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# ENTHALPY AND VOLUME CHANGES ACCOMPANYING ELECTRON TRANSFER FROM P-870 TO QUINONES IN *RHODOPSEUDOMONAS SPHAEROIDES* REACTION CENTERS

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**A capacitor microphone was used to measure the enthalpy and volume changes that accompany the electron transfer reactions, $PQ_A \xrightarrow{h\nu} P^+Q_A^-$ and $PQ_AQ_B \xrightarrow{h\nu} P^+Q_AQ_B^-$, following flash excitation of photosynthetic reaction centers isolated from *Rhodopseudomonas sphaeroides*. P is a bacteriochlorophyll dimer (P-870), and $Q_A$ and $Q_B$ are ubiquinones. In reaction centers containing only $Q_A$, the enthalpy of $P^+Q_A^-$ is very close to that of the $PQ_A$ ground state ($\Delta H_r = 0.05 \pm 0.03$ eV). The free energy of about 0.65 eV that is captured in the photochemical reaction evidently takes the form of a substantial entropy decrease. In contrast, the formation of $P^+Q_AQ_B^-$ in reaction centers containing both quinones has a $\Delta H_r$ of $0.32 \pm 0.02$ eV. The entropy change must be near zero in this case. In the presence of *o*-phenanthroline, which blocks electron transfer between $Q_A^-$ and $Q_B$, $\Delta H_r$ for forming $P^+Q_A^-Q_B$ is $0.13 \pm 0.03$ eV. The influence of flash-induced proton uptake on the results was investigated, and the $\Delta H_r$ values given above were measured under conditions that minimized this influence. Although the reductions of $Q_A$ and $Q_B$ involve very different changes in enthalpy and entropy, both reactions are accompanied by a similar volume decrease of about 20 ml/mol. The contraction probably reflects electrostriction caused by the charges on $P^+$ and $Q_A^-$ or $Q_B^-$.**

## Introduction

The primary photochemical reaction in bacterial photosynthsis is electron transfer from a bacteriochlorophyll complex (P-870 or P) to an initial acceptor complex (I), and then to a quinone ($Q_A$). From $Q_A^-$, an electron passes to a second quinone ($Q_B$), while $P^+$ oxidizes a *c*-type cytochrome [1–3]. Thermodynamic properties of these electron-transfer processes have been studied by several methods.

The midpoint redox potentials ($E_m$) of the photosynthetic electron carriers have been measured by monitoring photochemical activity as a function of the ambient redox potential. P has an $E_m$ value of approx. +480 mV [1,4]. It does not depend strongly on pH [5,6]. The apparent $E_m$ of $Q_A$ ranges from −50 to −100 mV at pH 7, depending on bacterial species (see Ref. 1 for tabulation). In chromatophores, the $E_m$ of $Q_A$ depends on pH, as though the reduced quinone can bind a proton. Since electron transfer from $Q_A^-$ to $Q_B$ appears to be faster than proton uptake, the effective $E_m$ of $Q_A$ is probably that for forming the anionic semiquinone, about −180 mV [7,8]. This means that the free energy of the $P^+Q_A^-$ radical pair is approx. 0.65 eV above that of the $PQ_A$ ground state. The $E_m$ of $Q_A$ does not depend on pH in isolated reaction centers [9,10]. The reason for the different behavior in chromatophores and isolated raction centers is not clear. The $E_m$ of $Q_B$ has been measured in chromatophores of *Chromatium vinosum* [5] and of *Rhodopseudomonas viridis* [11]. Case and Parson [5] used a double-flash technique and ob-

Abbreviation: Aces, *N*-(2-acetamido)-2-aminomethanesulfonic acid.

tained $E_m$ values of +80 or −90 mV at pH 7.7, depending on the experimental conditions. Rutherford and Evans [11] titrated an EPR signal that probably represents the $Q_B^-$ semiquinone anion [12]. They obtained $E_m$ values of 67 and −15 mV at pH 8.0 and 10.0, respectively, for single reduction, and of −155 mV for double reduction at pH 10.0. Kinetic studies on isolated reaction centers suggest that the $E_m$ of $Q_B$ is about 80 mV more positive than that of $Q_A$ [13].

It is of interest to resolve the free energy difference between $P^+Q_A^-$ and $PQ_A$ into underlying changes in enthalpy and entropy. This is particularly pertinent to theoretical work on the rates of electron-transfer reactions, because most of the theory has considered only differences in energy, not free energy [3]. From measurements of the temperature dependence of the $E_m$ values, Case and Parson [5] estimated the enthalpy changes for reduction or oxidation of P, $Q_A$, $Q_B$ and cytochrome *c* in *C. vinosum* chromatophores. They concluded that most free energy stored in the $P^+Q_A^-$ and $P^+Q_B^-$ states takes the form of an entropy decrease. The enthalpies of these states appeared to be close to that of the ground state (PQ). Carithers and Parson [14] measured the temperature dependence of delayed fluorescence from *Rps. viridis* chromatophores in the presence of *o*-phenanthroline. An Arrhenius plot of the intensity of delayed fluorescence indicated that $P^+Q_A^-$ lay approx. 12 kcal/mol (0.54 eV) lower in enthalpy than the excited singlet state, $P^*Q$. Taking the excitation enthalpy of $P^*$ in *Rps. viridis* as 1.26 eV, the enthalpy of $P^+Q_A^-$ can be calculated to be approx. 0.7 eV above the ground state. This value does not agree with the potentiometric titrations that were done in *C. vinosum*. From similar experiments at low temperature, Fleischman [15] reported an even higher value for the energy of $P^+Q_A^-$.

Measurement of heat released or absorbed during a reaction is the most direct method to determine the enthalpy change in the reaction. However, few calorimetric experiments have been done with photsynthetic systems. Callis et al. [16] used a capacitor microphone transducer to measure the volume change accompaning the primary photochemical process in chromatophores from *C. vinosum*. The amount of heat released or absorbed can be calculated from the volume change due to thermal expansion or contraction of the medium. Callis et al. [16] concluded that light-driven electron transfer from cytochrome *c*-555 to $Q_B$ does not cause a significant enthalpy change. Most of the free energy stored in the product of the photochemical reaction appeared to be a negative entropy. This agreed with the results from the temperature dependence of $E_m$ [5]. Ort and Parson [17–19] have made similar calorimetric measurements in purple membrane fragments of *Halobacterium halobium*.

In view of the discrepancies between the potentiometric measurements and the studies of delayed fluorescence, there is a need for further studies using different techniques and a variety of photosynthetic samples. One possible source of different results from different methods is interactions between the electron donor and acceptor [3,5]. Interactions between $P^+$ and $Q_A^-$, for example, cannot be studied by potentiometric titrations because $Q_A$ is titrated only with P reduced and P is titrated only with $Q_A$ oxidized; neither of these titrations gives the state $P^+Q_A^-$. Another limitation of chemical titrations is that redox components must be allowed to equilibrate with the medium. Transient states formed during the photochemical process might be different from the states in the final equilibrium.

The present paper describes measurements of the enthalpies of the $P^+Q_A^-$ and $P^+Q_B^-$ staes in isolated reaction centers, using an improved model of the capacitor microphone.

## Materials and Methods

Reaction centers of *Rhodopseudomonas sphaeroides* strain R-26 were prepared essentially as described by Clayton and Wang [20]. For the experiments on reaction centers with two quinones, excess ubiquinone-10 (about 30 mol per equivalent of reaction centers) was added in ethanolic solution to suspensions of reaction centers in 10 mM Tris-HCl or phosphate, pH 8, and 0.1% lauryldimethylamine oxide. After incubation for 1–2 h at room temperature followed by 12–20 h at 4°C, the unbound quinone was removed from the reaction centers by DEAE-cellulose chromatography. For preparation of reaction centers with one quinone, reaction centers (in 10 mM Tris-HCl buffer) were incubated with 1% lauryldimethylamine oxide and 1 mM *o*-phenanthro-

line for 3–6 h at room temperature [21] and purified again by DEAE-cellulose chromatography.

Volume change measurements were made with the capacitor microphore described elsewhere [17,22]. Samples were illuminated by 588 nm, 0.5 $\mu$s flashes from a rhodamine-6-G dye laser. The intensity of the flash was attenuated using neutral density filters. To get a good signal-to-noise ratio, the illumination flashes were repeated at about 15-s intervals, and 16 signals were averaged.

**Data Analysis and Calibration**

A volume change due to a photochemical reaction can arise in two ways [16–19]. First, there may be a volume difference, $\Delta v_r$, between reactants and products. Second, the solution may expand or contract through heating or cooling. The heat absorbed or released is equal to the enthalpy change of the reaction, $\Delta H_r$. Thus, the volume change after flash excitation is:

$$\Delta V = (n_e E_e - n_r \Delta H_r)\alpha/\rho C + n_r \Delta v_r \quad (1a)$$

or

$$\Delta v \equiv \Delta V/n_e = (E_e - \phi \Delta H_r)\alpha/\rho C + \phi \Delta v_r \quad (1b)$$

where $n_e$ is the number of einsteins of photons absorbed, $n_r$ is the number of moles of product generated, $\phi$ ($= n_r/n_e$) is the quantum yield of the photochemical reaction, $E_e$ is the energy of the photon (per einstein), $\Delta H_r$ is the (partial molar) enthalpy of the products relative to the reactants, $\Delta v_r$ is the difference in molar volume between reactants and products, $\alpha$ is the thermal expansion coefficient of the solution, $C$ is the heat capacity of the solution at constant pressure, and $\rho$ is the density of the solution. The quantum yield in bacterial photosynthesis is essentially unity if the intensity of the exciting light is low enough [23].

If the photochemically active sample is replaced by a solution of an inert, non-fluorescent absorber, all of the energy of the absorbed photons is converted to heat. The volume change $\Delta V_{h\nu}$ then becomes:

$$\Delta V_{h\nu} = n_e E_e \alpha/\rho C \quad (2a)$$

or

$$\Delta v_{h\nu} \equiv \Delta V_{h\nu}/n_e = E_e \alpha/\rho C \quad (2b)$$

The difference between $\Delta v$ and $\Delta v_{h\nu}$ is:

$$\Delta v' = \Delta v - \Delta v_{h\nu} = -\phi \Delta H_r \alpha/\rho C + \phi \Delta v_r \quad (3)$$

The term $-\phi \Delta H_r \alpha/\rho C$ in Eqn. 3 depends strongly on temperature because of the temperature dependence of $\alpha$, while the term $\phi \Delta v_r$ is generally relatively independent of temperature. The volume difference $\Delta v_r$ frequently is due mainly to a change in the number of electrically charged species in the system. Charged groups cause an ordering (electrostriction) of molecules in their vicinity. Volume changes due to electrostriction usually do not depend strongly on temperature [24].

If $\phi$ and $\Delta H_r$ do not depend on temperature, one can estimate the value of $\Delta H_r$ by measuring $\Delta v$ and $\Delta v_{h\nu}$ at two different temperatures, $T_1$ and $T_2$:

$$\Delta v'(T_1) - \Delta v'(T_2) = -\phi \Delta H_r \{\alpha(T_1) - \alpha(T_2)\}/\rho C \quad (4)$$

In practice, the value on the left in Eqn. 4 is divided by the difference between $\Delta v_{h\nu}$ at two temperatures:

$$\frac{\Delta v'(T_1) - \Delta v'(T_2)}{\Delta v_{h\nu}(T_1) - \Delta v_{h\nu}(T_2)} = -\phi \Delta H_r/E_e \quad (5)$$

To obtain a more reliable value, one can measure the volume changes at several temperatures and determine $\partial \Delta v/\partial T$:

$$\frac{\partial \Delta v/\partial T - \partial \Delta v_{h\nu}/\partial T}{\partial \Delta v_{h\nu}/\partial T} = \frac{\partial \Delta v'/\partial T}{\partial \Delta v_{h\nu}/\partial T} = -\phi \Delta H_r/E_e \quad (6)$$

If $\Delta H_r$ depends on temperature, Eqn. 6 would be true for a temperature where $\alpha = 0$.

Since $E_e$ and the values of $\alpha$, $\rho$ and $C$ for pure water are all known, Eqn. 2b allows one to calibrate the signals in ml/einstein absorbed. As an inert absorber, we used bromcresol purple in very dilute buffer (less than 0.5 mM phosphate, pH about 8). Fig. 1A shows the volume change measured at 24°C with a flash strength of 0.016 J, and Fig. 1B shows that the volume change was proportional to the intensity of the flash. This demonstrates the linear

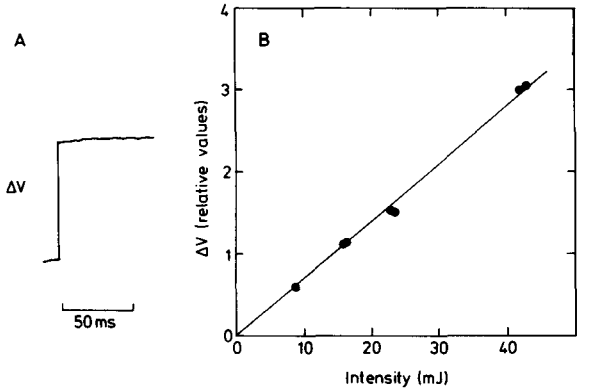


Fig. 1. Flash-induced volume changes of bromcresol purple solution. A, time courses measured with a flash intensity of 0.016 J; B, the initial expansion as a function of flash intensity. Temperature 24°C; $A_{588} = 0.51$ cm$^{-1}$ (the optical path length in the microphone cell is about 2 cm). The solution contains about 0.4 mM phosphate buffer.

response of the capacitor microphone to the volume change. $\alpha$, $\rho$ and $C$ of pure water at 24°C are $2.27 \cdot 10^{-4}$ K$^{-1}$, 1.00 g/cm$^3$ and 1.00 cal/K per g, respectively. The energy of the photon of 588 nm is 49 kcal/einstein. Thus, the observed expansions correspond to $(49 \times 10^3) \times (2.27 \times 10^{-4})/(1.00 \times 1.00) =$ 11.1 ml/einstein. Although the calibration does not require knowing the absolute number of absorbed photons, it was necessary to monitor the laser flash and correct for fluctuations in the intensity. The calibration would not be necessary to calculate the enthalpy change according to Eqn. 5, if the apparatus were stable enough. However, we routinely made calibrations before and after each determination of $\partial\Delta v/\partial T$ or $\partial\Delta v_{h\nu}/\partial T$, using a bromcresol purple solution with approximately the same absorbance as the sample at 588 nm. This minimized the effects of any drift in the apparatus between the two determinations. The work done in moving the microphone diaphragm is negligible, relative to the energy of the flash; this can be calculated from $\Delta V$, $\alpha$, and the isothermal compressibility of water.

## Results

With the capacitor microphone, one can measure the volume change in the interval between 100 $\mu$s and 1 s after the flash. Reaction centers that contain both $Q_A$ and $Q_B$ (2-Q reaction centers) complete the electron transfer from P to $Q_B$ within 1 ms [25–27]. The state $P^+Q_B^-$ then decays slowly, with half-time of approx. 1 s [28,29]. Therefore, one can measure $\Delta H_r$ for the formation of $P^+Q_B^-$ using 2-Q reaction centers. Addition of *o*-phenanthroline [30,31] or extraction of $Q_B$ from the reaction center [28] blocks electron transfer from $Q_A$ to $Q_B$. State $P^+Q_A^-$ relaxes with a half-time of 60–80 ms [28,29]. These samples allow one to measure $\Delta H_r$ for forming $P^+Q_A^-$.

Fig. 2 shows typical time courses of the volume change after flash excitation. Traces 1 and 2 are the volume changes of a bromcresol purple solution, which is susposed to be photochemically inactive. Trace 1 is a measurement at 22°C; the expansion is due to the release of the energy of the absorbed photons as heat. It relaxes slowly, depending on the capacitance of the microphone, and dissipation of heat from the cuvette or dissipation of pressure through the valve of the cuvette. Trace 2 is a measurement at 3.6°C, where $\alpha$ of the buffer is close to zero. Little or no volume change is observed at this temperature. Traces 3 and 4 are the volume changes of reaction centers with one quinone. The reaction center solutions and the bromcresol purple had the same absorbance at the excitation wavelength. At 3.6°C (trace 4), the reaction centers contract rapidly and then relax with a half-time of about 80 ms. The decay kinetics correspond to those of $P^+Q_A^-$. The initial contraction is due to a volume difference between $PQ_A$ and $P^+Q_A^-$ ($n_r\Delta v_r$ in Eqn. 1a). The volume change at 22°C (trace 3) reflects both the contraction $n_r\Delta v_r$ and an expansion due to release of heat. The difference between traces 3 and 1 gives the time course of $n_r\Delta v'$ at 22°C (dotted line). Its initial value is approximately the same as $\Delta V$ at 3.6°C, implying that little or no enthalpy is stored in state $P^+Q_A^-$. The decay of $n_r\Delta v'$ is slower at 22°C ($t_{1/2} = 120$ ms) than at 3.6°C, as expected from the temperature dependence of the rate of the $P^+Q_A^- \rightarrow PQ_A$ electron transfer reaction (see ref. 3 and references listed therein).

Fig. 3 shows the time courses of the volume change of 2-Q reaction centers at 3.6°C. Rapid contraction is followed by slow relaxation with a half-time of about 0.7 s (curve 1). In the presence of 2 mM *o*-phenanthroline (curve 2), the relaxation becomes as fast as it is with 1-Q reaction centers ($t_{1/2}$ = 70 ms), as expected if electron transfer to $Q_B$ is blocked.

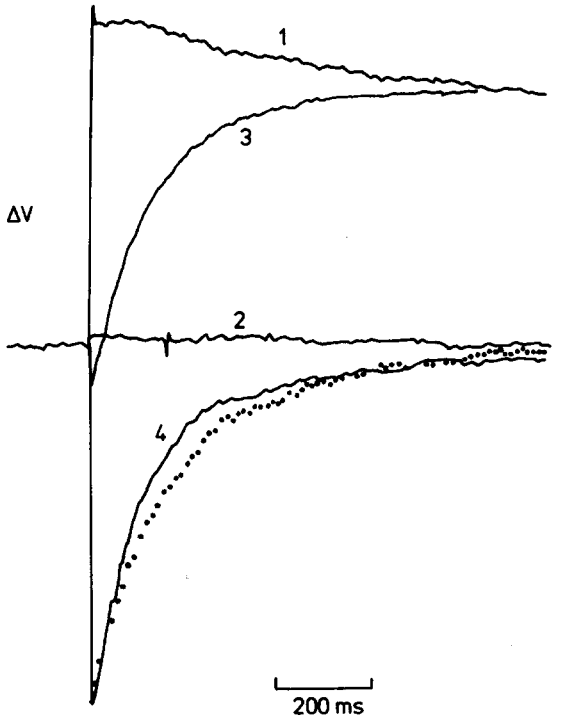


Fig. 2. Flash-induced volume changes of 1-Q reaction centers or bromcresol purple solution. Traces 1 and 2, bromcresol purple; traces 3 and 4, reaction centers. Temperature was 22°C for traces 1 and 3, and 3.6°C for traces 2 and 4. Dotted line, trace 3 minus trace 1. Buffer solution was 10 mM phosphate, pH 8.0, with 0.05% Triton X-100. Flash intensity, 0.02 J; $A_{588} = 0.52\ cm^{-1}$.

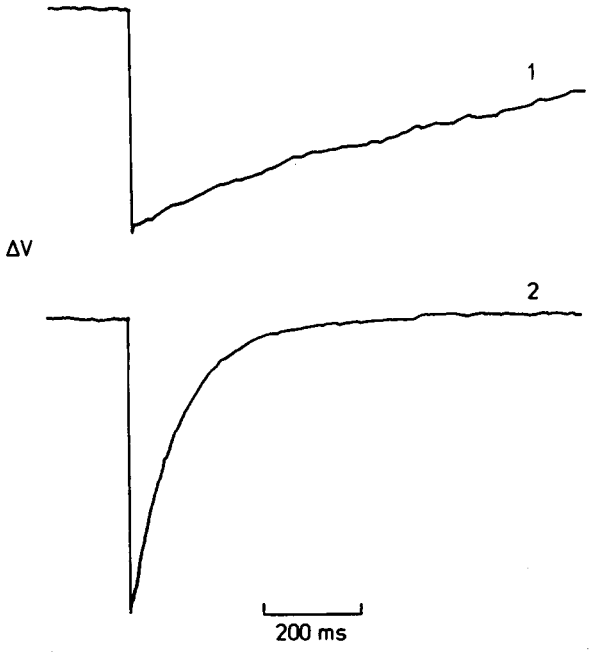


Fig. 3. Flash-induced volume change of 2-Q reaction centers. Reaction centers were suspended in 10 mM phosphate buffer, pH 7.7, with 0.1% lauryldimethylamine oxide and 100 mM NaCl. Trace 1, without *o*-phenanthroline; trace 2, with 2 mM *o*-phenanthroline. Temperature, 3.4°C; flash intensity, 0.02 J; $A_{588} = 0.49\ cm^{-1}$.

In Fig. 4, the initial values of $\Delta V_{h\nu}$, and $\Delta V$ of 2-Q and 1-Q reaction centers are plotted against the intensity of the exciting flash. The volume changes of bromcresol purple solution ($\Delta V_{h\nu}$) are linear in the flash intensity. The slope gives the value of $\Delta v_{h\nu}$ (Eqn. 2b). The contraction of the reaction centers saturates at high intensity. The slope in the range where $\Delta V$ is proportional to the flash intensity gives the value of $\Delta v$ (Eqn. 1b). In 1-Q reaction centers, the saturation curves of $n_r \Delta v' (= \Delta V - \Delta V_{h\nu})$ at 3.6 and 22°C are approximately the same. On the other hand, in 2-Q reaction centers, $n_r \Delta v'$ is more negative at 22°C than it is at 3.6°C. The difference indicates storage of enthalpy in the state $P^+Q_B^-$ (Eqn. 5).

The temperature dependences of $\Delta v_{h\nu}$ and of $\Delta v$ for 1-Q reaction centers are shown in Fig. 5. The figure gives the initial volume changes, in ml/einstein absorbed. $\Delta v_{h\nu}$ was measured with two different intensities (about 0.02 and 0.008 J). It increased almost linearly with increasing temperature, as expected from the known temperature dependence of $\alpha$ of water; this indicates that the sensitivity of the microphone itself does not depend on the temperature. The temperature where no volume change is observed (the temperature where $\alpha = 0$) was somewhat lower (2.4°C) than that of pure water, because the buffer solution (10 mM phosphate, pH 8.0, with 0.1% lauryldimethylamine oxide and 100 mM NaCl) contains electrolytes. $\Delta v$ of reaction centers was measured with two different flash strengths (about 0.008 and 0.0037 J), which are in the range where the volume changes are approximately proportional to the flash strength (see Fig. 4). The slope $\partial \Delta v / \partial T$ that gives the least-squares fit was calculated for each data set and is indicated in the figure. The results are also summarized in Table I.

The circles and triangles in Fig. 5 represent the volume changes in phosphate buffer and Tris-HCl buffer, respectively. The contractions are larger with phos-

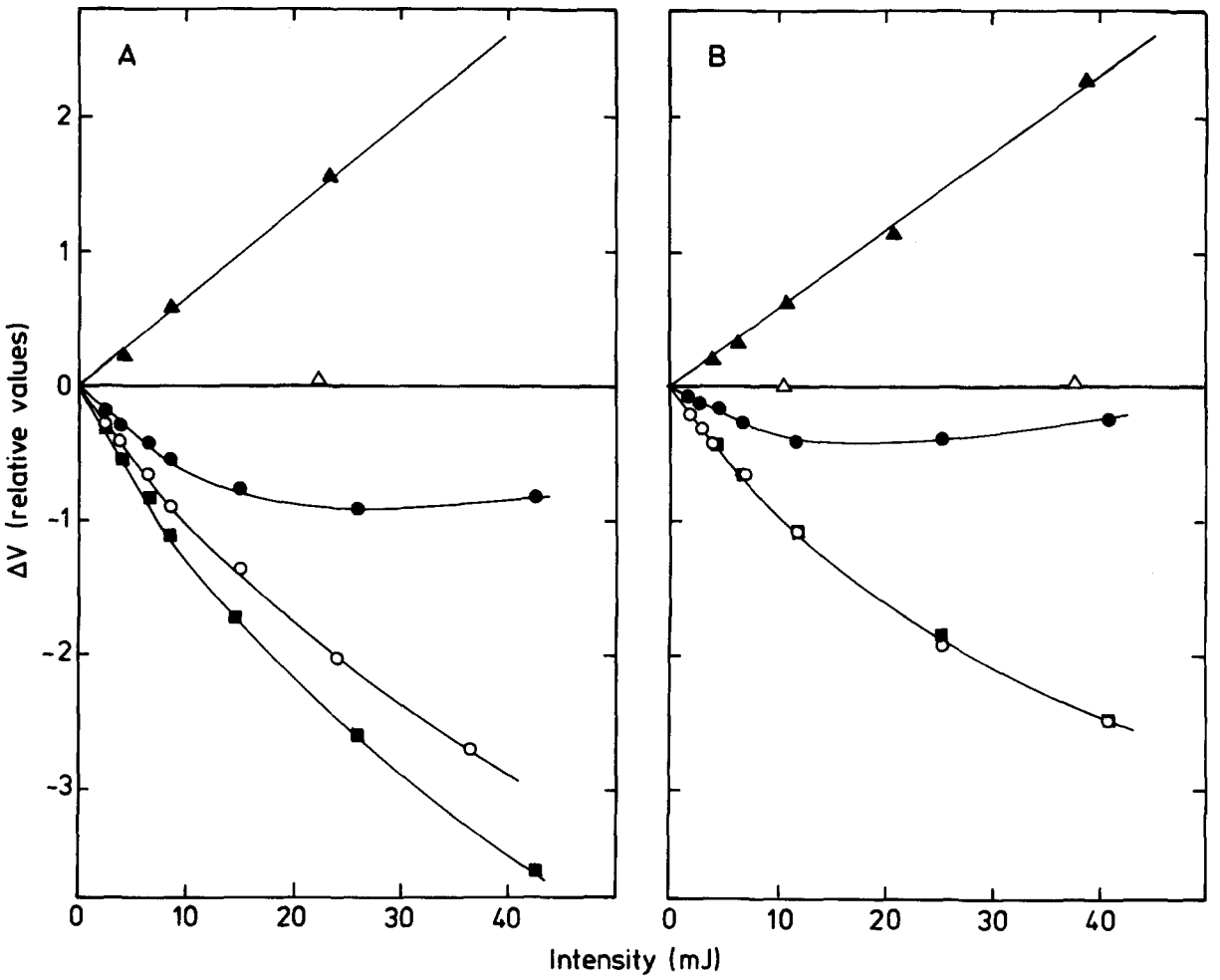


Fig. 4. Volume changes as a function of flash intensity. △ and ▲, $\Delta V_{h\nu}$ measured with bromcresol purple solution; ○ and ●, $\Delta V$ measured with 2-Q (A) and 1-Q (B) reaction centers; ■, $n_r\Delta v'(= \Delta V - \Delta V_{h\nu})$. ○ and △, 3.6°C; ●, ▲ and ■, 22°C. Buffer solution was 10 mM phosphate, pH 8.0, with 0.1% lauryldimethylamine oxide. $A_{588}$, 0.69 $cm^{-1}$ for 2-Q reaction centers and 0.52 $cm^{-1}$ for 1-Q reaction centers.

phate buffer than they are with Tris-HCl, and the temperature dependences are slightly steeper. These differences are probably caused by uptake of protons. This needs to be considered in some detail, before we used Eqn. 6 with the data of Fig. 5 to calculate $\Delta H_r$. If the photochemical reaction is accompanied by proton uptake, $\Delta v'$ becomes:

$$\Delta v' = -\phi_r(\Delta H_r^\circ + n_p\Delta H_r^p)\alpha/\rho C + \phi_r(\Delta v_r^\circ + n_p\Delta v_r^p) \quad (7)$$

where $\Delta H_r^\circ$ and $\Delta v_r^\circ$ are the enthalpy change and volume change due to the photochemical charge separation, $\Delta H_r^p$ and $\Delta v_r^p$ are those associated with the transfer of protons from the buffer to the reaction center, and $n_p$ is the number of protons taken up per photochemical reaction. $\Delta H_r^p$ and $\Delta v_r^p$ depend on the nature of the buffer [17,18]. $\Delta v_r^p$ could be written as:

$$\Delta v_r^p = \Delta v_p(R) - \Delta v_p(B) \quad (8)$$

where $\Delta v_p(R)$ and $\Delta v_p(B)$ are the volume changes associated with the protonation of the reaction center and the buffer, respectively, by $H_3O^+$. The protona-

TABLE I

VOLUME CHANGE, PROTON UPTAKE AND ENTHALPY CHANGE ASSOCIATED WITH FLASH-INDUCED CHARGE SEPARATION IN 1-Q REACTION CENTERS

The values of $\Delta\nu_r$ and $\partial\Delta\upsilon/\partial T$ were obtained from least-squares fits to data sets similar to those shown in Fig. 5. The buffer solutions (10 mM) contained 0.1% lauryldimethylamine oxide and 100 mM NaCl. n.d., not determined.

| pH | Buffer | Intensity [a] | $\Delta\upsilon_r$ [b] (ml/einstein) | $n_p$ [c] | $\partial\Delta\upsilon/\partial T$ (ml/einstein per K) | $\partial n_p/\partial T$ [d] ($K^{-1}$) | $\frac{\partial\Delta\upsilon'/\partial T}{\partial\Delta\upsilon_{h\nu}/\partial T}$ [e] | $\Delta H_r$ [f] (eV) |
|---|---|---|---|---|---|---|---|---|
| 8.0 | $P_i$ | high | −20.2 ± 0.74 | – | 0.62 ± 0.018 | – | −0.031 ± 0.029 | 0.07 ± 0.06 |
| | Tris | | −13.4 ± 0.51 | – | 0.51 ± 0.015 | – | – | – |
| | $P_i$ − Tris | | −6.8 ± 0.90 | 0.27 ± 0.04 | 0.11 ± 0.023 | 0.0057 | – | – |
| 8.0 | $P_i$ | low | −21.8 ± 1.26 | – | 0.68 ± 0.026 | – | 0.063 ± 0.041 | −0.13 ± 0.09 |
| | Tris | | −15.3 ± 0.96 | – | 0.67 ± 0.023 | – | – | – |
| | $P_i$ − Tris | | −6.5 ± 1.58 | 0.26 ± 0.06 | 0.01 ± 0.035 | 0.0017 | – | – |
| 6.5 | $P_i$ | high | −19.3 ± 0.47 | – | 0.63 ± 0.012 | – | −0.016 ± 0.020 | 0.03 ± 0.04 |
| | Aces | | −22.0 ± 1.70 | – | 0.67 ± 0.042 | – | 0.047 ± 0.066 | −0.10 ± 0.14 |
| | $P_i$ − Aces | | 2.7 ± 1.76 | −0.11 ± 0.07 | – | – | – | – |
| 6.5 | $P_i$ | low | −21.2 ± 0.53 | – | 0.61 ± 0.013 | – | −0.047 ± 0.021 | 0.10 ± 0.04 |
| | Aces | | −22.5 ± 1.79 | – | n.d. | – | – | – |
| | $P_i$ − Aces | | 1.3 ± 1.87 | −0.05 ± 0.07 | – | – | – | – |
| Mean [g] | | | | | | | | 0.05 ± 0.03 |

[a] High and low intensities mean approx. 0.008 and 0.0037 J, respectively.

[b] $\Delta\upsilon_r$; $\Delta\upsilon$ at the temperature where $\alpha = 0$.

[c] $$n_p = \frac{\Delta\upsilon_r(P_i) - \Delta\upsilon_r(\text{Tris})}{\Delta\upsilon_p(P_i) - \Delta\upsilon_p(\text{Tris})} = \{\Delta\upsilon_r(P_i) - \Delta\upsilon_r(\text{Tris})\}/25.$$

In the calculation for $n_p$ at pH 6.5, $\Delta\upsilon_p$(Aces) is supposed to be the same as $\Delta\upsilon_p$(Tris).

[d] $$\partial n_p/\partial T = \frac{\{\partial\Delta\upsilon(P_i)/\partial T - \partial\Delta\upsilon(\text{Tris})/\partial T\} - n_p[\{\Delta H_p(P_i) - \Delta H_p(\text{Tris})\}/E_e]\partial\Delta\upsilon_{h\nu}/\partial T}{\Delta\upsilon_p(P_i) - \Delta\upsilon_p(\text{Tris})}$$

$$= \{\partial\Delta\upsilon(P_i)/\partial T - \partial\Delta\upsilon(\text{Tris})/\partial T + 0.19 n_p \partial\Delta\upsilon_{h\nu}/\partial T\}/25.$$

[e] $\partial\Delta\upsilon'/\partial T = \partial\Delta\upsilon/\partial T - \partial\Delta\upsilon_{h\nu}/\partial T$; $\partial\Delta\upsilon_{h\nu}/\partial T = 0.64 \pm 0.005$, measured with bromcresol purple solution in 10 mM phosphate buffer (pH 8.0) with 0.1% lauryldimethylamine oxide and 100 mM NaCl.

[f] $$\Delta H_r = -E_e \frac{\partial\Delta\upsilon'/\partial T}{\partial\Delta\upsilon_{h\nu}/\partial T} = -2.11 \frac{\partial\Delta\upsilon'/\partial T}{0.64}.$$

[g] In the calculation of the mean, each value of $\Delta H_r$ was weighted inversely by its own variance. This would not take into account systematic errors in $\Delta H_r$.

tion of phosphate ($HPO_4^{2-}$) by $H_3O^+$ causes the loss of a negative charge on phosphate buffer and of the positive charge of $H_3O^+$. This process is accompanied by an expansion ($\Delta\upsilon_p(P_i) = 24$ ml/mol [32]). Protonation of Tris buffer is accompanied by a much smaller volume change ($\Delta\upsilon_p(\text{Tris}) = -1$ ml/mol [32]), since the number of charges in the solution remains constant. The difference in $\Delta\upsilon'$ at the temperature where $\alpha = 0$ is, therefore:

$$\Delta\upsilon'(P_i) - \Delta\upsilon'(\text{Tris}) = -\phi n_p\{\Delta\upsilon_p(P_i) - \Delta\upsilon_p(\text{Tris})\}$$

$$= -25\phi n_p(\text{ml/einstein absorbed}) \quad (9)$$

The results shown in Fig. 5 (a difference of about 6.5–6.8 ml/einstein absorbed at 2.4°C) correspond

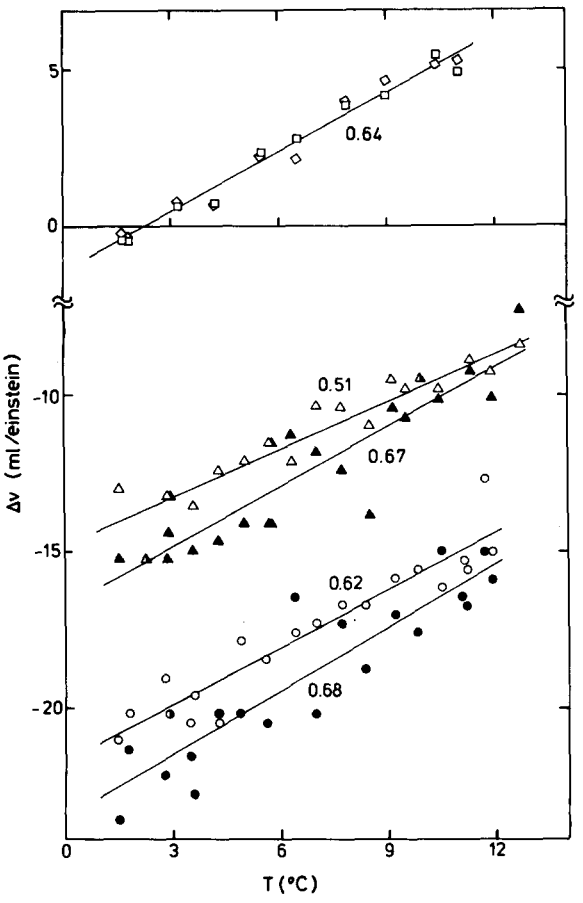


Fig. 5. Volume change of 1-Q reaction centers as a function of temperature. □ and ◇, $\Delta v_{h\nu}$ measured with bromcresol purple solution; ○, ●, △ and ▲, $\Delta v$ measured with reaction centers. □, ◇, ○ and ●, phosphate buffer; △ and ▲, Tris buffer. Flash strength, approx. 0.02 J (□), 0.008 J (◇, ○ and △) or 0.0037 J (● and ▲). Buffer solutions (10 mM, pH 8.0) contained 0.1% lauryldimethylamine oxide and 100 mM NaCl. $A_{588} = 0.49$ cm$^{-1}$. Lines that give the least-squares fit are shown. The numbers indicate the slopes of the lines (in ml/einstein per K).

to $n_p = 0.26$–$0.27$ protons taken up per photon absorbed.

If $n_p$ depends on temperature, the slope $\partial\Delta v'/\partial T$ becomes:

$$\partial\Delta v'/\partial T = \phi(\Delta H_r^\circ + n_p\Delta H_r^p)(1/\rho C)\partial\alpha/\partial T - \phi\Delta H_r^p(\alpha/\rho C)\partial n_p/\partial T + \phi\Delta v_r^p\partial n_p/\partial T \quad (10)$$

The term $-\phi\Delta H_r^p(\alpha/\rho C)\partial n_p/\partial T$ is negligible at the temperatures where $\alpha$ is close to zero. $\Delta H_r^p$ can be divided into the protonation enthalpies of the reaction center, $\Delta H_p(R)$, and the buffer, $\Delta H_p(B)$:

$$\Delta H_r^p = \Delta H_p(R) - \Delta H_p(B) \quad (11)$$

Thus, the difference between the slopes $\partial v'/\partial T$ with phosphate and Tris buffer at the temperature where $\alpha = 0$ is:

$$\partial\Delta v'(P_i)/\partial T - \partial\Delta v'(\text{Tris})/\partial T = \phi n_p\{\Delta H_p(P_i) - \Delta H_p(\text{Tris})\}(1/\rho C)\partial\alpha/\partial T - \phi\{\Delta v_p(P_i) - \Delta v_p(\text{Tris})\}\partial n_p/\partial T \quad (12)$$

$\Delta H_p$ for phosphate and Tris buffer are $-1.8$ and $-11.3$ kcal/mol, respectively [33]. $(1/\rho C)\partial\alpha/\partial T$ is given from the slope with the bromcresol purple solution. Using the value 0.26 for $n_p$, $\partial n_p/\partial T$ is calculated to be $-0.0057$ K$^{-1}$ from the slope of data obtained with the stronger flashes (Fig. 5) and $-0.0017$ from those obtained with the weaker flashes. Although the values are very small, the term $\phi\Delta v^p(\partial n_p/\partial T)$ in Eqn. 10 may not be negligible, depending on the value of $\Delta v_r^p$. Information about $\Delta v_p(R)$ thus is necessary.

In Fig. 6, faster time courses of the volume changes in the presence of different buffers are shown. The measurements of rapid time courses are difficult because a sudden volume change causes the signal to oscillate with a period of about 120 $\mu$s for 2–3 ms. This is probably caused by pressure waves in the cuvette. The time courses shown in the figure were obtained by taking the centers of the oscillation. The initial contractions that occured within 100 $\mu$s were approximately the same in phosphate and Tris buffer. In Tris the contraction was followed by an expansion with a time constant of about 0.5 ms, while no corresponding expansion was observed in phosphate buffer. Similar observations have been made previously in chromatophores of *C. vinosum* [16]. The expansion in Tris buffer is presumably caused by proton uptake. It accounts for the fact that the net volume decrease measured on a slower time scale is smaller in Tris than it is in phosphate (Fig. 5). The transfer of protons between phosphate and the reaction center evidently is not accompanied by a significant volume change. This means that $\Delta v_p(R)$ is approximately the

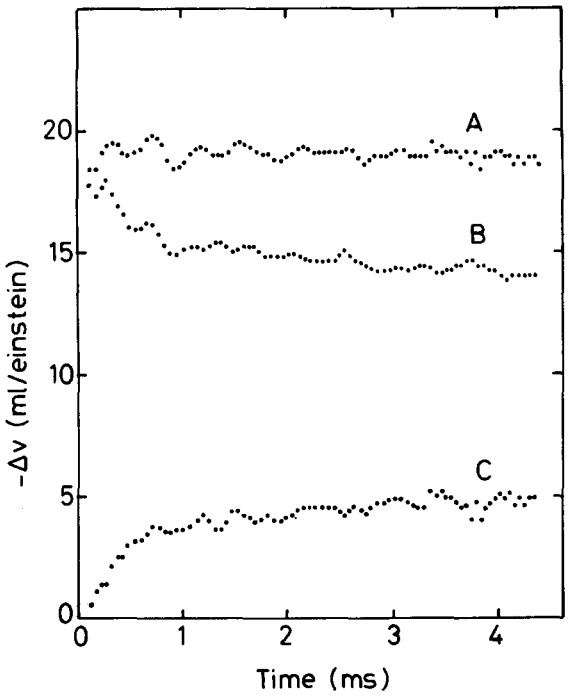


Fig. 6. Rapid time courses of the volume change of 1-Q reaction centers. A, in phosphate buffer; B, in Tris buffer; C, A minus B. Flash strength, approx. 0.08 J; $A_{588} = 0.40$ cm$^{-1}$. The buffer solutions (10 mM, pH 8.0) contained 0.1% lauryldimethylamine oxide and 100 mM NaCl. Volume decreases are plotted upwards in this figure.

same as $\Delta v_p(P_i)$. Thus, the third term in Eqn. 10 also is probably negligible in phosphate buffer, so that one can simply use Eqn. 6 to calculate $\Delta H_r$.

The values of 0.62 ml/einstein per K (higher intensity) and 0.68 ml/einstein per K (lower intensity) for $\partial v/\partial T$ in phosphate buffer give enthalpy changes of 0.07 ± 0.06 and −0.13 ± 0.09 eV, respectively. The values contain the enthalpy change associated with deprotonation of the buffer. However, the protonation enthalpy of phosphate is relatively small (−1.8 kcal/mol). Its contribution to the enthalpy change measured would be about 0.5 kcal/einstein or 0.02 eV, which is not considerable.

The calculations described above are collected in Table I, along with similar calculations based on measurements at lower pH (pH 6.5). Phosphate and Aces buffers were used in the latter case. Although $\Delta v_p$ of Aces is not known, it is probably similar to that of Tris. At pH 6.5, the volume changes of 1-Q reaction centers measured in phosphate and Aces were same within experimental error. Light-induced proton binding evidently does not occur at this pH. $\partial \Delta v/\partial T$ measured with either buffer can be used to calculate the enthalpy change. The calculations show that $\Delta H_r$ for the formation of $P^+Q_A^-$ at pH 6.5 is similar to that seen at pH 8.0. The mean value of all of the measure-

TABLE II

VOLUME CHANGE, PROTON UPTAKE AND ENTHALPY CHANGE ASSOCIATED WITH FLASH-INDUCED CHARGE SEPARATION IN 2-Q REACTION CENTERS

Experimental conditions and calculations are as in Table I. n.d., not determined.

| pH | Buffer | Intensity | $\Delta v_r$ (ml/einstein) | $n_p$ | $\partial \Delta v/\partial T$ (ml/einstein per K) | $\frac{\partial \Delta v'/\partial T}{\partial \Delta v_{h\nu}/\partial T}$ | $\Delta H_r$ (eV) |
|---|---|---|---|---|---|---|---|
| 7.7 | $P_i$ | high | −18.8 ± 0.70 | – | 0.51 ± 0.017 | −0.203 ± 0.028 | 0.43 ± 0.06 |
| | Tris | | −15.1 ± 0.42 | – | n.d. | – | – |
| | $P_i$ – Tris | | −3.7 ± 0.82 | 0.15 ± 0.03 | – | – | – |
| 7.7 | $P_i$ | low | −20.5 ± 0.79 | – | 0.51 ± 0.018 | −0.203 ± 0.029 | 0.43 ± 0.06 |
| | Tris | | −16.9 ± 0.54 | – | n.d. | – | – |
| | $P_i$ – Tris | | −3.6 ± 0.93 | 0.14 ± 0.04 | – | – | – |
| 6.4 | $P_i$ | high | −19.9 ± 0.83 | – | 0.52 ± 0.020 | −0.188 ± 0.032 | 0.40 ± 0.07 |
| | Aces | | −18.6 ± 0.42 | – | 0.51 ± 0.011 | −0.203 ± 0.019 | 0.43 ± 0.04 |
| | $P_i$ – Aces | | −1.3 ± 0.93 | 0.05 ± 0.04 | – | – | – |
| 6.4 | $P_i$ | low | −22.2 ± 0.99 | – | 0.56 ± 0.022 | −0.125 ± 0.035 | 0.26 ± 0.07 |
| | Aces | | −20.3 ± 0.50 | – | 0.50 ± 0.011 | −0.219 ± 0.19 | 0.46 ± 0.04 |
| | $P_i$ – Aces | | −1.9 ± 0.11 | 0.08 ± 0.04 | – | – | – |
| Mean | | | | | | | 0.42 ± 0.02 |

TABLE III

VOLUME CHANGE AND ENTHALPY CHANGE ASSOCIATED WITH FLASH-INDUCED CHARGE SEPARATION IN 2-Q REACTION CENTERS IN THE PRESENCE OF *o*-PHENANTHROLINE

Experimental conditions and calculations are as in Table I. Phosphate buffer is used. *o*-Phenanthroline, 2 mM.

| pH | Intensity | $\Delta v_r$ (ml/einstein) | $\partial \Delta v / \partial T$ (ml/einstein per K) | $\frac{\partial \Delta v'/\partial T}{\partial \Delta v_{h\nu}/\partial T}$ | $\Delta H_r$ (eV) |
|---|---|---|---|---|---|
| 8.0 | high | −22.5 ± 1.15 | 0.61 ± 0.026 | −0.047 ± 0.041 | 0.10 ± 0.09 |
| | low | −24.8 ± 1.26 | 0.59 ± 0.026 | −0.078 ± 0.041 | 0.16 ± 0.09 |
| 6.5 | high | −20.4 ± 0.48 | 0.60 ± 0.011 | −0.063 ± 0.019 | 0.13 ± 0.04 |
| | low | −22.5 ± 0.72 | 0.60 ± 0.015 | −0.063 ± 0.025 | 0.13 ± 0.05 |
| Mean | | | | | 0.13 ± 0.03 |

ments with 1-Q reaction centers is $\Delta H_r = 0.05 \pm 0.03$ eV.

Table II shows the results with 2-Q reaction centers. The measurements were made at pH 7.7 and 6.4. At pH 7.7, $\Delta v_r$ is larger in phosphate buffer than in Tris buffer; the difference corresponds to $n_p = 0.14$–0.15 protons taken up per photon absorbed. At pH 6.4, $\Delta v_r$ is approximately the same in phosphate and Aces buffer, indicating that the amount of proton uptake decreases at low pH, as it does in 1-Q reaction centers. These results agree with measurements of proton uptake using pH indicators [34]. At pH 7.7, the rapid time courses of the volume change of 2-Q reaction centers were similar to those of 1-Q reaction centers; i.e., proton uptake causes contraction in Tris buffer but not in phosphate buffer (data not shown). Therefore, $\partial \Delta v / \partial T$ measured with phosphate buffer can be used to calculate $\Delta H_r$. We can also use the values with Aces buffer at lower pH, where little or no proton uptake occurs. The calculated $\Delta H_r$ is 0.42 ± 0.02 eV, independent of pH.

In the presence of *o*-phenanthroline, 2-Q reaction centers showed an enthalpy change of 0.13 ± 0.03 eV (Table III). This is similar to, but slightly more positive than that measured in 1-Q reaction centers.

## Discussion

The results obtained with both 1-Q reaction centers and 2-Q reaction centers in the presence of *o*-phenanthroline show that the enthalpy of the $P^+Q_A^-$ radical pair is approximately the same as that of the $PQ_A$ ground state. This agrees with the conclusion reached from the temperature dependence of the $E_m$ values of P and $Q_A$ in *C. vinosum* chromatophores [5]. The free energy stored in the radical pair appears to take the form of decreased entropy. The $E_m$ values of P and $Q_A$ at 295 K are +480 mV [1] and −180 mV [8], respectively, and if the free energy of interaction between $P^+$ and $Q_A^-$ is negligible, the standard free energy change for the formation of $P^+Q_A^-$ is about +0.65 eV. From this, the entropy change ($\Delta S_o$) for the formation of $P^+Q_A^-$ is calculated to be about −50 cal/K per mol. The enthalpy of $P^+Q_A^-$ could depend on whether or not the reaction center has the second quinone, but the results obtained with the *o*-phenanthroline-treated 2-Q reaction centers suggest that the difference is at most 0.1 eV.

On the other hand, our results do not agree with the large, positive $\Delta H_r$ values obtained from the temperature dependence of delayed fluorescence from *Rps. viridis* chromatophores [14,15]. This could reflect differences between the two species of bacteria, or between chromatophores and isolated reaction centers. The $E_m$ of P decreases with increasing temperature in *C. vinosum* [5], while it appears to be less dependent on temperature in *Rps. viridis* [14]. The $E_m$ of $Q_A$ is pH dependent in chromatophores, but is independent of pH in isolated reaction centers [9,10]. However, the discrepancy between the $\Delta H_r$ value calculated from the delayed fluorescence measurements and that obtained calorimetrically probably does not reflect differences in the amount of proton uptake that occurs during the two types of measurement. The delayed fluorescence measurements and our measurements at low pH both were

made under conditions that gave little or no proton binding. Another possibility is that the delayed fluorescence from chromatophores is influenced by an electrical potential difference between the positions of $P^+$ and $Q_A^-$. This difference might depend on the integrity of the chromatophore membrane. The intensity of the delayed fluorescence also depends on the quantum yield of the photochemical reaction, which in *Rps. viridis* is only 40–50% [14,35]. A temperature dependence of the quantum yield could influence the estimation of $\Delta H_r$.

For the formation of $P^+Q_B^-$, we obtained an enthalpy change of 0.42 ± 0.02 eV. This contrast markedly with the results on $P^+Q_A^-$, and also with the previous conclusions from the measurements of the temperature dependence of $E_m$ values [5] and from the calorimetry with *C. vinosum* chromatophores [16]. In the calorimetric measurements on chromatophores [16], the experimental conditions were such that cytochrome *c*-555 could donate electrons to $P^+$ rapidly. The electron transfer from cytochrome to P could be the origin of the different results. Another possible interpretation is that the enthalpy decrease is caused by proton uptake, which occurs in *C. vinosum* chromatophores [16] but not in reaction center preparations from *Rps. sphaeroides* strain R-26 after a single-turnover flash [34]. The amount of proton uptake appears to vary among different bacterial species and strains [36]. Reaction centers do bind protons after a single flash at high pH, but the amount of proton uptake is relatively small (0.14–0.15 proton per absorbed photon at pH 7.7). Also, it is possible that the binding site is different from that of the proton uptake in *C. vinosum* chromatophores or of the proton uptake that follows two successive flashes [34].

Because the preparation of 2-Q reaction centers involves the addition of exogenous Q, it is possible that an electron moves from $Q_B^-$ to a different quinone in these. This possibility was minimized by chromatographing the reaction centers to remove excess Q. In addition, the flash repetition rate was kept low enough so that $P^+Q_B^-$ could decay completely between flashes. Vermeglio [37] and Wraight [38] have shown that transfer of electrons to bulk quinones occurs only after both $Q_A$ and $Q_B$ have been reduced by excitation with two flashes spaced more closely together.

Recent studies of the decay kinetics of $P^+Q_A^-$ and $P^+Q_B^-$ [13,29] indicate that the standard free energy of $P^+Q_B^-$ is probably about 0.08 eV below that of $P^+Q_A^-$, or about 0.57 eV above the ground state. This would mean that the entropy change associated with formation of $P^+Q_B^-$ is about −7 cal/mol per K. Redox titrations have suggested that the $E_m$ of $Q_B$ is approx. +80 mV at pH 7 [5,11]. This would put the free energy of $P^+Q_B^-$ about 0.40 eV above that of the ground state, and would mean that the entropy change is essentially zero. However, these measurements were made in chromatophores of *C. vinosum* and *Rps. viridis*, and the $E_m$ values were pH dependent. The values presumably represent the standard free energy for the reduction of $Q_B$ to $Q_BH$. In any case, it seems clear that the enthalpy and entropy changes that accompany the formation of $P^+Q_B^-$ are very different from those associated with $P^+Q_A^-$. Since $Q_A$ and $Q_B$ are both ubiquinone in *Rps. sphaeroides*, these differences must result from differences in the interactions with $P^+$, Fe, or other components of the reaction center.

Electron transfer from $Q_A^-$ to $Q_B$ involves an enthalpy increase of about 0.35 eV; this means that the activation enthalpy of the electron transfer is at least 0.35 eV. The activation enthalpy of this reaction has been measured by several investigators. Parson [25] reported an activation enthalpy of 9.3 kcal/mol (0.37 eV) for *C. vinosum* chromatophores. Chamorovsky et al. [26] reported activation enthalpies of 12.4 kcal/mol (0.55 eV) and 9.9 kcal/mol (0.33 eV) for chromatophores of *Ectothiorhodospira shaposhnikovii* and *Rhodospirilum rubrum*, respectively. Petty and Dutton [39] measured the temperature dependence of the rate of proton uptake by *Rps. sphaeroides* chromatophores and obtained an apparent activation enthalpy of 10.5 kcal/mol (0.46 eV). They concluded that this value arose from the activation enthalpy of a rate-limiting step in electron transfer from $Q_A$ to membrane ubiquinone. All of these values are consistent with our results.

The accuracy of our measurements is limited by the fact that the formation of $P^+Q_A^-$ or $P^+Q_B^-$ causes a contraction ($\Delta V_r$) that is large, relative to the volume change due to the release of heat. One way to overcome the problem would be to increase the temperature range over which the measurements are made. However, this would increase the chance of other

sources of error. Ort and Parson [19] observed that the volume change of purple membrane fragments of *H. halobium* showed abnormal temperature dependence around 17°C.

Although we do not have any direct information on the origin of $\Delta v_r$, it is reasonable to ascribe the contraction to an electrostrictive effect of the positive charge of $P^+$ and the negative charge on $Q_A^-$ or $Q_B^-$. In this respect, it is interesting that, although the enthalpy and entropy changes associated with reducing $Q_A$ and $Q_B$ are very different, $\Delta v_r$ is about the same for the formation of $P^+Q_A^-$ and that of $P^+Q_B^-$.

**Note added in proof** (Received April 8th, 1981)

Recent measurements of delayed fluorescence from reaction centers suggest that $\Delta H_r$ for $P^+Q_A^-$ is much larger than the value obtained here (Arata, H. and Parson, W.W., unpublished results).

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