Volume Contraction on Photoexcitation of the Reaction Center from *Rhodobacter sphaeroides* R-26: Internal Probe of Dielectrics

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ABSTRACT Reaction centers of *Rhodobacter sphaeroides* undergo a ~20 A^3/mole volume contraction in <50 ns after excitation. The rapid volume change is tentatively assigned to electrostriction. From its magnitude, we infer that the effective dielectric coefficient is 10–15 if the compressibility of the reaction center is similar to that of globular proteins. The volume contraction is not sensitive to replacement of the natural ubiquinone at the Q_A site by other quinones or to the occupancy of the Q_B site. The quenching caused by pressure on the reaction centers most likely occurs on a faster time scale than that of electron transfer.

INTRODUCTION

The isolation of the reaction center from photosynthetic bacterial system (Reed and Clayton, 1968) initiated the era of rapid progress in understanding the primary reactions of photosynthesis (Feher et al., 1989; Gunner, 1991). These studies gained added significance with the determination of the crystal structure of the reaction center because this was the first membrane protein to have its structure known at atomic resolution (Deisenhofer et al., 1985). The protein serves to store the energy of a photon via a sequence of charge separating electron transfer reactions. These reactions all occur by electron tunneling (Devault, 1980; Marcus and Sutin, 1985).

Spectroscopic studies have determined the kinetics of the sequence of reactions in photosynthetic reaction centers over the range of femtoseconds to seconds and have provided information about the energetics of the reactions (Woodbury et al., 1986; Arata and Parson, 1981a; Chidsey et al., 1985). Two stages of electron transfer are found. The first generates an oxidized bacteriochlorophyll dimer and reduced bacteriopheophytin $(BChl)_2^+BPh^-$ in 3 ps followed by reduction of a quinone to form $(BChl)_2^+Q_A^-$ in 200 ps. In principle, the kinetics and energetics of these steps can also be investigated by pulsed, time-resolved photoacoustic measurements (Braslavsky and Heibel, 1992). In the liquid phase, photoacoustic measurements are sensitive not only to the enthalpy of the reaction, detected by the thermal expansion of the solution but also to the volume change of the reaction itself, which is directly detected. On making pulsed, photoacoustic measurements on reaction centers, we observed a large negative (contraction) signal in striking contrast to the positive (expansion) signal of the reference compound (a copper porphyrin) that degrades the absorbed light to heat on the picosecond time scale. Further experiments showed the contraction to be caused by a volume change, not by a positive enthalpy (absorption of heat), and that it occurs in <50 ns. This observation confirms the earlier finding of Arata and Parson (1981b) who, using a capacitative cell, observed a negative volume change of 36 A^3 in reaction centers of *R. sphaeroides* on the 10 ms time scale. We report our results here and give a tentative explanation of their origin. It is possible to remove both the primary and secondary quinones and to reconstitute the primary quinone with a large variety of other quinones (Gunner et al., 1982, 1986a; Gunner and Dutton, 1989; Franzen et al., 1990). The ability to exchange the quinones allows further testing of the requirements for the volume contraction.

MATERIALS AND METHODS

Preparation of chromatophores and reaction centers

Isolated RC samples were prepared (Clayton and Wang, 1971) with 3.35 mM RC, 10 mM Tris-HCl pH 8.0, and 0.01% lauryldimethylamine oxide (LDAO) (the residual detergent from diluting the RCs), Q_A and/or Q_B were removed (Woodbury et al., 1986; Okamura et al., 1975) and 22 to 34 μM of the various quinones were added (see Gunner et al., 1986a; Gunner and Dutton, 1989). Quinones were obtained from Fluka Chemie AG (Buchs, Switzerland) and Aldrich Chemical Co. (Milwaukee, WI).

The electron transfer activity of all samples was tested by monitoring the change in optical absorbance after a 10 μs flash in a University of Pennsylvania Biomedical Instrument Shop Flash Photolysis Spectrophotometer. The reaction was followed at 425 or 860 nm, regions sensitive to the oxidation state of $(BChl)_2^+$. These optical signal amplitudes before and after the photoacoustic measurements differed by less than 10%.

Pulsed, time-resolved photoacoustic measurements

The methodology has been described elsewhere (Feitelson and Mauzerall, 1993; Mauzerall et al., 1994). A 7 ns pulse of 532 nm light from a

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Q-switched, frequency-doubled NdYag laser is shaped into a uniform slit that illuminates a 1×1 cm cuvette. The area exposed is ≈0.1 cm^2, and the energy is 2–20 μJ, a flux that is well below the multiphoton region. The crucial reference solution is copper uroporphyrin (CuURO, obtained from turaco flight feathers) adjusted to the same absorbancy as the sample at 532 nm. Copper porphyrins degrade absorbed photons to heat in <10 ps. The reference solutions had the same buffer composition and detergent as the RC sample solutions. Changes in detergent up to 0.1% had no effect on the measurements. The acoustic detector is homemade and sits in the solution 2–3 mm above and parallel to the slit illumination. It consists of piezoelectric film (Kynar, 28 μ) sandwiched between a stainless steel outer jacket (ground) and an inner rod leading coaxially to a high impedance, low noise, ns preamplifier (Amptek 250). The signal is then filtered (1 MHz) and further amplified (Stanford Research Instrument 560) before being digitized (Tek 710) and stored in a computer (Hewlett Packard 340) for analysis. Programs written by D. M. Detectors (Molectron JD3–09) measure the light pulse energy before and after the cell and transfer (Molectron JD-2000) the digitized data to the computer. The cuvette is equipped to allow solution replacement, N_2 purge, and temperature measurement. It is contained in a thermostated block.

RCs containing both Q_A and Q_B have a slow turnover time of ≈1 s. It was determined that the photoacoustic signal is negative at 16 s per repetitive pulse but positive at 0.5 s per pulse. On averaging over many pulses, most RCs are in the $P^+Q_B^-$ state and most of the absorbed light is degraded to heat.

RESULTS

Reaction centers

Sign and amplitude of signal

On illumination of reaction centers of *R. sphaeroides* mutant R-26 containing native Q_A and no Q_B with a 532 nm, 7 ns, weak (i.e., linear region) pulse of light, a negative (contractive) photoacoustic signal is observed, in contrast to the positive (expansive) signal from the reference solution (Fig. 1).

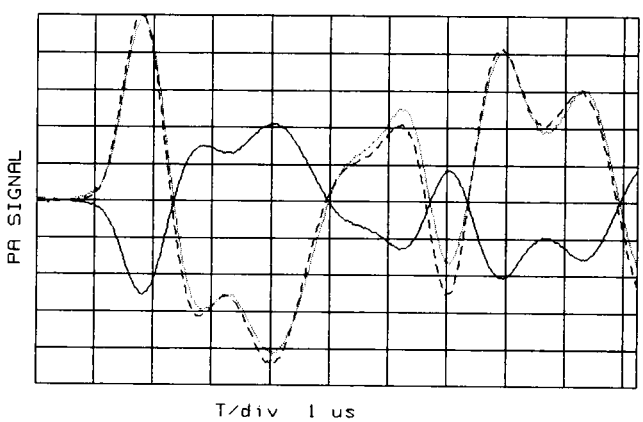

FIGURE 1 The amplitudes of the photoacoustic signals are plotted versus time. The dashed line first peak positive signal is the reference, CuURO. The solid line first peak negative signal is the reaction centers with Q_A. The dotted line is the reaction center data multiplied by −2. The apparent lag of the reaction center data was not reproduced in other expanded time scale measurements. The large negative component is seen to be as fast as the impulse response,(<50 ns), but the poor match at later times shows evidence of millisecond components that can be fit by convolution. The temperature was 27°C, the wavelength was 532 nm, the absorbancy was 0.34 cm^{-1}, the incident energy was about 20 mJ in an area of 0.1 cm^2, and 128 pulses were averaged.

The excitation at 532 nm (2.3 eV) must liberate 40% of the photon energy on a sub-picosecond time scale on dropping to the excited state of the trap at 860 nm (1.38 eV), and a remaining half of the remaining energy is lost on conversion to the stable state: $P^+Q_A^-$ (Arata and Parson, 1981a; ~0.6 eV stored). Thus, on energetic grounds, a positive signal at 70% of the amplitude of the reference is expected. The observed negative signal must be much diminished by the presence of a large positive signal because most of the absorbed light is degraded to heat. This is confirmed by lowering the temperature to that of the maximum density (≈3°C) of the solution, where there is no change in solution volume when the solution is heated. At this temperature the negative reaction center signal *increases* twofold, whereas that of the reference shrinks to zero (Fig. 2). The volume change of the RC is obtained directly by this measurement. An estimate of the molar thermal volume, ΔV_{th}, for the reaction is obtained from: $\Delta V_{th} = R\alpha h\nu/\rho C_p$, where R is the ratio of the photoacoustic signal of the sample at maximum density temperature to that of the reference at temperature T, and α, ρ, and C_p are the thermal expansivity, the density, and the heat capacity, respectively, of the solvent at temperature T; h and ν are Plank's constant and the frequency of the absorbed light, respectively (Braslavsky and Heibel, 1992). The thermal volume equivalent, at 20°C is 18 A^3/532 nm photon. The volume decrease in the RC so obtained, ΔV_{th}, is 20 ± 2 A^3/mol RC at ≈3°C, assuming a quantum yield of unity. This is about 0.02% of the RC volume. A volume change of <1 A^3 can be detected. It should be noted that Wraight and Clayton measured a quantum yield of unity at 860 nm, but only 0.83 at 700 nm, where the absorption is largely caused by bacteriopheophytin, as it is at 532 nm; thus, the yield may be less than unity and the estimated ΔV_{th} is a minimum value.

Photoacoustic data were obtained for both reference and sample solutions between 0 and 25°C. The lines fitting the amplitudes of the first peak versus temperature in both sets of measurements were almost linear, but the slope of the sample line was less then that of the reference because of

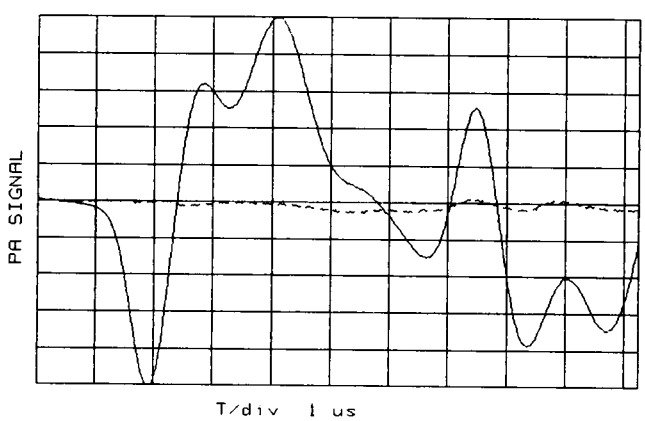

FIGURE 2 The amplitudes of the photoacoustic signals are plotted versus time. The large amplitude solid line is the reaction centers with Q_A at the temperature of maximum density, ~3°C. The dashed line is the reference compound, CuURO. Other conditions as in Fig. 1.

energy storage. This supports the reasonable hypothesis that the signal from the RCs is composed of a temperature-independent volume term and a thermal term varying as α. The other parameters and the response of the pyroelectric film are only weakly dependent on this small temperature change.

Kinetics

The use of resonant acoustic detectors results in data having good signal-to-noise ratio but severely restricts the bandwidth of the data, precisely by the "Q" of the resonance that enhances the sensitivity. The advantage of the wide bandwidth detectors is that data analysis by convolution can be meaningfully applied. Because the acoustic pressure signal is caused by the rate of heat production, the amplitude of the measurement is weighted by the rate constant of the heat producing step (Feitelson and Mauzerall, 1993). Thus, rapid reactions are more readily observed than slow reactions. The time window available in the present experiments is 0.05–10 μs.

Convolution of the sample signal with that of the reference shows that the volume contraction occurs in <50 ns (Fig. 3). A fit via convolution of the first peak alone shows no components with lifetime >20 ns (one sample channel). The fit of the entire time course indicates no components of greater than a few percent amplitude between this lower limit and 1 μs. There are indications of changes on the several microsecond time scale, but these will not be discussed here.

Quinone dependence

Irradiation of centers containing both Q_A and Q_B at a period of 16 s per pulse, which provides sufficient time for charge recombination between flashes, produces a signal that is negative as found for RCs containing only Q_A. The amplitude is approximately the same as with Q_A alone. Thus, the decrease in volume in the RCs with Q_B removed is not caused by a hole at the Q_B site.

Replacement of ubiquinone in the Q_A site with various quinones (Gunner and Dutton, 1989) produced a similar volume contraction that averaged 20% less than that of reaction centers with the native Q_A ubiquinone-10 present (Table 1). The quinones used have quantum yields of ionization >0.9 (Gunner and Dutton, 1989).

Reaction centers without either Q_A or Q_B produced a positive signal of fast heat liberation of about one-third the amplitude of the reference. These centers gave a negative signal at ≈3°C that correlated with the 5% (Table 1) and 15% residual Q_A found in two different preparations. However, the amplitude is fourfold larger than expected given the fraction of Q_A determined by flash photolysis.

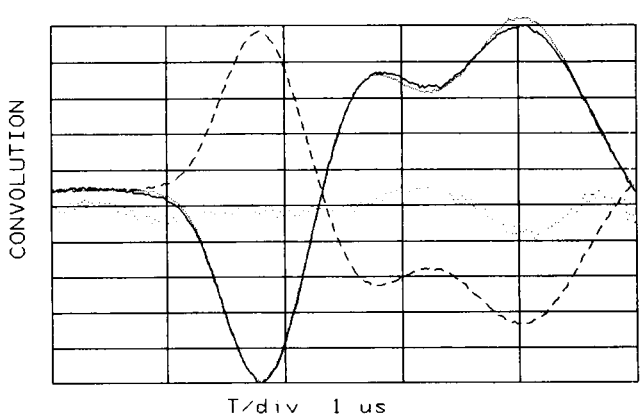

FIGURE 3 Deconvolution of the signal from reaction centers with Q_A (——) by re-iterative convolution (· · · · ·) with the reference signal (– – – –). The sparse dots are the residuals of the fit on a fivefold expanded amplitude scale with zero at center scale. The residuals average <1% and show that the major negative signal has no components of lifetime >50 ns. A fit of the first negative peak alone shows no components with lifetime >20 ns.

CONCLUSION

The most likely cause of the volume contraction on photoexcitation of reaction centers is electrostriction, a phenomenon that should yield a negative volume change and occur on the time scale of electron transfer, <1 ns. Thus, the hypothesis accounts for both the sign and the kinetics of the observed changes. Our data show that the time of formation of the negative signal is conservatively <50 ns, the present time resolution of the measurements (Fig. 3). The observed volume change could occur either during the initial stage of charge separation that forms P^+H^- (t = 3 ps) and/or during charge separation to $P^+Q_A^-$ (t = 200 ps). It would be interesting to study these effects with higher time resolution. The rapid attenuation of the sound wave in hydrogen-bonded liquids limits the bandwidth to ≈1 GHz but, combined with established deconvolution methods, it may be possible to separate the effect of charge transfer to pheophytin (3 ps) from that to quinone (200 ps).

The ability to exchange the quinone at site A furnishes support for the hypothesis that the volume contraction is caused by electrostriction. The volume change is not a simple movement into any "void" left by the phytol tail-less quinones because the natural ubiquinone-10 containing RC shows the same or larger negative volume change than RC with tail-less replacement quinones. A simple conformation change by itself will not lead to a volume change. The

TABLE 1 Volume contraction on excitation of reaction centers containing differing quinones

Quinone	ΔV, A^3
Ubi-	−20
2-Chloro-9,10-Anthra-	−14
9, 10-Anthra-	−15
2,3-Dimethyl-9,10 Anthra-	−14
None*	−8

The error in the measurement is ±2 A^2. The variable ΔH of the various quinone reactions does not affect the measurement of ΔV.
*This sample had 5% ubiquinone present as determined by flash photolysis.

resulting structure must be more, or less, closely packed than before to cause a volume change. This conclusion is similar to that reached with samples containing Q_A versus those containing Q_A plus Q_B noted above.

The rather large volume contraction seen on irradiating RC without quinones (Table 1) is puzzling. The triplet state is formed in only 30% yield (Norris et al., 1982); thus, its molar contraction would have to be as large as that of the P^+Q^- state to explain the observation. This may be possible if the dimer state is highly polarized, i.e., $^3(P^+P^-)$. It is possible that our estimate of Q_A present by flash photolysis is too low.

The volume contraction reported by Arata and Parson (1981b), 33 A^3, is larger than claimed here. Possibly, a further decrease occurs on a very slow time scale, or the quantum yield may differ at the different wavelengths: 532 nm (Bph) and 588 nm (Bchl). A recent paper (Malkin et al, 1994) reports photoacoustic measurements on RC of *R. sphaeroides* on the 1 μs time scale with a resonant detector. The reported volume contraction is 12 A^3 per Einstein absorbed, but the occupancy of the Q_A and Q_B site is completely unspecified. The authors observed no contraction for a quinone-less sample (with no criteria of occupancy) and about 25 A^3 for RC of mutant Y210W. The complex method they use to obtain the volume change is open to large errors.

If the reaction center resembles a polar liquid (Treutlein et al., 1992), one can use the Drude-Nernst Eq. 1 (Drude and Nernst, 1894; Hamann, 1974) on its centenary to estimate the volume contraction (ΔV). Electrostriction is caused by the lowering of the energy of a dielectric in an electric field. This energy is proportional to the product of the field and the local polarizability, measured by the dielectric coefficient. In an electric field gradient, this results in a force, or pressure, on the dielectric. For a spherically symmetric univalent ion in a homogeneous dielectric,

$$\Delta V = -\left(\frac{e^2}{2r\epsilon}\right)\frac{\partial \ln \epsilon}{\partial P} = -\left(\frac{e^2}{2r\epsilon^2}\right)\frac{\partial \epsilon}{\partial P}. \qquad (1)$$

In Eq. 1, e is the electronic charge, r is the ion radius, ϵ is the dielectric coefficient, and P is the pressure. For oppositely charged P^+ and Q_A^- with a separation distance of 29 A (center to center), there will be, in addition to the terms of Eq. 1 for the individual ions, a similar term where r is now the center-center distance of the ions (D. Mauzerall and Evans, unpublished data). This will diminish the volume contraction by 10–15% and is neglected for the present. Allowing a 5 A radius for the bacteriochlorophyl cation and 3 A for the quinone anion, the estimate of ΔV by summing the individual ion contributions is about an order of magnitude too large if benzene ($\epsilon = 2$) is used as the "solvent," but is close for methanol ($\epsilon = 33$). It is fivefold too small if one assumes water ($\epsilon = 80$) is the "solvent." Parameters were obtained from Hamann (1974).

What if proteins are not polar liquids? The pressure variation of the dielectric coefficient is linearly related to the compressibility (Owen and Brinkley, 1943). The compressibility of globular proteins (Gekko and Noguchi, 1979) varies from that of organic solvents ($10^{-4}bar^{-1}$) to one-tenth that value, typical of organic crystals (King, 1986). Freiberg et al. (1993) have estimated the compressibility of the light-harvesting pigment-protein complex of *Rhodospirillum rubrum* to be $1–2 \times 10^{-5}bar^{-1}$ via pressure-induced shifts of absorption spectra. Assuming a value of $10^{-5}bar^{-1}$, the roughly 10-fold decrease in this parameter from that of organic liquids will cause a threefold decrease in the estimated dielectric coefficient (ΔV observed $\propto \epsilon^{-2}$ (Eq. 1), and it is assumed that the volume and pressure changes remain in the linear range) to 10–15. This suggests that the effective dielectric coefficient for detergent solubilized RCs is larger than is often assumed for protein interiors. This is not unexpected because even interior sites of the RC are less than a Coulomb radius ($e^2/\epsilon kT \approx 15$ nm for $\epsilon = 4$) from water, $\epsilon = 80$. Classical electrostatics then causes an increase in the effective dielectric coefficient in the lower dielectric in a position sensitive way (Mauzerall and Drain, 1992.) DELPHI calculations (Sharp and Honig, 1990; Gunner and Honig, 1991) of the interaction between P^+ and Q_A^- within the reaction center structure yield interaction energies that would correspond to an effective dielectric coefficient of approximately 10 (M. Gunner, unpublished observation). This calculation places the protein ($\epsilon = 2$) within a slab modeling the membrane ($\epsilon = 2$) surrounded by water ($\epsilon = 80$). Thus, smaller interaction between the charges, expressed here as a higher effective value for ϵ, can be due to the impact reaction field of the surrounding water. In addition, the response of polar residues within the protein itself may contribute to higher internal dielectric coefficients (Warshel et al., 1989).

Stark effect spectral shifts provide another means of monitoring the effective dielectric constant between a charge and a chromophore. Steffin et al. (1994) suggest that the dielectric constant between the P and the L branch bacteriopheophytin is 7, somewhat lower than the value reported here for interaction of P^+ and Q_A^-. These authors also report that the effective dielectric constant is only 2.1 for the response of the M branch bacteriopheophytin to the charge on P^+. The asymmetry of the dielectric constant on L and M branches of the R_cs can also be estimated by measurements of electrostriction accompanying formation of and $P^+Q_B^-$. Little difference between the volume contraction for the two states was seen here or in previous measurements (Arata and Parson 1981b). Electrostriction may be a good method to determine the effective dielectric coefficient surrounding charges in a protein.

The observation of a stable volume contraction within 20 ns of charge separation suggests that either the rate of the primary charge separation (forming P^+H^-) or the next electron transfer step (forming $P^+Q_A^-$) should increase with pressure. Because the yield at 1 atm is near unity (Wraight and Clayton, 1973), little effect would be predicted. In fact, Clayton and DeVault (1972) observed that

the quantum yield of forming P^+ in reaction centers decreased with pressure from unity to 0.4 at 6000 atm and recovered to 0.8 on removing the pressure. The negative ΔV of reaction we have observed implies that their result shows a non-Le Chatlelier behavior and supports their suggestion that high pressure introduces a quenching process. With our present knowledge, the quenching under high pressure is most likely to occur in the dimer bacteriochlorophyll excited state because the loss must occur before the volume contraction.

Although photoacoustic measurements are often used to determine the enthalpy of photo-initiated reactions, the accuracy of the present measurements is not sufficient to determine fully the enthalpy of the $P^+Q_A^-$ state. This is in good part caused by the excessive prompt heat liberated by the short wavelength excitation and will be improved when excitation in the 860 nm band is possible.

The discovery of this fast photocontraction of the bacterial reaction centers opens a new approach to the understanding of the workings of this remarkable molecular system. It will be of interest to expand these measurements to other photosynthetic systems.

Note added in proof—We have now observed a volume contraction of 0.8 ± 0.05 A^3 on forming the triplet state of zinc uroporphyrin in water. This result supports our interpretation of the effect observed in the RC. (Mauzerall and Feitelson, 1995, in press).

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