The Enthalpy and Entropy of Reaction for Formation of $P^+_{\text{QA}^-}$ from Excited Reaction Centers of *Rhodobacter sphaeroides*

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**Abstract:** The enthalpy and volume changes for the charge-transfer reaction between excited donor and ionized donor and acceptor in bacterial reaction centers were determined using pulsed photoacoustics. Excitation in the lowest absorption band of the centers at 860 nm minimized the thermal signal caused by degradation of excess energy. Knowing the free energy of this reaction, $-0.86$ eV, the determination of the enthalpy, $-0.44$ eV, fixes the entropy at $25°C$ as about one-half ($\Delta S = +0.42$ eV) of the free energy for the normal ubiquinone-10 containing centers. This is a larger contribution than anticipated from previous estimates of the enthalpy. The unexpected sign of the entropy is assigned to the release of counterions from the reaction center surfaces when the charge transfer cancels the dominant opposite charges at the interfaces. The enthalpy and entropy of six reaction centers containing exchanged quinones did not correlate with their free energies. The volume contractions ranged from $-28$ to $-42$ Å$^3$ and roughly correlated with the size of the quinone as expected from electrostriction.

**Introduction**

The kinetics of the electron transfer steps in bacterial reaction centers have been thoroughly investigated from femtoseconds to seconds, and thus the reaction sequence is well characterized. However, the thermodynamic properties of these intermediates are far less well-known. The sequence begins, following excitation of the donor dimer bacteriochlorophyll (P), with a rapid (3 ps) electron transfer to the bacteriopheophytin (H) in the L branch of the reaction center and is followed by a slower (200 ps) step to the primary quinone (QA). The free energies of these steps have been obtained as the separate redox potentials of the donor and acceptor. Arata and Parson have measured the free energy ($-0.86$ eV) of the excited state to donor cation acceptor anion reaction from the ratio of delayed to prompt fluorescence and the enthalpy ($-0.7$ eV) by use of the temperature dependence of the kinetics of delayed light emission. These and other measures of $\Delta H$ will be discussed in the body of this paper.

Photoacoustic (PA) methodology allows a direct measure of the enthalpy of reaction plus changes in the reaction volume in a photochemical sequence. The two contributions can be separated by measurement at the temperature of maximum density of water where only changes in volume contribute to the PA signal. Our previous measurements on reaction centers gave a volume change of $-22$ Å$^3$ for the formation of $P^+_{ qual}\text{QA}^-$. This contraction was assigned to electrostriction and was used to obtain an estimate of the effective dielectric coefficient of the protein. Measurements by several workers have reported volume changes ranging from $-12$ to $-34$ Å$^3$ and values of the enthalpy change varying from $-0.44$ to $-1.33$ eV.

Photoacoustic data for *Rhodobacter sphaeroides* reaction centers have been obtained previously using 532 nm excitation. At that wavelength, ca. 30% of the photon energy is degraded to heat instantly and adds a large background to the measurement. With the tuning ability of optical parametric oscillator technology, we can now excite the reaction centers near their trap energy, avoiding this excess heat. We set out to obtain $\Delta V$, $\Delta H$, and, using the literature value of $\Delta G^\circ$, $\Delta S^\circ$ of this reaction.

**Experimental Section**

*R. sphaeroides* reaction centers (RC’s) were isolated following standard procedures using lauryl dimethylamine oxide (LDAO) extraction and purified using ammonium sulfate and DEAE (diethylaminoethyl) chromatography. These RC’s have Q$_A$ occupancy $>95\%$ and $\sim5\%$ Q$_B$ occupancy. Solutions of OD$^{660} = 1$ in 1 cm ($\sim7 \mu$M RC) in 10 mM Tris pH 8 were deoxygenated by stirring a thin layer in

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a small flask under flowing helium for 15 min. They were then introduced anaerobically into the PA cell via syringes. Visible absorption spectra, corrected for scatter if present, were checked before and after experiments and agreed within 2%. Experiments were done in a dark room, with care to avoid exposure to light, which can cause photodegradation of the reaction centers.

Reaction centers with substituted quinones were prepared by established procedures.\textsuperscript{15,16} Solutions of RC’s contained 10 mM Tris pH 8, <0.025% LDAO, <1 mM ethylenediaminetetraacetic acid (EDTA), and one of the following: 30 μM 2,3-dimethylphthalonitrile (2,3-Me-NQ) and 0.3% ethanol; 20 μM 2-chloroanthraquinone (2-Cl-AQ) and 0.4% DMSO; 27 μM 2,3-dihydroxyanthraquinone (2,3-Me-AQ) and 0.4% dimethyl sulfoxide (DMSO); 50 μM duroquinone (DQ) and 0.3% ethanol; 50 μM ubiquinone 1 (UQ₁); and 0.3% ethanol; or 50 μM menaquinone-4 (MK₄) and 0.3% ethanol. The MK₄ is a close analogue of menaquino-4 having one isoprenyl unit and three isoprenyl units in the tail. Quantum yield was assumed to be unity for each substituted RC.

A Nd:YAG laser (Surelite II, Continuum) and optical parametric oscillator (OPO) were used to produce light of 860 nm. The idler beam of the OPO was used; a red filter at the output removed traces of visible light. The beam was conditioned by focusing through a 1 mm hole and recollimated. Neutral density filters were used to obtain the desired photon flux at the cell. The photon flux from a beam pick-off was measured with a Molecron 2000 detector and J3-09 probe following a 1.2 cm diameter iris. The pick-off was calibrated by measurement of the light incident on the cell with a J2SLP-2 probe. The pick-off value was read by the computer during data acquisition. The temperature was controlled to ±0.1 °C (Neslab RTE-5)\textsuperscript{17} and was measured using a type T thermocouple inserted in the brass cell holder via a Keithley 181 nanovoltmeter and was also read into the computer (HP 360).\textsuperscript{18}

The PA cell had a 1 mm spacer and followed the design of Arnout et al.\textsuperscript{19} The PA cell was equipped with a dielectric mirror (99% reflection for 700–950 nm, Newport). A 110 μm piezoelectric film was assembled against a stainless steel holder which was acoustically coupled to the dielectric mirror with grease. The voltage produced by the piezoelectric film was amplified by a lnthaco 1201 preamplifier, filtered to pass 3 kHz to 400 kHz, and had a gain typically of 1000. The signal was digitized with a Tektronix RTD 710 and read into the computer. The PA signal, light energy, and temperature were measured in batches of 32 signals.

The calorimetric reference, which degrades absorbed light to heat in less than the resolving time, was MontBlanc ink (old stock, West Germany). No volume change is observed with the reference compound, as evidenced by the zero intercept at 4 °C where α = 0 (α is the thermal expansion coefficient of water).\textsuperscript{20} The α of water is acceptable for use with dilute salts (here the ionic strength is ~5 mM). The reliability of MontBlanc ink as a reference was verified by comparison with CuSO₄.\textsuperscript{21} While the thermophysical properties of water are severely affected by 0.1 M CuSO₄ needed to obtain OD₆₅₀ = 1 in 1 cm, the photoacoustic signal of MontBlanc ink in 0.1 M MgSO₄ has the same amplitude and shape (±2.5%) as that of 0.1 M CuSO₄ with matching OD₆₅₀. Use of high concentrations of transition metal salts e.g., CoCl₂, has led to serious errors in PA measurements.\textsuperscript{22} For the experiments here, the MontBlanc ink was in 10 mM Tris buffer pH 8.4, 0.1% LDAO, OD₆₅₀ matching the RC solution. The PA signal for this reference yielded a linear response versus photon flux over the same range as used in the RC, over the time range of interest, the PA signal contains only fast components (<100 ns), i.e., it gives the same time response as the reference signal:

\[
PA_{\text{ref}} = \frac{nF \alpha E_{\text{n}}}{\kappa} f(t) \quad n = E_{\text{0}}(1 - 10^{-3}) \quad (1)
\]

where \(E_{\text{0}}\) is photon flux, A is the absorbance per 2 mm of solution (since the light passes through the 1 mm cell a second time after being reflected by the mirror), \(n\) is photons absorbed, \(F\) = piezo film sensitivity, \(\alpha\) = thermal expansivity/heat capacity × density, \(\kappa\) = compressibility, and \(f(t)\) = impulse response. In the RC, over the time range of interest, the PA signal contains only fast components (<100 ns), i.e., it gives the same time response as the reference signal:

\[
PA_{\text{RC}} = \frac{nF \alpha E_{\text{n}}}{\kappa} f(t) \left[\alpha'Q_{\text{RC}} + \Delta V\right] \quad (2)
\]

where \(Q_{\text{RC}}\) includes the enthalpy change and other rapidly released heat (see Scheme 1), and \(\Delta V\) is the volume change. The first term in the parentheses of eq 2, due to the thermal signal, disappears at the temperature of maximum density, \(T_m\), near 4 °C, leaving the second (volume) term.

The volume is obtained by conversion of energy to volume by \(\alpha\) at 25 °C and correcting for the change in compressibility of water between 4 and 25 °C.

\[
\Delta V = \frac{P_{\text{f}}}{P_{\text{ref}}} \kappa^{25} (14.2 \text{ Å}^3) \quad (3)
\]

where 14.2 Å³ is the volume change, via \(\alpha\), at 25 °C for each 860 nm photon absorbed. In eq 3, \(\kappa^{25}\) and \(\kappa^{25}\) are the compressibility of water at the temperature of maximum density (in dilute Tris, \(\alpha = 0 \text{ at } 4 \text{ °C}\)) and at 25 °C. Assuming \(\alpha\) is constant, this term, in units of energy, can be subtracted from the signal at 25 °C to obtain the thermal signal at that temperature (eq 4). By normalizing the \(P_{\text{RC}}\) signal to that found for the reference signal, the thermal term is obtained directly in units of the photon energy \(E_{\text{0}}\), and can be scaled to molar quantities since the solutions are dilute. A better way to separate the thermal and volume terms is to use the ratio of the slopes d(PA/\(E_{\text{0}}\))/dt for RC and for the reference compound over the range of 1–25 °C (eq 5).
The slope of the plot yields $Q_{RC}$ and the intercept $\Delta V$. This method uses all the available data, enhancing the signal-to-noise ratio and averaging any variation in the parameters over the temperature range. No signal was observed when the light pulse was blocked; therefore dark subtractions were not used. Water in the cell yielded a signal, PA$_{w}$, whose magnitude was $\sim$0.15 that of the reference signal at an absorbency of 0.1 in 1 mm at 860 nm at the same temperature. This is in agreement with the weak absorption by water at this wavelength. All reported data were corrected by subtracting the slope of $k$-PA$_{w}$ versus $\alpha$ from the sample or reference slopes. The quoted errors are the sum of the standard deviations of the components of eq 3 or 5. Signals were collected in batches of 32, with up to 512 averages for absorbency of 0.1 in 1 mm at 860 nm at the same temperature. This is an average of the results of three analyses from further consideration.

If the sample is normalized to the reference at the same temperature, both the $P$ and $x$ terms cancel and the $\Delta V$ and $\Delta H$ are directly obtained from a plot of the normalized $PA_{RC}$ signal versus $\alpha$. However, the error increases greatly at lower temperatures as $\alpha$ approaches zero and the thermal component of the signal decreases accordingly. We have analyzed the data this way and found similar ($\pm$5%) values of $\Delta V$, but the values of $\Delta H$ are about 0.1 eV more negative than the values given by eqs 4 and 5 in four of the seven cases. Since the reference data were of insufficient S/N close to $T_m$, we have omitted the results of these analyses from further consideration.

Tris, 10 mM, pH 8.4, was used as buffer. It has a small change of pH with temperature, $\Delta p$H/$\Delta T = -0.028$ K$^{-1}$,$^{21}$ amounting to 0.6 pH unit over the 21 $\degree$C temperature change. This has no significant effect on the quantum yield or kinetics of the reaction.

Pulse energies of up to 200 $\mu$J cm$^{-2}$ at the 860 nm band ($E_{860} = 1.28 \times 10^3$ M$^{-1}$ cm$^{-1}$, $\sigma_{860} = 4.86$ Å$^2$) could be used with negligible (0.5%) excitation of the oxidized center, assuming Poisson target theory.$^{23}$ A pulse frequency of 1 Hz was used because of the slow ($\sim$100 ms) 10 (UQ$_{10}$) recharge recombination time.

Correction of measured $\Delta H$ and $\Delta V$ must be made to account for the ca. 5% (determined by flash photolysis) of reaction centers lacking $Q_a$ or containing $Q_b$. The corrections are $-0.03$ eV for $\Delta H$ and $-1.5$ Å$^2$ for $\Delta V$; they are at the experimental error.

The pulse saturation curve was determined at 4 $\degree$C to avoid contribution to the thermal signal by residual (12%)$^{22}$ absorption of oxidized RC, P$^+$, at 860 nm. Single shots were used at the higher photon fluxes because repetitive shots caused bleaching of the reaction centers requiring minutes of recovery time. The PA signals were fit to the equation $\Delta V = \Delta V_0(1 - e^{-\alpha t})$ by nonlinear least squares.

Since the air microphone photoacoustic signal is sensitive to thermal changes alone, it was used to verify the values of $\Delta H$. A differential microphone situated equidistant between two identical cells$^{24}$ was used to obtain $\Delta H$ for the RC containing UQ$_{10}$. A 100 $\mu$m layer of solution of RC in the thermostated cell and light of 600, 734, and 828 nm were used (the OD at each of these three wavelengths was 0.5 in 1 cm). The energy difference between these excitation wavelengths and the trap energy is rapidly degraded to heat and thus serves as an internal energy impulse response. Signals were normalized to equal number of photons and subtracted in pairs. The resulting difference signals were scaled to

\[
\Delta H = (E_{860} - E_{860}) - \left(\frac{P_{860}}{E_{860}}\right) (1.44 \text{ eV})
\]

\[
\Delta V = (E_{860} - E_{860}) - \left(\frac{d(PA\alpha)}{d\alpha}\right) (1.44 \text{ eV})
\]

(4)

(5)

Figure 1. Photoacoustic spectra for reaction centers containing 2,3-Me-NQ and MontBlanc ink calorimetric reference: OD$^{100}$ = 1 per cm, 55 $\mu$m cm$^{-1}$, 1 Hz pulse frequency, 128 averages, 25 $\degree$C, gain 500; (dotted line) MontBlanc reference; (heavy solid line) reaction center; (light solid line) fit by convolution (fast fraction $\sim$0.84, $\tau = 0.04$ ms, amplitude $\times 0.01$); The large negative signal from the volume contraction conceals the smaller positive signal from the enthalpy.

the photon interval energy and used as the impulse response and calibration for analysis at each wavelength.

Results

Shown in Figure 1 are typical photoacoustic spectra for 2,3-MeNQ containing reaction centers (RC) and MontBlanc ink (MB) at 25 $\degree$C along with a fit by convolution. No component of time constant $>0.05$ ms and amplitude $>5\%$ was detected. The striking negative signal from the RC at room temperature is evident.

The pulse saturation curve obtained at 4 $\degree$C using 860 nm light, shown in Figure 2A, was fit with a cross section $\sigma = 1.7 \pm 0.4$ Å$^2$ assuming $\phi = 1$ and a volume change $\Delta V = -25.3 \pm 2.2$ Å$^3$. Shown in Figure 2B is the pulse saturation curve at 4 $\degree$C using 788 nm photons; an isobestic point exists at this wavelength, such that absorbency remains the same during saturation, as opposed to 860 nm where P absorbs much more than P$^+$. The pulse saturation curve at 788 nm was fit with $\sigma = 1.9 \pm 0.3$ Å$^2$ (\(\phi = 1\)) and $\Delta V = -27.5 \pm 1.3$ Å$^3$. Calculated $\sigma$ is 4.9 Å$^2$ at 860 nm$^{22}$ and 5.5 Å$^2$ at 788 nm. The poor fit to the shape of the curve, particularly at 860 nm, is probably the result of inhomogeneous light distribution. This has a larger effect on the estimated cross section than on the $\Delta V$ at saturation. The results clearly show that the measured $\Delta V$ is independent of multiple excitations of charge-separated centers, which are 10-fold more prevalent at 788 nm than at 860 nm.

Shown in Figure 3 is PA$\alpha$ vs $\alpha$ for RC and MB. The data yield a good fit to a straight line with slopes of standard deviation $\pm$0.01 eV. The slopes (eq 5) were used to obtain $\Delta H$, and the intercept (eq 3) of the RC plot was used to obtain $\Delta V$. Since this is a linear system, one can rescale the scale variable to per molecule or per mole.

The results clearly show that the measured $\Delta V$ is independent of other values from the literature. The very first preparation gave low values of $\Delta V$, but similar values of $\Delta H$ as later preparations, for unknown reasons. We believe that $-28$ Å$^3$ is the best value for the volume change because two preparations produced this value and it agrees with those in refs 12 and 13.

The values of $\Delta H$ are presented in Table 2 along with literature values. The energy of the 860 nm photon, 1.44 eV, is 0.06 eV greater than the 0$\rightarrow$0 transition energy, 1.38 eV, Scheme 1, and the measured heat is so corrected (eq 5). Heat emitted


observed for the quinone-substituted RC's, even larger than the 

\[ \frac{\text{formation of P}^+}{\text{charge formation}} \]

Figure 2.

The observation that heat and volume changes are measured as fast responses (\( \tau < 100 \) ns) agrees with the known time constant of 200 ps for the formation of the charge separated species \( \text{P}^+\text{Q}_A^- \).

**Volume Changes.** The volume change obtained for RC's containing \( \text{UQ}_{10} \) from the pulse saturation curve, \( \Delta V = -28 \text{ Å}^3 \) (Figure 2), agrees with that obtained from measurements exciting only 10% of the RC's, \(-28 \text{ Å}^3 \) at 4 °C (Table 1). These methods use completely different calculations to obtain the volume change. The value, \(-22 \text{ Å}^3 \), obtained in our previous measurements with 532 nm excitation and a different photoacoustic setup, is less accurate. However, our value disagrees with that of \(-12 \text{ Å}^3 \) obtained by Malkin et al.\(^{11,12} \) and closer to \(-32 \text{ Å}^3 \) of Puchenkov et al.\(^{12} \) and \(-35 \text{ Å}^3 \) of Arata and Parson.\(^{13} \) These discrepancies might be caused by several factors. Malkin et al. did not correct for the change of compressibility of water between 4 and 25 °C. In addition, they used CuCl\(_2\) as the reference; for OD = 0.2 per cm at 590 nm, this requires \(~200 \text{ mM CuCl}_2\), which will substantially change the thermophysical properties from those of pure water (see Experimental Section). The value \(-32 \text{ Å}^3 \) was obtained using CoCl\(_2\), known to yield a nonlinear PA response.\(^{19} \) The value \(-35 \text{ Å}^3 \) was obtained using bromocresol purple, which is believed to be an adequate reference.\(^{20} \)

The skewed shape of the pulse saturation curves (Figure 2A,B) probably arises from inhomogeneous light distribution across the illuminated area. This has the greatest effect on \( \sigma \), while having little or no effect on the volume change obtained by fitting the saturated portion of the function.

**Enthalpy Change.** There is significant variability in the reported values of \( \Delta H \) for the \( \text{P}^+\text{Q}_A^- \) reaction (Table 2). Our values are the lowest at \(-0.44 \text{ eV} \). Some older work has been carried out with excess fluorescence at 41-13 or at a repetition rate that did not allow sufficient time for the centers to recover.\(^{11,12} \) Either of these will yield unproductive multiplets excitation which will cause more heat to be emitted. The reference compounds may have been inadequate.\(^{19,25} \) In addition, all previous measurements excited the RC's in the 500–600 nm region. This requires a large correction for the rapid heat loss to attain the 1.4 eV excited state. In contrast, the work reported here used direct excitation into this state. Absorption by water at this near-infrared wavelength is significant, but is easily corrected.

There are also significant differences between the \( \Delta H \) obtained by the PA method and that obtained by measurements of delayed fluorescence. The latter method assumes equilibrium between a single charge-separated state \( \text{P}^+\text{Q}^- \) and the excited P state on the 100 ns time scale while the PA method measures, in the present case, the heat released on the <100 ns time scale. There could be a relaxation in the charge-separated state in the time interval between these measurements such as observed on freezing preparations in the dark and in the light.\(^{30} \) An excellent summary of the evidence for such relaxations of the protein


**Table 1.** $\Delta V$ for Formation of $P^+Q_A^-$ from $PQ_A$ in $R. Sphaeroides$ Reaction Centers Containing $UQ_{10}$

<table>
<thead>
<tr>
<th>$\Delta V/\text{Å}^3$</th>
<th>$\lambda_{e\ell}$ nm</th>
<th>Energy/µJ cm$^{-2}$</th>
<th>Technique</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-18$</td>
<td>860</td>
<td>180</td>
<td>PA</td>
<td>this work$^a$</td>
</tr>
<tr>
<td>$-28 \pm 1$</td>
<td>860</td>
<td>40</td>
<td>PA</td>
<td>this work$^b$</td>
</tr>
<tr>
<td>$-25 \pm 2$</td>
<td>860</td>
<td>pulse saturation</td>
<td>PA</td>
<td>this work$^b$</td>
</tr>
<tr>
<td>$-28 \pm 1$</td>
<td>860</td>
<td>pulse saturation</td>
<td>PA</td>
<td>this work$^b$</td>
</tr>
<tr>
<td>$-12 \pm 2$</td>
<td>590</td>
<td>500; and pulse saturation</td>
<td>PA</td>
<td>this work$^b$</td>
</tr>
<tr>
<td>$-32 \pm 1$</td>
<td>532</td>
<td>$\sim$500</td>
<td>PA</td>
<td>11</td>
</tr>
<tr>
<td>$-35 \pm 2$</td>
<td>588$^b$</td>
<td>1000</td>
<td>capacitor microphone</td>
<td>13</td>
</tr>
<tr>
<td>$-22 \pm 2$</td>
<td>532$^b$</td>
<td>200</td>
<td>PA</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ Based on 14.2 Å$^3$ per 860 nm photon. Obtained from amplitude analysis of data. Conditions were 10 mM Tris pH 8.4, OD$^{560} = 1$ per cm. $^b$ 10 mM Tris pH 8.0, 0.1% LDAO, OD$^{588} = 1$ per cm. $^c$ 3.35 µM RC, 10 mM Tris pH 8.0, 0.01% LDAO. OD$^{532} = 0.34$ per cm. Corrected for change in compressibility of water between 4 and 25 °C.

**Table 2.** $\Delta H$ of Formation of $P^+Q_A^-$ from $PQ_A$ in $R. Sphaeroides$ Reaction Centers Containing $UQ_{10}$

<table>
<thead>
<tr>
<th>$\Delta H/\text{eV}$</th>
<th>$\lambda_{e\ell}$/nm</th>
<th>Energy/µJ cm$^{-2}$</th>
<th>Technique</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>$-0.44 \pm 0.03$</td>
<td>860</td>
<td>40–185</td>
<td>PA</td>
<td>this work$^b$</td>
</tr>
<tr>
<td>$-0.34 \pm 0.02$</td>
<td>600, 734, 828</td>
<td>35</td>
<td>air microphone, internal reference</td>
<td>this work$^b$</td>
</tr>
<tr>
<td>$-0.44^c$</td>
<td>532</td>
<td>200</td>
<td>PA</td>
<td>10</td>
</tr>
<tr>
<td>$-1.33^c$</td>
<td>588</td>
<td>1000</td>
<td>capacitor microphone</td>
<td>13</td>
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<td>$-0.75 \pm 0.01$</td>
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<td>500</td>
<td>delayed fluorescence</td>
<td>8</td>
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<td>$-0.55$</td>
<td>590</td>
<td>$\sim$500</td>
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<td>11</td>
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<tr>
<td>$-0.82 \pm 0.04$</td>
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<td>12</td>
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<tr>
<td>$-0.76 \pm 0.1^d$</td>
<td>590</td>
<td>100</td>
<td>PA</td>
<td>25</td>
</tr>
</tbody>
</table>

$^c$ Based on 1.44 eV per 860 nm photon and $0–0$ transition = 1.38 eV. Conditions, unless otherwise noted, were 10 mM Tris pH 8.4, OD$^{560} = 1$ per cm. $^d$ 3.35 µM RC, 10 mM Tris pH 8.0, 0.01% LDAO. OD$^{532} = 0.34$ per cm, 128 pulses. Corrected for change in compressibility of water between 4 and 25 °C. $^e$ 10 mM Tris pH 8.0, 0.1% LDAO, OD$^{588} = 1$ per cm. $^f$ *Rhodopseudomonas rubrum* was used.

**Table 3.** $\Delta G^e$ in Formation of $P^+Q_A^-$ from $PQ_A$ ($G+Q_A^- \rightarrow G_{\text{pQ}_A}$) in $R. Sphaeroides$ Reaction Center

<table>
<thead>
<tr>
<th>$\Delta G^e$/eV</th>
<th>Technique</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-0.79$</td>
<td>redox potentials</td>
<td>13</td>
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<tr>
<td>$-0.86 \pm 0.02$</td>
<td>fast vs delayed fluorescence</td>
<td>8</td>
</tr>
<tr>
<td>$-0.87 \pm 0.01$</td>
<td>direct voltammetry (films)</td>
<td>7</td>
</tr>
<tr>
<td>$-0.85$</td>
<td>redox titration</td>
<td>5</td>
</tr>
<tr>
<td>$-0.87$</td>
<td>redox titration</td>
<td>6</td>
</tr>
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</table>

**Table 4.** Thermodynamic Parameters of the Reaction $PQ_A$ to $P^+Q_A^-$ at 25 °C

<table>
<thead>
<tr>
<th>$\Delta V/\text{Å}^3$</th>
<th>$\Delta H/\text{eV}$</th>
<th>$\Delta S$ (in situ)/eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$UQ_{10}$</td>
<td>$-28 \pm 1$</td>
<td>$-0.44 \pm 0.06$</td>
</tr>
<tr>
<td>$UQ_{10}$, 0.2M NaCl</td>
<td>$-30 \pm 1$</td>
<td>$-0.51 \pm 0.06$</td>
</tr>
<tr>
<td>$UQ_{10}$</td>
<td>$-42 \pm 4$</td>
<td>$-0.63 \pm 0.1$</td>
</tr>
<tr>
<td>duroquinone, 0–15 °C</td>
<td>$-40 \pm 2$</td>
<td>$-0.9 \pm 0.2$</td>
</tr>
<tr>
<td>2.3-Me-NQ</td>
<td>$-39 \pm 2$</td>
<td>$-0.34 \pm 0.08$</td>
</tr>
<tr>
<td>MK$_4$</td>
<td>$-38 \pm 1$</td>
<td>$-0.39 \pm 0.06$</td>
</tr>
<tr>
<td>2-Cl-AQ</td>
<td>$-31 \pm 1$</td>
<td>$-0.48 \pm 0.06$</td>
</tr>
<tr>
<td>2,3-Me-AQ</td>
<td>$-29 \pm 1$</td>
<td>$-0.36 \pm 0.04$</td>
</tr>
</tbody>
</table>

The $\Delta G^e$ is the trap energy assumed to be 1.38 eV. The errors quoted for $\Delta H$ are the sum of the standard deviations of the individual contributions to the calculation and were doubled to cover the possible systematic errors. As noted in the text, uncertainty in the value of $k/e$ for the RC protein could maximally change the magnitude of $\Delta V$ by $\pm 10\%$ and increase (decrease) $\Delta H$ thus decreasing (increasing) $\Delta T\delta$ by roughly 0.2 eV.

Following charge transfer and a general model for these effects has been published.$^{31}$ The analysis indicates that over 80% of the relaxation would occur in $<10$ ns in water at 300 K. This agrees with our observation of no $\Delta H$ or $\Delta V$ contribution of amplitude $>$5% in our time window of $\approx$50 ns to 3 µs (Figure 1 and ref 10). However, slower processes such as proton uptake near $Q_A^-$ and release near $P^+$ which occur on the 100 µs time scale$^{25}$ could affect the measurement of delayed fluorescence.


$^{32}$ Maroti, P.; Wright, C. A. Biophys. J. 1997, 73, 367–381.


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A problem may arise with the first-order treatment of the data where \( \Delta V \) is assumed to be independent of temperature. This assumption is used to correct the measured amplitudes at a temperature where \( \alpha \) and thus the measured heat are finite. If \( \Delta H \) is a function of temperature \( (T) \) and/or if \( \Delta V \) is a strong function of \( T \), we will see this by curvature in our \( \Delta V \), \( \alpha \) (T)-corrected plots of the PA amplitudes versus \( \alpha \). This is not observed (±2%). If, however, \( \Delta V \) is a linear function of \( \alpha \) (not \( T \)), it will fall onto the “\( \Delta H \) observed” line and thus cause an error in the assumed \( \Delta H \). This could occur if all of the volume contraction is caused by electrostriction and its temperature dependence follows that of \( \alpha \), not \( T \), which differ by ~20% over the 25 deg range. Both \( \kappa \) and \( \epsilon \) decrease about 10% over the 4–20 °C interval in the abnormal solvent water. Thus if estimated by the Drude–Nernst equation,

\[
\partial \Delta G_{el}/\partial P = \Delta V_{el} = (\epsilon^2 \kappa/2\epsilon)(\partial \ln \epsilon/\partial \ln V)
\]

where \( \Delta G_{el} \) is the Born charging energy, \( \Delta V_{el} \) (electrostriction) will not be temperature dependent. Here \( \kappa^2 \epsilon^2 \) is the charge on the sphere of radius \( \epsilon \). However, for most substances \( \kappa \) increases with temperature while \( \epsilon \) decreases, thus \( \Delta V_{el} \) will increase with increasing temperature, causing an error. Unfortunately, \( \epsilon \) and \( \kappa \) are only poorly known for proteins and their temperature derivatives are essentially unknown. The little data on change of compressibility with temperature are unreliable.

The compressibilities of many materials, including nonprotonic solvents and polymers, although different in their absolute values, increase by about 10% over a 20 °C range. The determination of compressibility of protein solutions by ultrasound velocity measures mostly hydration and relaxations, not the protein itself. However, Chalikian et al. claim to have separated the hydration and the intrinsic compressibilities of some 15 globular proteins and find that the intrinsic compressibility increases only 2% over a 20 °C range. Because the volume contraction can be twice the thermal effect at 25 °C, a 10% increase in amplitude of \( \Delta V \) would cause \( \Delta H \) calculated by the difference at only two temperatures (4 and 25 °C) to be underestimated by about 0.3 eV. However, since our slope analysis averages over this range and the increase in proteins may be smaller, the possible error is more likely to be 0.1 eV. Given the uncertainty of how \( \epsilon \) and \( \kappa \) will change with temperature, it is possible that the effect in proteins could even be in the opposite direction. A study of the same reaction in heavy water where the magic temperature is near 11 °C would allow a direct determination of the temperature variation of \( \Delta V \). Our preliminary analysis of data from the reaction of triplet ZnUP and ferricyanide in D2O also shows only a small effect as expected. As this 7 °C temperature change is one-third of the temperature difference between 4 and 25 °C, we assign an uncertainty of less than ±10% to the measured \( \Delta H \), given the ±2% linearity of our plots.

In the above we have assumed that \( \Delta H \) is independent of temperature. For complex systems this is not necessarily so. Apparent discrepancies between calorimetric enthalpies, such as determined by the PA method, and those derived from the variation of equilibrium constants with temperature, such as those determined by delayed fluorescence measurements, have been observed in protein systems. These can be caused by variations in the heat capacity and thus the enthalpy of these reactions.

**Entropy Change.** Taking the value of \( \Delta G \) for the native RC’s to be $-0.86 \text{ eV}$ for the production of P+Q− from P0Q0 and assuming a temperature independent \( \Delta V \), it is found (Table 4) to be half enthalpic $(0.44 \text{ eV})$ and half entropic $(T \Delta S = +0.42 \text{ eV})$. This is significant, as the entropic change is usually assumed to be zero in the interpretation of the free energy dependence of electron transfer rates. Thus standard formulations of Marcus theory assume that the vibrations coupled to electron transfer have the same frequency in reactant and product states, which implies that \( \Delta S \) is zero. Also treatments of the temperature dependence of the rates often assume that \( \Delta G \) is independent of temperature, again assuming that \( \Delta S \) is zero.

The sign of \( T \Delta S \) is also surprising since charge separation, which is accompanied by electrostriction around the new charge centers, is an ordering process for solvent dipoles for which one would expect $T \Delta S < 0$. Negative values for \( T \Delta S \) are indeed found for electron transfer from triplet Zn uroporphyrin to naphthoquinone sulfonate in water, $T \Delta S = -0.6 \text{ eV}$. In a protein, however, dipoles cannot freely reorient in the newly formed electric fields of cation and anion. Thus the orientational entropy may be considerably reduced. In thermodynamic terms,

\[
\Delta S_{el} = -\partial \Delta G_{el}/\partial T = \frac{\epsilon^2}{2 \epsilon e} \frac{\partial \ln \epsilon}{\partial T}
\]

where \( \Delta G_{el} \) is the Born charging energy of the dielectric, \( r \) is the radius of the ion and \( \epsilon \) is the dielectric coefficient. If \( \partial \ln \epsilon/\partial T \) is negative for practically all liquids since they expand on heating and \( \epsilon \) decreases with decreasing density. Again water at <4 °C is abnormal. Equation 7 qualitatively predicts the negative entropy usually observed. The entropic change measu...
Formation of \( P^+QA^- \) from Rhodobacter sphaeroides

\( \Delta H \) and \( \Delta S \) in RC’s with Different Quinones as QA’s. The free energy of the charge separation reaction can be changed by removing the native UQ\(_{10}\) and replacing it with a variety of other quinones.\(^{(26,27)}\) The various quinones used in these experiments have a range of reduction potentials. Both the midpoint potentials in solution (DMF) and those in situ are known (Table 5). \( \Delta V \) was determined with RC’s containing six different replacement quinones (Table 4). The two benzoquinones have \( \Delta V \) of \( \sim 40 \) \( \AA \), the two naphthoquinones, of \( \sim 38 \) \( \AA \), and the two anthraquinones, of \( \sim 28 \) \( \AA \). The \( \Delta V \) seems to vary with the number of rings and thus with the size of the quinone, as expected from the Drude–Nernst equation (eq 6). The size of the “tail” seems to have little effect. UQ\(_1\) with one isoprenoid unit and MK\(_4\) with four units have \( \Delta V \)’s that are similar to their tailless analogues DQ and 2,3-Me-NQ. However, the native UQ\(_{10}\) has a \( \Delta V \) similar to those of the largest quinones and most likely fills the binding site. The smaller, looser fitting quinones may increase the local compressibility, \( \kappa \), causing a larger \( \Delta V_{el} \). Plots of \( \Delta H \) versus \( \Delta V \) or \( \Delta G \) or of \( T\Delta S \) versus \( \Delta V \) or \( \Delta G \) show no definite trends.

The RC-containing duroquinone is the only one with a negative, albeit small, \( T\Delta S \) (Table 4). It is the smallest and the most symmetrical of the quinones and may be rotating in the QA pocket. On ionization, in addition to the electrostriction, such motion would be stopped and a further increase of negentropy would occur. However the \( \kappa PA \) versus \( \alpha \) plot was curved in this case and the \( \Delta H \) was calculated from the \( 4–15 \) \( ^\circ \)C data only.

Thus at this time there is no obvious correlation between the measured thermodynamic parameters and the free energy of reaction. Similar lack of correlation has been found in RC’s\(^{(52)}\) and in other protein systems.\(^{(53,54)}\) Future work is needed to resolve the underlying causes.

\textbf{Conclusion}

Photacoustic measurements have been used to determine the change in volume and enthalpy upon photoinduced charge separation in normal and quinone-substituted reaction centers. Combining the latter values with the free energy of the reaction, significant positive entropy of reaction is found.

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