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Pressure effects on the photochemistry of bacterial photosynthetic reaction centers from *Rhodobacter sphaeroides* R-26 and *Rhodopseudomonas viridis*

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Electron-transfer reactions and triplet decay rates have been studied at pressures up to 300 MPa. In reaction centers from *Rhodobacter sphaeroides* R-26, high pressure hastened the electron transfers from both the primary and secondary quinones (Q_A and Q_B) to the primary electron donor bacteriochlorophyll, P. Motion of Q_A between two sites, one nearer to P and the other nearer to Q_B, could account for these pressure effects. In reaction centers from *Rhodopseudomonas viridis*, charge recombination was slowed by high pressure. Decay rates were also studied for the triplet state, P^R. In *Rb. sphaeroides* R-26 with Q_A reduced with $Na_2S_2O_4$, the decay was hastened by pressure. This could be explained if P^R decays through a charge-transfer triplet state, or if the decay kinetics of P^R are sensitive to the distance between P and Q_A^-. In *Rps. viridis* reaction centers, and in *Rb. sphaeroides* reaction centers that were depleted of Q_A, the lifetime of P^R was not altered by pressure.

Introduction

The primary photochemical reaction of photosynthesis is charge separation that occurs in a special site called a reaction center. Reaction centers from the purple bacteria are composed of three protein subunits, four bacteriochlorophyll, two bacteriopheophytin and two quinone molecules and a non-heme iron atom [1]. Some preparations also include carotenoids and a tightly bound cytochrome *c*. Two of the bacteriochlorophyll molecules are closely associated with each other and function as the primary electron donor (P) [2]. Within 4 ps after excitation, an electron is transferred to an initial electron acceptor (I) that appears to be one of the bacteriopheophytin molecules [3–6]. In about 200 ps [7–9] it is further transferred to a quinone (Q_A), and in 6 μs to 60 μs to the second quinone (Q_B) [10,11]. When cytochrome *c* is present it can reduce the oxidized primary donor in 270 ns [9] to 30 μs [12] depending on the bacterial species. The crystal structure of the reaction center from *Rhodopseudomonas viridis* [13] indicates that the physical distance between the electron and hole increases with elec-

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Abbreviations: P, primary electron donor; I, initial electron acceptor; Q_A, primary quinone; Q_B, secondary quinone; P^R, triplet state; BPh, bacteriopheophytin; BChl, bacteriochlorophyll.

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tron transfer from the bacteriopheophytin (BPh) to Q_A and from cytochrome *c* to P and this hinders the wasteful charge recombination reaction.

The functions of two of the bacteriochlorophylls and one bacteriopheophytin are still not well known. One of these bacteriochlorophylls is associated with the bacteriopheophytin electron acceptor [14]. It may play a role in electron transfer between P and the bacteriopheophytin [15]. In isolated reaction centers from *Rps. viridis* or *Rhodobacter sphaeroides* illuminated under reducing conditions, either of the two bacteriopheophytins can be reduced [16,17]. This indicates the possibility that the additional pigment molecules may be involved in the normal charge separation reactions.

In the hope of gaining information on the function of these three pigments, we undertook to use pressure as a variable in a study of the photochemistry of the reaction center. Pressure can alter chemistry in several ways [18,19]. It can alter equilibria if there is a volume change in going from reactants to products. If a reaction proceeds via an intermediate state, the formation of which involves a change in entropy, ΔS, the rate of the reaction may vary with pressure in line with any corresponding volume change ('activation volume'). If pressure alters the distance or orientation between electron donor and acceptor, the rate of electron tunneling can be altered. Spectral properties can be affected by pressure through the increased dielectric constant of the compressed medium: this causes red shifts of absorption and emission bands. In these ways pressure may affect the kinetics of the electron-transfer processes in the reaction center and the spectra of the intermediate states.

The effects of pressure on proteins have been reviewed in several places [20–27]. Energy transfer between accessory pigments and chlorophyll in algae is inhibited by high pressure [28,29]. Cytochrome oxidation in chromatophores of the photosynthetic bacterium *Chromatium vinosum* also is inhibited by pressure [30]. Clayton and DeVault [31] used pressures up to 600 MPa * in a study of reaction centers from *Rb. sphaeroides*. The near infrared absorption spectrum at 600 MPa shows red shifts of the bands at 865, 800 and 760 nm of 380, 150 and 10 cm^{-1}, respectively. Clayton and DeVault also observed decreases in the quantum yields of fluorescence and of charge separation. Their results were probably complicated by the presence of several quinones per reaction center.

Kleinfeld [32] has presented preliminary results on the kinetics of the back electron-transfer reaction $P^+Q_A^- \rightarrow PQ_A$ in reaction centers of *Rb. sphaeroides*. The decay times at 0.1, 250, and 500 MPa were 124, 80, and 136 ms, respectively. Only one quinone per reaction center was present.

Here we describe the effects of pressure on the rates of one forward reaction and three back reactions in reaction centers of two species of photosynthetic bacteria. These reactions were chosen because the large absorbance changes associated with them make them easy to study. We chose to study *Rb. sphaeroides* because much is already known about its reaction centers, and *Rps. viridis* because its reaction centers have recently been crystallized [33,34].

Materials and Methods

Reaction centers, both from *Rb. sphaeroides* and *Rps. viridis*, were prepared essentially as described by Schenck et al. [35]. They were dialyzed to replace the detergent lauryldimethylamine oxide with Triton X-100. We found that lauryldimethylamine oxide at a concentration of 0.1% precipitates at pressures above 200 MPa. Triton X-100 at 0.05% did not precipitate at pressures up to 250 MPa. The reaction center solutions were buffered with 50 mM Tris-HCl at pH 8.0. Tris(hydroxymethyl)aminomethane ($pK_{A,25\,C} = 8.1$) was used because the dependence of its pK_A on pressure is small [36]. At 300 MPa, the value of pK_a increases by only 0.1.

Bacteriochlorophyll *a* used in this work was isolated from *Rhodospirillum rubrum* using two somewhat different methods, both similar to the method described by Strain and Svec [37]. The first method was described by Scherz and Parson [38]. The second method uses adsorption of the bacteriochlorophyll on powdered sugar in a centrifuge tube, followed by repeated washing with petroleum ether to remove carotenoids. The

* 100 MPa = 1 kbar = 14500 psi.

bacteriochlorophyll can be eluted with 10% ethanol, 90% petroleum ether. The product showed no more than the usual amount of absorbance at 680 nm, where the chlorin decomposition product absorbs [34], and at 760 nm and 530 nm, where bacteriopheophytin absorbs. Bacteriopheophytin *a* was prepared from bacteriochlorophyll *a* by treating an ethanolic solution with glacial acetic acid.

The high pressure apparatus consists of a hand pump to provide a fluid at high pressure and a bomb to contain a sample at high pressure. The pump and bomb are connected by stainless steel tubing and connectors purchased from Superpressure Inc. The hand pump used was model OH-102-60 of Pressure Products Industries. It delivers either water or hydraulic oil as the pressure fluid. Pressure was measured with an Astra gauge (Pressure Products Industries).

Two different bombs were used to obtain optical data at high pressure. The first, purchased from Superpressure, is rated to 340 MPa. It has two sapphire windows with optical apertures of 6 mm diameter separated by a distance of 3 cm. A glass cuvette with a 1 mm path length was used to contain the liquid sample, which was isolated from the high-pressure fluid by a latex rubber diaphragm. The second pressure bomb was designed and constructed at the Washington State University. It has four single-crystal sapphire windows with optical apertures of 6 mm, thus providing two orthogonal optical pathways. The light beams passing through each pair of windows intersect at the center of the bomb. The sample is contained in a glass cuvette, 12.5 mm square and 20 mm tall. Cuvettes were used with optical path length of either 10 or 6 mm. The bombs were filled with water to keep hydraulic oil out of the light path. All measurements were made at ambient temperature ($21 \pm 1°$ C).

Ground state absorption spectra were recorded with a Varian Cary 219 spectrophotometer using the Superpressure bomb. Samples with maximum absorbances of about 0.8 at 800 nm were used. After changing the pressure, a time of at least 2 min was allowed for the sample to come to equilibrium before making a measurement. Usually, spectra were recorded only after increasing the pressure, but a second spectrum at ambient pressure was taken after recording the spectra at high pressures to check for possible irreversible changes.

For measurements in the millisecond and microsecond regimes, the pressure bomb with four windows was used. The sample concentration was adjusted to give a maximum absorbance between 0.5 and 1.0 at 800 nm. Measurements were made both after increasing or decreasing the pressure. 1–2 min time was allowed between changing the pressure and making a measurement. Only in one case did this seem not to allow enough time for the sample to equilibrate.

For experiments with time resolution in the range of microseconds to seconds, a Q-switched ruby laser was used to pump a dye laser or to photolyze a sample directly. For some experiments we used Kodak IR-144 dye in dimethylsulfoxide to produce excitation at about 857 nm. The measuring light from a tungsten-iodine lamp passed through a monochromator (bandwidth, 6.6 nm) before interrogating the sample. The pump and probe beams passed through the sample at right angles. The probe light then passed through one or two smaller monochromators and filters to remove scattered laser light and finally was detected by a photomultiplier tube. The electrical signal from the photomultiplier tube was filtered and sent to a transient digitizer (Tektronix R7912, 7A16 or Biomation 802). The digital information was transferred to a microcomputer, where it was stored on floppy disks. 1–8 laser shots were averaged. The data were fit with a single or double exponential decay function.

Results and Discussion

Ground-state spectra

Spectra of reaction centers from *Rb. sphaeroides* R-26 show red shifts with increased pressure of the long-wavelength absorption bands, as has been observed previously [31,32]. When the pressure is increased from 0.1 to 300 MPa, the bands at 864 nm, 801 nm, and 758 nm, which are due mainly to bacteriochlorophyll dimer, bacteriochlorophyll monomers, and bacteriopheophytin monomers, shift by 177 cm^{-1}, 99 cm^{-1}, and 98 cm^{-1}, respectively *. Bandwidths are not altered. Low temper-

* In the neighborhood of 800 nm, a shift of 100 cm^{-1} is equivalent to about 6.4 nm.

ature shifts only the longest-wavelength band [40]. We also measured red shifts caused by pressure in the absorption bands of bacteriopheophytin *a* monomer and dimer in 0.03 M sodium dodecylsulfate. Dimers of bacteriochlorophyll [41] and bacteriopheophytin [42] have been observed previously in other mixed solvents. At low bacteriopheophytin cocentration, only one long-wavelength band, at 758 nm, is present, but as the concentration is increased, a band at 808 nm develops. Between 0.1 and 300 MPa, the bands at 758 nm and 808 nm shifted red by 61 cm^{-1} and 166 cm^{-1}, respectively. It is interesting that the dimers have greater red shifts than the monomers.

The theory of Bayliss [43] predicts the change in frequency of an electronic absorption band on bringing the molecule from the vacuum state to a condensed phase

$$\Delta\nu = -\frac{10.71\cdot 10^9 f}{\nu\alpha}\,\frac{n^2-1}{2n^2+1}$$

where n is the refraction index, f is the oscillator strength, ν is the frequency of the transition in a vacuum and α is the polarizability of the molecule. The derivative of this equation with respect to pressure is

$$\frac{d\Delta\nu}{dP} = -\frac{10.71\cdot 10^9 f}{\nu\alpha}\,\frac{6n}{(2n^2+1)^2}\,\frac{dn}{dP}$$

if one assumes that the oscillator strength and the polarizability are not affected by pressure. This equation shows that several factors could contribute to producing larger pressure-induced red shifts for the dimers. Firstly, since the dimers absorb at lower frequency and the refractive index, n, of the solvent decreases with decreasing frequency, the value of $6n/(2n^2+1)^2$ will be greater for the dimer, thus increasing the value of $d\Delta\nu/dP$. Secondly, the polarizability, α, is probably greater for the dimer, it being a larger molecule, but this would tend to decrease $d\Delta\nu/dP$. However, we believe that the dominant factor is most likely the increased oscillator strength of the transition in the dimer [14,44]. The value of f/ν in the above equation is directly proportional to the dipole strength of the transition:

$$|\mu|^2 = \frac{3h}{4\pi m}\,\frac{f}{\nu}$$

Since the value of $|\mu|^2$ for the dimer is approximately twice that of the monomer, the value of $d\Delta\nu/dP$ would be correspondingly increased for the dimer. Finally, it is possible that pressure alters the structure of the dimers by moving the monomers closer together, increasing the interactions between them and thus lowering the energy of the longest-wavelength electronic transition.

Charge recombination

The rates of electron transfer between the quinone acceptors and the primary donor

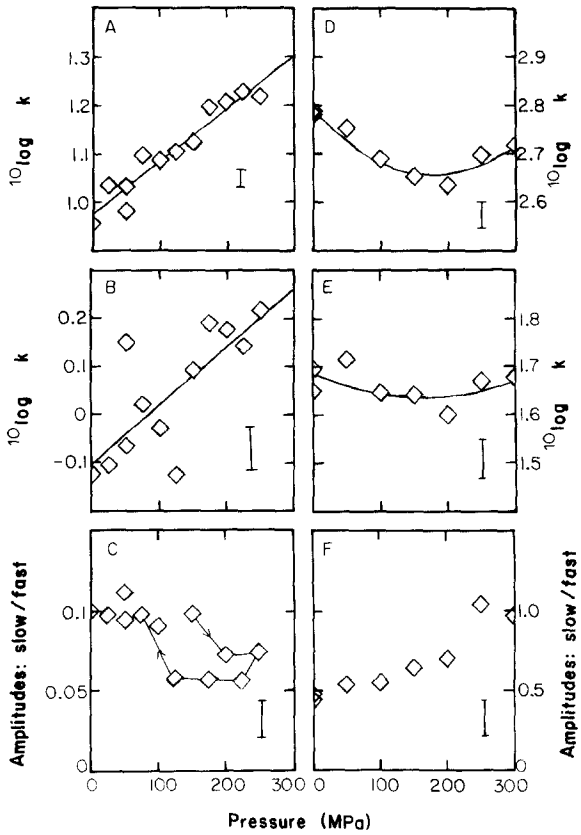

Fig. 1. Effect of pressure on the decay of P^+. A, B, and C, *Rb. sphaeroides* R-26, detected at 600 nm; D, E, and F, *Rps. viridis*, detected at 450 nm, with 75 μM $K_3Fe(CN)_6$ and 20 mM 1,10 *o*-phenanthroline present. Excitation was at 857 nm. A and D, decay of $P^+Q_A^-$ (fast component); B and E, decay of $P^+Q_B^-$ or other slow component; C and F, ratio of initial amplitudes of the slow component and fast component. Least-squares lines (A and B) or parabolas (D and E) are also shown. C also indicates the hysteresis in Q_B participation. The undertainty in the data is shown as an error bar, I, on each panel.

bacteriochlorophyll dimer were measured as a function of pressure in reaction centers from *Rb. sphaeroides* R-26 and *Rps. viridis*. The results are shown in Fig. 1.

The rate constants, their slopes with pressure, and activation volumes are included in the tabular summary of kinetic parameters in Table I. The activation volume ΔV^* is obtained from the variation of the rate constant with temperature, using the relation: $(\partial \ln k/\partial P)_T = -\Delta V^*/RT$. This expression is derived from the well-known Arrhenius relation $\ln K_{eq} = -\Delta G/RT$, recognizing that $(\partial \Delta G/\partial P)_T = \Delta V^*$ and using transition state theory [19]. In the latter, an equilibrium is presumed to exist between the reactants and the transition state (activated complex). The measured rate constant is the product of the equilibrium constant and the frequency factor. The frequency factor, which gives the rate at which the transition state forms products, is presumed to be independent of pressure. Thus, just as the temperature dependence of the rate constant gives an activation energy, the pressure dependence leads to an activation volume, ΔV^*.

The interpretation of such an activation volume is that it represents the volume difference between the transition state and the reactants. A positive value implies that a swelling occurs as the reactants assume the configuration of the transition state (rate constant falls with increasing pressure), while a negative value implies a shrinkage (faster rate as pressure increases). Measurement of a non-zero activation volume for a particular electron-transfer step in the photosynthetic reaction center would indicate then that changes in separation and/or relative orientation of the donor-acceptor pair are occurring during the process of electron transfer. By comparing the activation volume with the final vlume change for a reaction, one can infer the relative position of the transition state along the reaction coordinate.

The *Rb. sphaeroides* reaction centers had between one and two quinones per reaction center, as indicated by the biphasic nature of the P^+Q^- decay kinetics. This allowed us to determine the electron-transfer rates from both the primary, Q_A, (Fig. 1A and D), and secondary, Q_B, quinones (Fig. 1B and E) in a single experiment. Pressure increased the rate of electron transfer from either of the quinones of the primary donor. It is believed that the electron transfer from the secondary quinone proceeds mainly by thermal activation of the electron to the primary quinone [45,46]. The electron transfer from Q_A^- to P^+ is the rate-limiting step in the decay of $P^+Q_B^-$. Thus the activation volume of the decay of $P^+Q_B^-$ should be equal to the activation volume of the decay of $P^+Q_A^-$ (-6.1 ± 0.06 cm^3/mol) plus the volume change for the electron transfer from Q_B to Q_A. Our results indicate that this last volume change is -0.8 ± 2.5 cm^3/mol. Arata and Parson [47] found that reduction of Q_A or Q_B produced volume contractions of about 20 cm^3/mol in either case. Our results confirm that little change in volume is occasioned by the electron transfer between Q_A and Q_B.

It is less clear how the increase with pressure of the rate of electron transfer from Q_A to P should be interpreted. Kleinfeld's preliminary results, which were similar to ours, were discussed in terms of a decrease in the distance between P and Q_A. This is a possible explanation, but our differing results for reaction centers from *Rps. viridis* indicate that the explanation is not generally applicable. In that species, we found that the reaction rate decreased with increasing pressure.

One expects proteins to compress under high pressure, but it is not clear to what extent this should occur. The compressibilities of globular proteins have been found to be only 10%–20% as great as that of water [48,49], which has a relatively low compressibility for a liquid. It is not clear how much of the measured compressibility should be attributed to the protein and how much to the water near the surface of the protein. It could be, as Weber and Drickamer [23] suggest, that proteins compress very little because their shapes are limited by the strength of many covalent bonds. This picture of protein structure implies a low thermal expansion coefficient for proteins, for the same reason that the compressibility would be low. It also advises caution in accepting the idea that proteins contract at low temperature, which has been proposed as an explanation for electron-transfer reactions that increase in rate as the temperature decreases [50,51].

Despite the above considerations, there might still be a change in the distance between P and Q_A

or in their relative orientation at high pressure or low temperature. A change in the protein conformation or in the solvent packing could induce either P or Q to change its position. It is possible that changes in the position of Q_A are an essential feature of normal operation of the reaction center. Kleinfeld et al. [52] have provided evidence that a conformational change occurs when Q_A receives an electron from P. Reaction centers cooled to 77 K in the dark had higher rates for $P^+Q_A^- \rightarrow PQ_A$ than did reaction centers cooled under constant illumination. Reaction centers cooled in the dark were unable to form the state $P^+Q_B^-$, but those cooled under illumination formed that state with a rate constant greater than 10^3 s^{-1}. This suggests to us that in the dark, Q_A is trapped nearer to P and further from Q_B, while in the light, Q_A is trapped nearer to Q_B and further from P. This model would explain the temperature dependences of the electron-transfer reactions, since movement of Q_A would be thermally activated.

The results of our pressure studies appear to be consistent with the above model, as follows. In *Rb. sphaeroides*, pressure could slow the movement of Q_A^- between its two sites, or could stabilize Q_A in the site nearer to P, in much the same manner as low temperature does in the Kleinfeld experiments. Either possibility is consistent with the observed increase in the back reaction rate. Also, our data (Fig. 1C) show that the ability to form $P^+Q_B^-$ decreased at high pressure. This is also consistent with the model, but the observed hysteresis in Q_B participation suggests an alternative explanation that a slower process, possibly the dissociation and rebinding of Q_B, could be responsible. By contrast, no hysteresis was observed in the decay of $P^+Q_A^-$ or P^R.

Reaction centers of *Rps. viridis* were examined with $K_3Fe(CN)_6$ present to oxidize the bound cytochrome and with *o*-phenanthroline present to inhibit electron transfer from Q_A to Q_B. A biphasic decay of P^+Q^- was observed. The fast component presumably represents electron transfer from Q_A^- to P^+. The slower component is probably due to reaction centers in which Q_A^- is oxidized by $Fe(CN)_6^{3-}$, as described by Shopes and Wraight [53].

In *Rps. viridis*, pressure slowed electron transfer from Q_A^- to P^+ (Fig. 1D). The rate of this process has been found to depend on pH [53]. At pH 9.0, the rate constant is 910 s^{-1}, and at pH 6.0, 500 s^{-1}. Our experiments were at pH 8.0, near the inflection point. The solution was buffered with Tris, which undergoes little change in its pK_a with pressure [36]. Our results could be explained if pressure decreases the dissociation constant of the weak acid involved in the pH-dependent kinetics. If so, that acid is probably a cation. Neutral or anionic acids would be expected to have electrostrictive* volume decreases associated with their dissociation. Pressure would enhance their dissociation. Pressure did not significantly change the rate of the slow component (Fig. 1E), but did increase its relative amplitude (Fig. 1F).

Triplet states

The lifetime of the triplet states of three reaction center samples and of bacteriochlorophyll *a* in pyridine were measured as a function of pressure. The triplet states of the reaction center, P^R, were produced after reducing Q_A with $Na_2S_2O_4$ or extracting Q_A with 1% LDAO. This blocks the electron transfer from the intermediate acceptor (I) to Q_A. P^R is formed with a low yield (5–10%) by charge recombination. P^R is a triplet state of the bacteriochlorophyll dimer, although other triplet states involving charge separation (see below) may also be important. The reaction center solutions were not degassed, but O_2 has been found not to quench the triplet state of the reaction center of *Rb. sphaeroides* R-26 [54]. In any case, the $Na_2S_2O_4$ present in the solution should effectively remove O_2. The triplet state of bacteriochlorophyll in pyridine is readily formed on photo-excitation. The results of measurements at several pressures are shown in Fig. 2. The triplet decay rate constants and activation volumes are given in the table.

Reaction centers from *Rb. sphaeroides* R-26 with Q_A^- showed a definite decrease in the lifetime of P^R at high pressure. This could be explained on the hypothesis [54,55] that P^R is an equilibrium mixture of two states, of which one is the triplet state of the bacteriochlorophyll dimer, ^3P, and the other is a charge-transfer triplet state in which an

* Electrostriction is the phenomenon of decrease in separation distance or volume occasioned by the attractive Coulomb force between oppositely charged species.

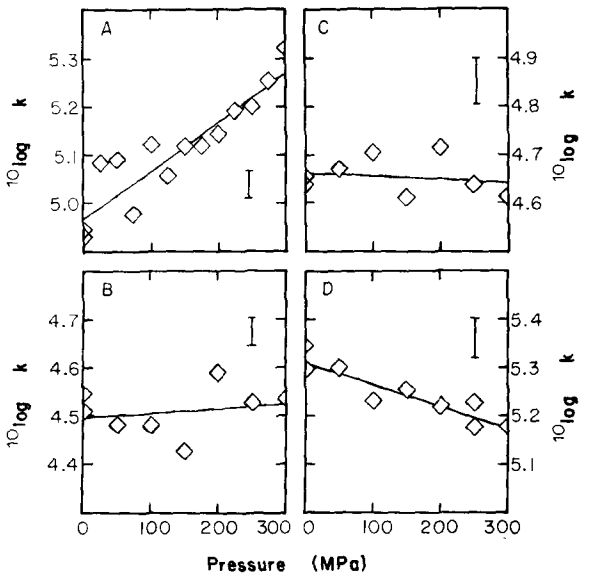

Fig. 2. Effect of pressure on triplet decay rates. A, *Rb. sphaeroides* R-26 reaction centers reduced with $Na_2S_2O_4$; B, *Rb. sphaeroides* R-26 reaction centers depleted of quinone; C, *Rps. viridis* reaction centers reduced with $Na_2S_2O_4$; D, bacteriochlorophyll *a* in pyridine. Excitation was at 857 nm, A, or at 694 nm, B, C, and D. Detection was at 680 nm, A; 420 nm, B; 570 nm, C; or 530 nm, D. The uncertainty in the data is shown as an error bar, I, on each panel.

electron is transferred to one of the monomeric bacteriochlorophylls, $^3(P^+B^-)$. The latter state was estimated to be higher in energy by about 0.03 eV and to have a decay rate constant 33-times greater than that of the former. The effect of increased pressure on such a system would be to favor the charge-transfer state, which would have the smaller volume due to the greater electrostriction associated with separated charges. This would lead to an increasing triplet decay rate as the pressure increased, which is what we observed. Thus the -6 cm^3/mol activation volume could be the volume difference between the localized triplet and the charge transfer triplet.

The proposal that P^R is an equilibrium mixture of a localized triplet state and a charge-transfer state was advanced originally to account for the temperature dependence of absorption changes associated with the formation of P^R, and for the temperature dependence of the decay kinetics of P^R [54,55]. More recently, Hoff et al. [56] have argued that the temperature dependence of absorption changes can be explained simply by a sharpening of the absorption bands with decreasing temperature. Recent picosecond spectroscopic studies and molecular orbital calculations also have cast doubt on the formation of $BChl^-$ in the reaction center [57–59]. To explain the temperature dependence of the P^R decay kinetics in reaction centers that are depleted of Q_A, Chidsey et al. [60] have suggested that P^R decays by way of the $^3(P^+I^-)$ radical pair, where I is the bacteriopheophytin that acts as an initial electron acceptor; and they have shown that the decay of P^R can be

TABLE I

KINETIC PARAMETERS: RATE CONSTANTS, SLOPES AND ACTIVATION VOLUMES

Values and standard deviations were calculated from linear least-squares analyses of the rate vs. pressure data for all plots except 1D, for which a parabolic least-squares fit was used. Values of $\partial k/\partial P$ can be readily obtained by forming the product, $k \cdot \partial \ln k/\partial P$.

Reaction	Figure	k (at 1 atm) (s^{-1})	$\partial \ln k/\partial P$ ($\times 10^9$)	ΔV^* (cm^3/mol)
Rb. sphaeroides R-26				
$P^+Q_A^- \rightarrow PQ_A$	1A	9.5 ± 0.3	2.5 ±0.2	−6.1±0.6
$P^+Q_B^- \rightarrow PQ_B$	1B	0.80± 0.09	2.8 ±0.8	−6.9±1.9
P^R decay (Q_A^-)	2A	(9.3 ± 0.5)·10^4	2.3 ±0.3	−5.7±0.8
P^R decay (no Q_A)	2B	(3.15± 0.22)·10^4	0.2 ±0.4	−0.5±1.0
Rps. viridis				
$P^+Q_A^- \rightarrow PQ_A$	1D	621.0 ±35	−3.5 ±0.4 [a]	+8.5±0.9 [a]
P^R decay (Q_A^-)	2C	(4.6 ± 0.5)·10^4	−0.16±0.3	+0.4±0.8
Bacteriochlorophyll *a* (pyridine)				
Triplet decay	2D	(2.1 ± 0.2)·10^5	−1.1 ±0.2	+2.6±0.4

[a] Figure 1D shows that the activation volume decreases and probably changes sign at the higher pressures. The values given are for the initial half of the range which is almost linear.

inhibited by weak magnetic fields, which disturb the conversion of $^3(P^+I^-)$ to the singlet radical pair, $^1(P^+I^-)$. Our results could be interpreted on this model if increased pressure favors the formation of $^3(P^+I^-)$ from P^R, again because of the greater electrostriction associated with the radical pair. However, magnetic fields have no effect on the decay kinetics of P^R in reaction centers that contain Q_A in the reduced form [59]. In contrast, elevated pressures accelerate the decay of P^R in reaction centers containing Q_A^-, but not in reaction centers lacking the quinone (Table I). Perhaps the simplest interpretation of our observations is that the decay of p^R is facilitated by a magnetic interaction between the triplet state and the Q_A^- radical. Such an interaction could be enhanced if Q_A^- moves closer to P.

In *Rps. viridis* reaction centers reduced with $Na_2S_2O_4$, the decay rates of P^R were largely unaffected by pressure. This could mean that in these reaction centers elevated pressure does not decrease the distance between P and Q_A^-.

The solution of bacteriochlorophyll *a* in pyridine was bubbled with argon gas in the pressure bomb for 3 h before the bomb was sealed. However, we evidently were not able to remove O_2 completely from the sample, for the observed triplet lifetime was about 5 μs, much less than the 57 μs lifetime observed by others [57]. The observed effect of pressure on this solution is probably ascribable to an increase in viscosity, which would decrease the diffusion-controlled second-order quenching rate constant.

Acknowledgments

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