q_a^- \) and the proton binding site was estimated to be larger than \(-5 \text{ Å}\).

The absorption of light by photosynthetic reaction centers (RCs) leads to a separation of charge between the electron donor, \( \text{D} \) (bacteriochlorophyll dimer), and the primary (quinone) acceptor, \( Q_a^- \), forming \( \text{D}^+ Q_a^- \) (for review, see references 1 and 2). Here we examine the protonation events accompanying the charge separation. Redox titrations of the \( Q_a^-/Q_a \) couple (3–5; for review, see reference 6) indicate that \( Q_a^- \) associates with a proton (\( pK_a = 9.8 \)). The same conclusion was reached from studies on the kinetics of electron transfer between \( Q_a^- \) and the secondary acceptor, \( Q_b \) (7). Spectroscopic measurements, however, indicate that the proton is not directly bound to \( Q_a^- \).

The optical absorption spectrum of \( Q_a^- \) at neutral \( pH \) (8–10) resembles that of the unprotonated semiquinone anion (11, 12). Similarly, the EPR spectrum of \( Q_a^- \) (13–16) is characteristic of the unprotonated anion (16).

To probe for a protonation site in the vicinity of \( Q_a^- \), we studied the charge recombination kinetics \( \text{D}^+ Q_a^- \rightarrow \text{DQ}_a \) with anthraquinone (AQ) replacing the native ubiquinone (UQ). With AQ, as opposed to UQ, the charge recombination rate, \( k_{\text{obs}} \), is sensitive to small perturbations in the free energy of \( Q_a^- \) (17, 18). Thus \( k_{\text{obs}} \) should be sensitive to a shift in the energy of \( Q_a^- \) caused by the electrostatic interaction with a nearby proton. A preliminary account of this work has been presented (19).

The charge recombination process of \( \text{D}^+ Q_a^- \) is schematically illustrated in Fig. 1; \( k_{\text{on}}^q \) and \( k_{\text{off}}^q \) are the rate for proton binding and release, and \( k_{\text{on}}^q \) and \( k_{\text{off}}^q \) are the recombination rates in the presence and absence of a proton, respectively. The electron on \( Q_a^- \) recombines with \( \text{D}^+ \) via thermal repopulation of the state \( \text{D}^+ I^- Q_a^- \), where \( I \) is the intermediate acceptor (2). The recombination rate depends on the free energy difference, \( \Delta G^0 \), between \( \text{D}^+ I^- Q_a^- \) and \( \text{D}^+ Q_a^- \) (18). The shift in this energy difference, \( \Delta G^0 \), caused by the binding of a proton, results in a change in the rate given by (18):

\[
k_{\text{obs}} = k_{\text{obs}}^0 e^{-\Delta G^0/k_BT},
\]

where \( k_s \) is Boltzmann's constant and \( T \) the absolute temperature.

The time dependence of the recombination kinetics, \( k_{\text{obs}} \), will depend on whether the proton equilibration rate, \( k_{\text{on}}^q + k_{\text{off}}^q \) (Fig. 1), is fast or slow with respect to the charge recombination rates \( k_{\text{on}}^q \) and \( k_{\text{off}}^q \). If the proton equilibration rates are fast, the states \( (\text{D}^+ Q_a^-)H^+ \) and \( \text{D}^+ Q_a^- \) are in equilibrium on the time scale of the charge recombination; the observed kinetics will follow a single exponential decay, with \( k_{\text{obs}} \) given by the sum of \( k_{\text{on}}^q \) and \( k_{\text{off}}^q \), weighted by the fraction of unprotonated and protonated RCs, respectively, i.e.,

\[
k_{\text{obs}} = \frac{k_{\text{on}}^q + 10(pK_a - pH) k_{\text{off}}^q}{1 + 10(pK_a - pH)}.
\]

where

\[
(pK_a - pH) = \log \left( \frac{[\text{D}^+ Q_a^- H^+]}{[\text{D}^+ Q_a^-]} \right) = \log \left( \frac{k_{\text{on}}^q}{k_{\text{off}}^q} \right).
\]

When the proton equilibration rate is slow compared with \( k_{\text{on}}^q \) and \( k_{\text{off}}^q \), the states \( (\text{D}^+ Q_a^-)H^+ \) and \( \text{D}^+ Q_a^- \) do not interconvert on the time scale of the charge recombina-

---

\*These states contain the intermediate state, \( I \), which we have omitted for the sake of simplicity.
induced charge separation was accomplished with a pulsed dye laser ($\lambda_0 = 584$ nm, 0.4 $\mu$s pulse width, 0.2 J/pulse). Changes in optical absorption were recorded with a spectrophotometer of local design (7). Experimental conditions were as described (7).

The recovery kinetics $D^+ \rightarrow D$ are shown in Fig. 2. The decrease in the recovery rate with decreasing pH implies that $D^+Q^-\text{aq}$ is stabilized by the presence of a proton. The kinetics followed a single-exponential decay at all pH values (Fig. 2), implying that the proton binds rapidly compared with $k_{\text{obs}}$ i.e.,

$$k_{\text{on}}^+ + k_{\text{off}}^+ \gg k_{\text{obs}} \sim 10^2 \text{s}^{-1}.$$  (4)

This limit is consistent with the results of proton uptake measurements (21–23); the stoichiometry of the measured uptake is, however, controversial (21–24).

The pH dependence of $k_{\text{obs}}$ is shown in Fig. 3. The value of $k_{\text{obs}}$ at low pH is in agreement with previous findings (17, 18, 25). To verify that the increase in $k_{\text{obs}}$ at high pH was not an artifact of the quinone removal and reconstitution process, measurements were performed with RCs reconstituted with UQ. The rate $k_{\text{obs}}$ changed by <25% over the range 6 $< pH < 11$, in agreement with results for native RCs (7). The solid line in Fig. 3 represents the best fit of the kinetics model (Eq. 2) to the data with $k_{\text{obs}}^{H^+} = 97 \text{ s}^{-1}$, $k_{\text{obs}}^{H^+} = 230 \text{ s}^{-1}$ and $pK_a = 9.8$. Note that the value of $pK_a$ matches that found from both redox titrations (3–5) and electron transfer (7).

The interaction energy of $Q^-\text{aq}$ with a nearby proton depends on $k_{\text{obs}}^{H^+}$ and $k_{\text{obs}}^{-}$ (see Eq. 1). The energy splitting $\Delta G^0$ is given by (Eq. 1 with $T = 21^\circ$C):

$$\Delta G^0 = k_\text{B}T \ln \frac{k_{\text{obs}}^{H^+}}{k_{\text{obs}}^{-}} = 22 \text{ meV}. \quad (5)$$

The magnitude of $\Delta G^0$ is approximately two orders of magnitude smaller than the optical transition energy of

![Figure 2](image2.png)  
Semilogarithmic plot of the change in optical absorption at 865 nm after a single, saturating flash of light (0.4 $\mu$s pulse width, 0.2 J/pulse) for different values of pH. The contribution to the kinetics from a residual (~10%) population of RCs containing UQ was measured before the addition of AQ and was subtracted in the data shown. The solid lines represent the best fit of the function $\Delta A_{865}(t)/\Delta A_{865}(0) = e^{-\lambda t}$ to the data. The observed kinetics were independent of the light intensity for $10^2 \text{ W/cm}^2 < I < 10^4 \text{ W/cm}^2$. Conditions: To 1.8 $\mu$M RCs depleted of UQ were added, 20 $\mu$M QA in either 10 mM PIPES (piperazine-N,N'-bis[2-ethanesulfonic acid]) (pH 7.1), 10 mM CHES (cyclohexylamino-ethanesulfonic acid) (pH 9.9) or 10 mM CAPS [3-(cyclohexylamino)-propanesulfonic acid] (pH 11.6), and 0.025% (wt/vol) LDAO (lauryl dimethylamine-N-oxide) at $T = 21^\circ$C.

![Figure 3](image3.png)  
The pH dependence of the charge recombination rate $k_{\text{obs}}$. The solid line (Model) was calculated using Eqs. 2 and 3 with $pK_a = 9.8$, $k_{\text{obs}}^{H^+} = 97 \text{ s}^{-1}$ and $k_{\text{obs}}^{-} = 230 \text{ s}^{-1}$. Conditions as in Fig. 2, except for varying buffers and pH.
occurs when the proton is situated about halfway between I− and OQ−, i.e., θ = 0. At this location, r_{HQ} ≈ 5 Å; the ratio r_{HQ}/r_{IQ} is essentially independent of εr_{IQ}.

In conclusion, we have shown that a proton is associated with Q_{A−} at a distance ≥ 5 Å. This relatively large distance may explain why the protonation has not been observed in either the optical or EPR spectrum of (Q_{A−})H^+*. The pK that we determined (9.8) is in agreement with the values found from redox (3–5) and electron transfer (7) studies.

We thank E. C. Abresch for isolating the reaction centers, B. Honig and A. Warshel for a stimulating discussion, and J. P. Allen for critical comments.

This work was supported by the National Science Foundation (PCM 82-02811) and the National Institutes of Health (GM 13191).

Received for publication 24 May 1985 and in final form 1 July 1985.

REFERENCES


KLEINFELD ET AL. Protonation of the Primary Acceptor in Photosynthetic Reaction Centers

851

Figure 4 The normalized proton to Q_A distance, (r_{HQ}/r_{IQ}), as a function of the angle, θ, between the I− − Q_A and the H+ − Q_A axes, for different values of r_{IQ}. The curves were calculated using Eqs. 5 and 6. Inset shows the geometrical arrangement of the reactants.

Q_{A−} (9–11). This may explain why the optical spectrum of Q_{A−} is essentially unaffected by the binding of a proton.

From the value of δG, one can make a rough estimate of the location of the proton binding site relative to Q_{A−}. We assume that the interaction of the proton with both Q_{A−} and I− is electrostatic in origin, as has been done to explain the effects of point charges in other photosynthetic systems (26–29). The change in energy δG is caused by the difference in the distance of the proton to Q_{A−} (i.e., r_{HQ}) compared with the distance to I− (i.e., r_{HI}). From Coulomb's law we obtain

\[ \delta G = -\frac{e^2}{r_{HI}} - \frac{e^2}{r_{HQ}} \]

\[ = -\frac{e^2}{r_{HQ}} \left[ \frac{1}{1 + (r_{HQ}/r_{IQ})^2} - 2(r_{HQ}/r_{IQ}) \cos \theta \right] \frac{1}{(r_{HQ}/r_{IQ})} \]

where e is the electronic charge, ε is the effective dielectric constant, r_{IQ} is the distance between the I− and Q_{A−} and θ is the angle between the I− − Q_{A−} and H+ − Q_{A−} axes (see insert in Fig. 4). The distance r_{HQ} has been estimated from spectroscopic measurements to be 8–12 Å (30,31). The value of ε is difficult to estimate when one is dealing with distances on the scale of atomic dimensions (see references 32–35). Consequently, we have not assumed a specific value of ε, but have calculated distances in terms of εr_{IQ}. By equating Eqs. 5 and 6, permissible combinations of (r_{HQ}/r_{IQ}) and θ were determined for the range of 15 Å ≤ εr_{IQ} ≤ 50 Å (Fig. 4). The minimum distance of the proton to Q_{A−}.

2Although δG represents the change in energy difference between two states (Fig. 1), the shifts in the individual energies are expected to be small with respect to the optical transition energy.

3We assume that the relative distances between the reactants D^+, I−, Q_{A−} do not change upon protonation.

4It will be of interest to obtain the value of ε when the distances have been determined from crystallographic and/or electron nuclear double resonance measurements.