Origin of Optical Activity in the Purple Bacterial Photoreaction Center†

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ABSTRACT: The photoreaction center (RC) of purple bacteria contains four bacteriochlorophyll (Bch) and two bacteriopheophytin (Bph) molecules as prosthetic groups. Their optical activity, as measured by circular dichroism (CD) spectroscopy, is largely increased *in situ* as compared to organic solutions. The all-exciton hypothesis posits that this enhanced optical activity is entirely due to excitonic interactions between the electronic transitions of all six bacteriochlorin molecules. Using the simple exciton theory, this model predicts that the near-infrared CD spectra should be conservative. The fact that they are not, whether the special pair of Bch (SP) that constitutes the primary electron donor is reduced or oxidized, has been explained by hyperchromic effects. The present work tests this hypothesis by successively eliminating the absorption and, therefore, the optical activity of the Bphs and of the non-special-pair (non-SP) Bchs. This was accomplished by trapping these pigments in their reduced state. RC preparations with the four non-SP bacteriochlorins trapped in their reduced state and, therefore, with an intact SP displayed conservative CD spectra. RC preparations with only the electronic transitions of SP and of one non-SP Bch also showed conservative CD spectra. These conservative CD spectra and their corresponding absorption spectra were simulated using simple exciton theory without assuming hyperchromic effects. Bleaching half of the 755-nm absorption band by phototrapping one of the two Bph molecules led to the complete disappearance of the corresponding CD band. This cannot be explained by the all-exciton hypothesis. These results suggest that the optical activity of the SP alone, or with one non-SP Bch, is due to excitonic interactions. They also suggest that the optical activity of the other three bacteriochlorins is due to other factors, such as pigment−protein interaction.

Thanks to X-ray crystallography, we now have a good understanding of the three-dimensional arrangement of the four bacteriochlorophyll (Bch)[1] and two bacteriopheophytin (Bph) molecules that serve as prosthetic groups in the photoreaction center (RC) of purple photosynthetic bacteria (Deisenhofer *et al.*, 1985; Allen *et al.*, 1987; Chang *et al.*, 1986). The six bacteriochlorins are arranged around a pseudo C_2 symmetry axis that traverses a special pair of Bch molecules (SP) located on the periplasmic side of the membrane and an iron atom on the cytoplasmic side. The two non-SP Bchs, the two Bphs, and two quinone (Q) molecules are disposed on each side of this axis along two potential branches of electron transfer designated as A and B. The primary photochemical process involves the transfer of an electron from the SP through one of the two Bph molecules, Φ_A, to the terminal electron acceptor, Q_A, giving rise to the radical pair $P^+-Q_A^-$. In both branches, a non-SP Bch molecule (B_A or B_B) is placed between the SP and Φ_A or Φ_B.

Paradoxically, in spite of this detailed knowledge of their structural arrangement, we have an only imperfect understanding of the *in situ* circular dichroism spectra (CD) of the prosthetic groups. While Bch and Bph are only slightly optically active in organic solution (Philipson & Sauer, 1972), they are highly so when they are embedded in the RC protein matrix (Sauer *et al.*, 1968; Reed & Ke, 1972). This phenomenon has led to the speculation that their near-infrared optical activity is due to the excitonic interactions of their Q_Y electronic transition dipoles (Sauer *et al.*, 1968; Shuvalov & Asadov, 1979; Knapp *et al.*, 1986; Vasmel *et al.*, 1986; Won & Friesner, 1988; Pearlstein, 1988). This model can be tested: it predicts (Tinoco, 1962; Knapp *et al.*, 1985, 1986; Vasmel *et al.*, 1986; Won & Friesner, 1988; Pearlstein, 1988) a null sum for the positive and negative rotational strengths of the bacteriochlorins' Q_Y CD bands. In other words, this model predicts "conservative" Q_Y CD spectra.

However, this is not the case: the near-infrared CD spectra are not conservative, whether the special pair is reduced (SP) or oxidized (SP^+) (Philipson & Sauer, 1973; Shuvalov & Asadov, 1979). This discrepancy prompted a more plausible interpretation that takes into account possible hyperchromic effects arising from pigment−pigment interactions. A complete exciton model based on this interpretation predicted nonconservative spectra (Parson & Warshel, 1987; Scherz & Parson, 1984).

According to X-ray crystallography, the six bacteriochlorins are arranged around a C_2 symmetry axis. This information allowed another test of the all-exciton hypothesis. If the CD signal was due entirely to excitonic interaction, it should disappear when measured along the symmetry axis (Tinoco, 1962; Muccio & Cassim, 1979). The experimental results did not confirm this expectation: while the rotational strength of the positive 870-nm band and of the negative 810-nm band did decrease, those of the positive 795-nm band and the negative 750-nm band underwent a small increase, instead (Mar and Gingras, 1984). These results imply that

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[1] Abbreviations: Bch, bacteriochlorophyll; Bph, bacteriopheophytin; RC, photoreaction center; SP, special pair; B_A, B_B, Φ_A, Φ_B, Q_A, and Q_B, non-special-pair bacteriochlorophyll, bacteriopheophytin, and quinone in the A and B branches of the photoreaction center.

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the Bch CD bands at 810 and 870 nm are due to excitonic interaction, in contrast to those of Bch and Bph at 795 and at 750 nm, respectively. Picosecond CD spectroscopy confirmed this interpretation and indicated that excitonic interactions alone cannot account for the positive CD band at 795 nm since its spectrum is not conservative and displays nearly the same rotational strength whether the RC is in the $(Bch)_2^+\Phi_A$, $(Bch)_2^+\Phi_A^-$, or $(Bch)_2^+\Phi_A Q_A^-$ state (Xie & Simon, 1991). Recently, Steffen *et al.* (1994) argued, on the basis of Stark-effect spectroscopy, that the Bph and Bch absorption band positions could originate from electrochromic effects and not necessarily from excitonic interaction with the SP. These results indicate that excitonic interaction among the bacteriochlorins may not be the sole contributor to the RC's optical activity in the infrared.

In the present work, we took advantage of the possibilities offered by the RC of *Ectothiorhodospira* sp. where the Φ_A, Φ_B, B_A, and B_B pigments can be successively trapped as either singly or doubly photoreduced forms (Mar & Gingras, 1990, 1991, 1993). Those reduced forms display no Q_X or Q_Y absorption dipoles and, therefore, cannot undergo excitonic interactions. We used such RC preparations to deduce whether or not a given CD band is due to excitonic interaction. Although the CD spectra were nonconservative during the early events of phototrapping, they became conservative when interaction was limited to within the SP (a dimer), or between the SP and a single non-SP Bch (forming a trimer). The spectra can be matched by assuming excitonic interaction between the Q_Y transitions of these pigments, without invoking hyperchromic effects. The available evidence indicates that the negative 750-nm and the positive 795-nm CD bands, which contribute to the nonconservation of the CD spectrum, are not due to excitonic interaction.

MATERIALS AND METHODS

We extracted the photoreaction center from *Ectothiorhodospira* sp. (ATCC 31751) cells with dodecyl dimethylamine oxide (LDAO) according to Lefebvre *et al.* (1984). The RC was suspended in 50 mM Tris-HCl/Triton X-100 (0.1%, w/v, pH 7.4) after removing the LDAO. The sample in a 1-cm path length cuvette was made anaerobic by bubbling with nitrogen gas and layering paraffin oil on top of the sample. The suspension consisted either of 0.53 μM RC in 2.5 mL of 100 mM Tris-HCl (pH 8.0)/0.1% (w/v) Triton X-100/42.5 mM Na ascorbate or of 1.8 μM RC in 2.5 mL of 100 mM Tris-HCl (pH 8.0)/0.1% (w/v) Triton X-100/5.7 mM Na dithionite.

The absorption and CD spectra were recorded respectively with a Cary 2300 spectrophotometer and with a Jasco S-20 spectropolarimeter modified to be sensitive in the 800−900-nm range by the use of a Hamamatsu R669 photomultiplier tube. To measure CD spectra under actinic illumination, a 1-cm light pipe was placed on one side of the cuvette to permit cross-illumination of the sample. All spectra were recorded at room temperature.

For phototrapping experiments, the sample, kept anaerobic as described above, was illuminated with white light provided by a 500-W tungsten projection lamp. The beam was focused through a heat filter on a 1 cm diameter light guide placed to the side of the sample cuvette. The light intensity at the surface of the cuvette was 6.0×10^6 erg/cm^2 s. There were no measurable changes in the temperature of the sample following illumination. Appropriate light filters were used to prevent actinic light from reaching the photomultiplier tube.

Table 1: Singlet−Singlet Interaction Energies in cm^{-1} and Diagonal Energies[a] Relative to 12 500 cm^{-1} for the *Ectothiorhodospira* sp. RC

	SP_A	SP_B	B_A
SP_A	−401[a]	339	−54.8
SP_B	339	−401[a]	−146
B_A	−54.8	−146	40[a]

[a] These diagonal energies are not calculated, but are chosen to fit the data.

The experimental rotational (R) and dipole (D) strengths were obtained by integrating the circular dichroism and absorption spectra, respectively (Schellman, 1975).

$$R = 0.248 \int ((\epsilon_L - \epsilon_R)/\nu)\, d\nu \text{ (D magneton)} \quad (1)$$

$$D = 0.00918 \int (\epsilon/\nu)\, d\nu \text{ (D}^2\text{)} \quad (2)$$

where ν is the frequency (s^{-1}), ϵ is the extinction coefficient (M^{-1} cm^{-1}), and ϵ_L and ϵ_R are the extinction coefficients for left and right circular polarized light, respectively.

The theoretical rotational and dipole strengths were calculated from equations derived from the simple exciton theory (Cantor and Schimmel, 1980).

$$R_I = \frac{\pi}{\lambda} \sum_{i,j} C_{Ii} C_{Ij} ((\vec{r}_i - \vec{r}_j) \cdot \vec{\mu}_i \times \vec{\mu}_j) \quad (3)$$

$$D_I = 1 + 2\frac{\pi}{\lambda} \sum_{i,j} C_{Ii} C_{Ij} (\vec{\mu}_i \cdot \vec{\mu}_j) \quad (4)$$

where λ and μ are the peak wavelength and the dipole moment of the monomer band, respectively. The calculations used a dipole strength of 51 D^2 for monomeric Bch. The structure factors $(\vec{r}_i - \vec{r}_j) \cdot \vec{\mu}_i \times \vec{\mu}_j$ and $\vec{\mu}_i \cdot \vec{\mu}_j$ were obtained from the positions and orientations of the Q_Y absorption dipoles within the *Rhodopseudomonas* (*Rp.*) *viridis* RC (Deisenhofer *et al.*, 1985). The mutual orientation between the Q_Y absorption dipoles of the *Ectothiorhdospira* sp. SP was taken to be 133°. C_{Ii} is the amplitude coefficient of the exciton states $|I\rangle = \sum_i C_{Ii}|i\rangle$, where $|i\rangle$ is the excited state of the ith molecule. The C_{Ii} values are calculated from the Hamiltonian, which is represented by a symmetric Q_Y excitonic interaction matrix. The 3 × 3 interaction matrix for the two Bchs of SP and a non-SP Bch is given in Table 1. The Q_Y excitonic interaction energies are calculated from the point monopole expansion of Weiss (1972). The calculated C_{Ii} values for the RC's dimeric and trimeric Bchs are given in Table 2.

RESULTS

Absorption and CD Spectra with Primary Donor Oxidized or Reduced. The RC of *Ectothiorhodospira* sp. has three absorption bands in the near-infrared, with peaks at 755, 802, and 880 nm (Figure 1, top). We will designate these bands as P755, P802, and P880, respectively. Oxidation of the SP elicits a complete bleaching of P880, a slight hyper- and hypsochromicity of P802, and hyper- and bathochromicity

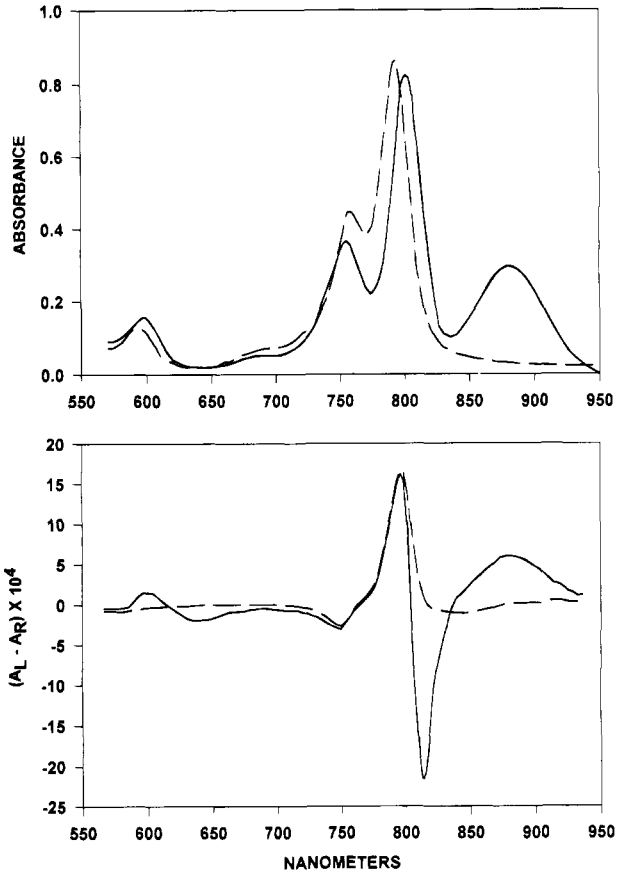

FIGURE 1: Absorption (top) and circular dichroism spectra (bottom) of *Ectothiorhodospira* sp. RC at room temperature, with the primary electron donor in the reduced (solid lines) or oxidized state (dashed lines). Oxidation was obtained with ferricyanide.

Table 2: Amplitude Coefficient Calculated from the Hamiltonian

Special-Pair Dimer

	$\|SP_A\rangle$	$\|SP_B\rangle$
$\|I\rangle$	0.707	0.707
$\|II\rangle$	0.707	−0.707

Special Pair Plus One Intact Non-Special-Pair Bch

	$\|SP_A\rangle$	$\|SP_B\rangle$	$\|B_A\rangle$
$\|I\rangle$	0.359	0.440	−0.821
$\|II\rangle$	0.692	−0.717	−0.085
$\|III\rangle$	0.626	0.537	−0.565

of P755. When the SP is reduced, the CD spectrum (Figure 1, bottom) displays bands with positive rotational strength at 800 and 880 nm and with negative rotational strength at 750 and 814 nm. The CD spectrum from 700 to 950 nm is nonconservative with a net rotational strength of +2.01 D magneton. Oxidation of the SP entails the disappearance of the 820- and 880-nm CD bands, leaving intact the bands at 750 and 800 nm. This loss of rotational strength is approximately the same for the positive (880 nm) and negative (814 nm) CD bands with the result that the CD spectrum remains nonconservative.

Absorption and CD Spectra in the Φ_A^- or Φ_BH_2 State Phototrapped with Dithionite. Very low intensity actinic illumination in the presence of dithionite leads to the trapping of Φ_A^- which, in the *Ectothiorhodospira* sp. RC, lasts only a few seconds at room temperature (Mar & Gingras, 1990). Under these conditions, there occurs a completely reversible

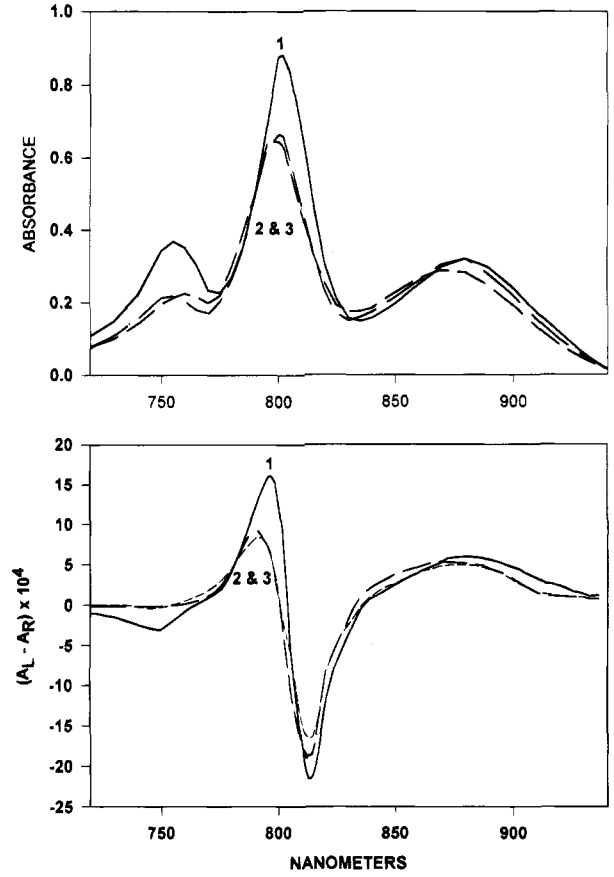

FIGURE 2: Room temperature absorption (top) and CD (bottom) spectra of *Ectothiorhodospira* sp. RC, untreated (1) or trapped in the Φ_A^- state (2) and in the Φ_BH_2 state (3). The sample was suspended in 50 mM Tris HCl/10^{-3} (v/v) Triton X-100/5.7 mM sodium dithionite. *To trap the Φ_A^- form*, the spectra were taken under actinic light (12 erg/cm^2 s) passed through a Corning CS 4-96 filter. The photomultiplier tube was protected from the actinic light with a Schott RG9 filter. *To trap the Φ_BH_2 form*, illumination was supplied as a 1-min pulse of white light at an intensity of 3.25 × 10^6 erg/cm^2 s. The CD spectrum was measured *after* actinic illumination.

bleaching of P540 (not shown), P802, and P755, the latter being only half bleached (Figure 2). The corresponding CD bands at 750 and 800 nm, measured under the same low-intensity illumination, are completely and partially bleached, respectively (Figure 2). The partial bleaching of P755 upon formation of Φ_A^- thus coincides with the complete disappearance of the 750-nm CD band. The first interpretation that comes to mind is that the 750-nm CD band is entirely attributable to Φ_A and not at all to Φ_B. Unexpectedly, that CD band also disappeared after the sole reduction of Φ_B.

A more intense illumination leads to the trapping of Φ_B in a doubly reduced form (Mar & Gingras, 1991), represented here as Φ_BH_2, which has a lifetime of days under reducing and anaerobic conditions. The absorption spectrum of the RC in the Φ_BH_2 state is characterized by a stable bleaching of the Φ_B bands at 530 and 755 nm. The corresponding CD spectrum does not display any 750-nm band and is very similar to the spectrum in the Φ_A^- state (Figure 2). Clearly, the CD band at 750 nm requires the presence of both Φ_A and Φ_B and is not due to their individual or added rotational strengths.

The reduction of either Φ_A or Φ_B has similar effects on the absorption and CD spectra of Bch: P800 in both the Φ_A^- and Φ_BH_2 states undergoes hypo- and hypsochromicity.

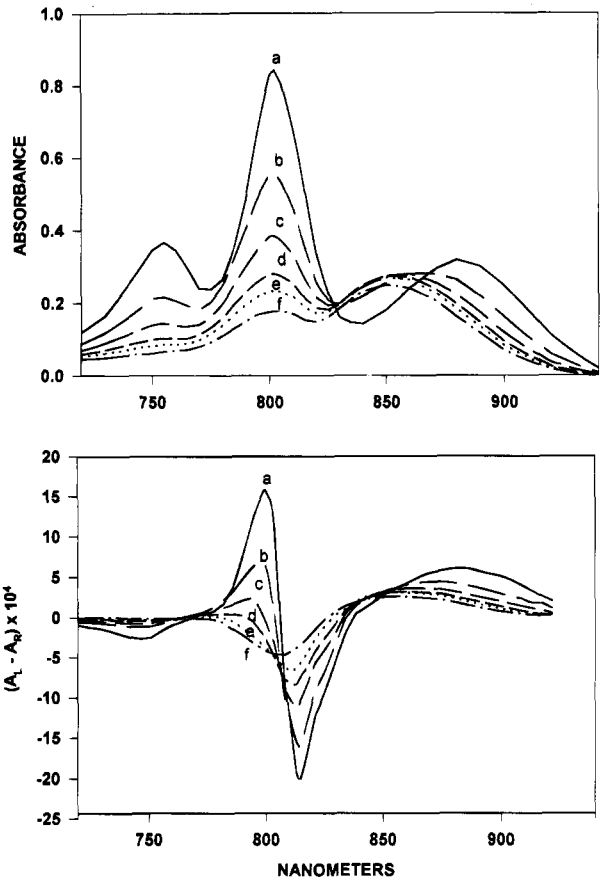

FIGURE 3: Absorption (top) and CD (bottom) spectra of *Ectothiorhodospira* sp. RC taken after different light exposures in the presence of 4.25 mM sodium ascorbate. The spectra were measured following illumination with 3.25×10^6 erg/cm^2 s of white light passing through a heat filter. Exposure periods in minutes: a = 0, b = 17.5, c = 34.5, d = 53.5, e = 69.5, and f = 117.5.

At the same time, the absolute value of the rotational strength shows large and small decreases at 795 and 810 nm, respectively. The CD spectra of both these states remain nonconservative but are more conservative than for state $\Phi_A\Phi_B$. This leaves the possibility that the Bch molecules contribute to the nonconservation of the CD spectrum.

Absorption and CD Spectra in the Φ_A^- or Φ_BH_2 State Phototrapped with Ascorbate. It is possible, after a long light exposure in the presence of dithionite, to obtain conservative CD spectra of the *Ectothiorhodospira* sp. RC (Mar & Gingras, 1988). To verify the generality of this observation, we replaced dithionite with ascorbate, a weaker reductant. As described previously, actinic illumination in the presence of ascorbate also allows the trapping of doubly reduced Bph and non-SP Bch (Mar & Gingras, 1991, 1993). Figure 3 shows the absorption and CD spectra taken after different light exposures in the presence of ascorbate. As with dithionite, P755 and P802 are photobleached, leaving two main bands with peaks at 800 and 850 nm. The phototrapping kinetics are different, however. With dithionite, P755 is bleached in two consecutive steps, the reduction of Φ_B preceding that of Φ_A (Mar & Gingras, 1991). In contrast, with ascorbate, P755 and the 750-nm CD band disappear simultaneously in a single slow step: Φ_AH_2 and Φ_BH_2 are phototrapped simultaneously (Figure 3). The cause of this difference may be that dithionite reduces all four hemes of the RC's cytochrome, whereas ascorbate reduces only the higher potential two C555 hemes (Lefebvre *et al.*,

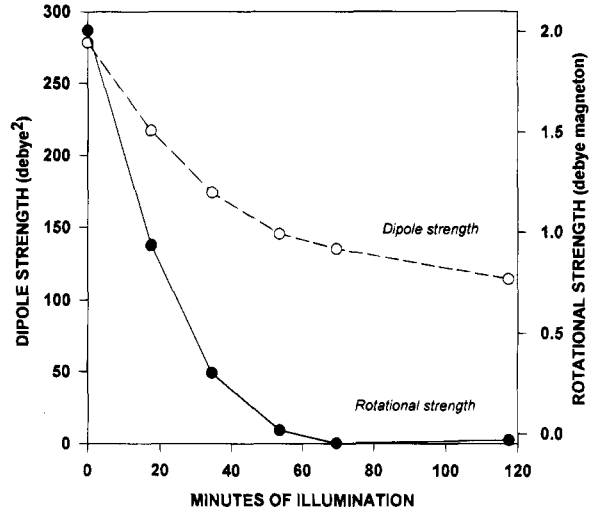

FIGURE 4: Dipole and rotational strengths of *Ectothiorhodospira* sp. RC as a function of light exposure. The rotational and dipole strengths at each light exposure were calculated by integrating the absorption and CD spectra of Figure 3 from 700 to 940 nm.

1984). We know that Φ_A^- is trapped first in both cases, so that with ascorbate, only two electrons per RC being available, Φ_AH_2 would necessarily be the first doubly reduced species to be trapped. Consequently, even if the trapping rate constant of Φ_BH_2 is larger than that of Φ_AH_2, as was shown for dithionite (Mar & Gingras, 1991), the trapping of Φ_AH_2 would become the rate-determining step. With dithionite, four electrons being available per RC, the difference in trapping rate constants would allow for the faster trapping of Φ_BH_2 than of Φ_AH_2. In that case, the four monomers would be trapped at their individual rates.

The total rotational strength, calculated by integrating the CD spectra from 700 to 940 nm, decreases to zero under continued illumination in the presence of ascorbate (Figure 4). As with dithionite, the CD spectra become conservative after a long period of illumination. At the same time, the total dipole strength, calculated by integrating the absorption spectra from 700 to 940 nm, decreases from 280 to 120 D^2 (Figure 4). As a first approximation, ignoring any intensity sharing with higher energy absorption bands, the total dipole strength of the RC is given by the sum of the dipole strengths of its individual Bph and Bch molecules. Assuming that the ratio of the dipole strengths of monomeric Bph/Bch in the RC is 0.65 as it is in acetone/methanol (7/2) (van der Rest & Gingras, 1974), we can attempt to predict how the trapping of these pigments would affect the value of the RC's dipole strength. The elimination of two of the six dipoles—those of Φ_A and Φ_B—would leave the preparation with a dipole strength of 211 D^2, the further elimination of one and then of two of the non-SP Bchs would leave the preparation with dipole strengths of 158 and 106 D^2, respectively. The value of 158 D^2 is reached after approximately 45 min of illumination. As shown in Figure 4, this time corresponds to a rotational strength (0.1 D magneton) approaching zero. After 8 more minutes of illumination, the rotational strength falls to zero. This is consistent with the interpretation that the CD spectrum becomes conservative when the two Bphs and one of the monomeric Bchs have been trapped in the doubly reduced state.

Continued illumination leads to a further bleaching of P800 and of the positive 795-nm and negative 810-nm CD bands. The CD spectra remain conservative throughout this further

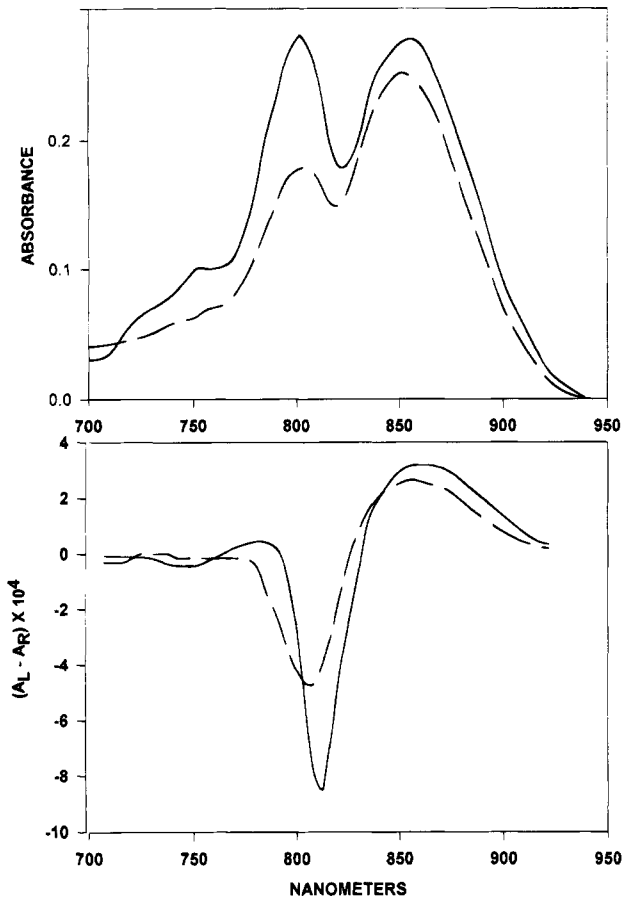

FIGURE 5: Absorption (top) and CD (bottom) spectra of *Ectothiorhodospira* sp. RC taken after 53.5 (solid line) and 117.5 min. (dashed line) of light exposure in the presence of 4.25 mM sodium ascorbate. Experimental conditions as in Figure 3.

bleaching (Figure 4). After 120 min, the bleaching of both the CD and the absorption bands reaches a saturation level that further illumination does not change significantly. According to the simple assumption made in the previous paragraph, this would correspond to the trapping of the two doubly reduced Bphs and of the two non-SP Bchs. Figure 5 compares the absorption and CD spectra taken after 53.5 and 117.5 min of illumination. As both the rotational and dipole strengths remain about the same after 117.5 min, we feel that the spectra taken at that time are due predominantly to the special pair. Note that the absorption spectrum taken at 53.5 min has a larger 800-nm band, which is in line with the idea that this spectrum is that of the SP plus one unreduced non-SP Bch. Both CD spectra are conservative, but the spectrum of the preparation illuminated for 53.5 min has a sharp negative band at 800 nm.

DISCUSSION

The greater rotational strength of the bacteriochlorins in the RC compared to organic solutions may be due to exciton interactions between their Q_Y transition dipoles. If these dipoles underwent only this type of interaction, their CD spectra would be conservative (Tinoco, 1962; Cantor & Schimmel, 1980). The nonconservation of the 750- and 800-nm CD bands, in both the SP and SP^+ states, suggests that the nonconservative component is due to other types of interactions. Such interactions could be with higher energy absorption dipoles, causing hypo- or hyperchromic effects (Scherz & Parson, 1984), or with absorption dipoles of the protein (Cantor & Schimmel, 1980).

Oxidation of the SP should interrupt the alleged excitonic interactions among the six bacteriochlorins, thus destroying the exciton spectrum. The nonconservation of the 750- and 800-nm CD bands in both the SP and the SP^+ states suggests that in both states the nonconservative CD component is due to one or all of the four monomeric Φ_A, Φ_B, B_A, and B_B moieties. Indeed, subtracting the CD spectrum of the SP^+ state from that of the SP state yields a nearly conservative difference CD spectrum (not shown). This suggests that the CD spectrum of the SP state contains a conservative component that is due to the strong excitonic interactions of the SP and that vanishes upon oxidation.

The negative CD band at 750 nm completely disappears when the RC has either one of its two Bphs reduced. *A priori*, this could mean that the 750-nm CD band is due to the interaction between Φ_A and Φ_B. However, the Q_Y interaction energy between them is so small (5 cm^{-1}) that the positive and negative CD bands due to this interaction would cancel out. The negative 750-nm CD band may be due to the rotational strength arising from the mixing of the Q_Y transitions of Bph and Bch. An all-exciton theory (Parson & Warshel, 1987) taking into account the interactions between all the pigments has not accurately predicted the CD spectrum, particularly the 750 nm band. As an added difficulty, this theory would not easily account for the complete cancellation of the contributions of the Bchs to the Bph optical activity, when only one Bph is present. This paper proposes that Bph−protein interaction, which was ignored in the all-exciton model, can account for the large optical activity at 750 nm when both Bphs are present and, also, for the lack of such activity when either one of the Bphs has been reduced.

We attribute the 795-nm CD band to one or both of the non-SP Bch molecules rather than to an exciton band. The decreased optical activity of this band, concomitant with the disappearance of the 750-nm CD band (Figures 2 and 3), probably reflects a RC protein conformational change induced by the reduction of Φ_A or Φ_B. Attributing the 795-nm CD band to protein−Bch interaction also offers an explanation for the nonconservative CD spectrum found in both the SP and SP^+ forms (Figure 1) or when either Φ_A or Φ_B is reduced (Figure 2). Furthermore, this hypothesis is consistent with the conclusions drawn from oriented and from picosecond CD spectroscopy (Mar & Gingras, 1984; Xie & Simon, 1991).

Eliminating the spectral contribution of Φ_A and Φ_B and of either B_A or B_B by trapping them in their doubly reduced state leads to a conservative CD spectrum (Figures 3, 4, and 5). This means that, in this case, the CD bands are due exclusively to excitonic interactions between the Q_Y absorption dipoles of the SP with B_A or B_B. This also implies that, at least in this case, the interaction with the other three excited transitions, Q_X, B_X, and B_Y, of the Bchs contribute very little to the Q_Y CD spectrum. If hypochromic effects are not important for three Bchs, then they should not be important for four. It would be fortuitous, indeed, if a fourth Bch, placed symmetrically with respect to the other three, induced large interactions between the Q_Y and the Q_X, B_X, and B_Y excited transitions of the neighboring Bchs. In other words, the nonconservation of the Q_Y CD spectra in intact RC are most likely due to causes other than pure excitonic interactions.

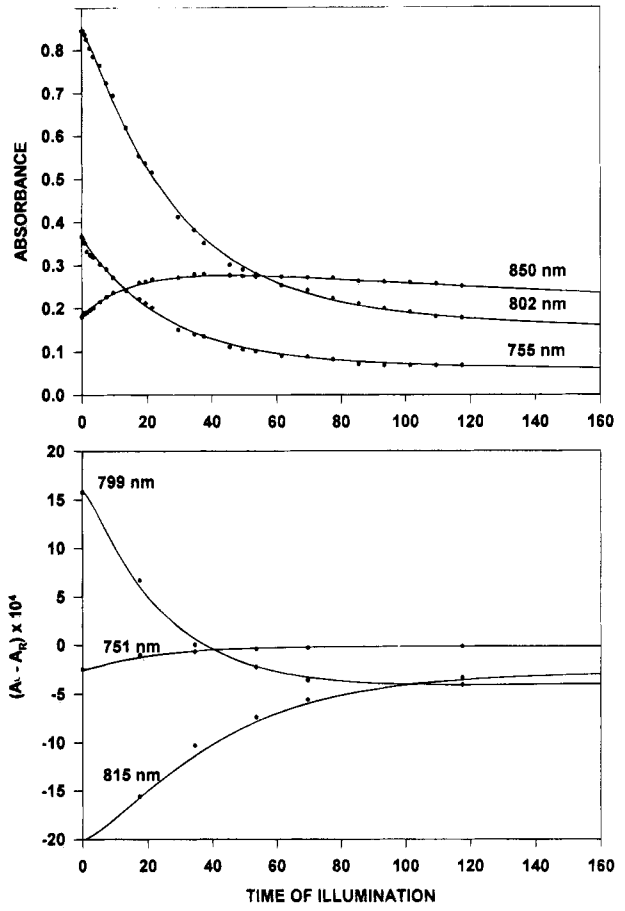

FIGURE 6: Absorption (top) and CD (bottom) kinetic curves of *Ectothiorhodospira* sp. RC taken as a function of the time of illumination (same conditions as in Figure 3). The points are experimental values for absorption and circular dichroism. The lines are calculated kinetic curves based on a model in which the Bph and Bch are phototrapped sequentially (see text).

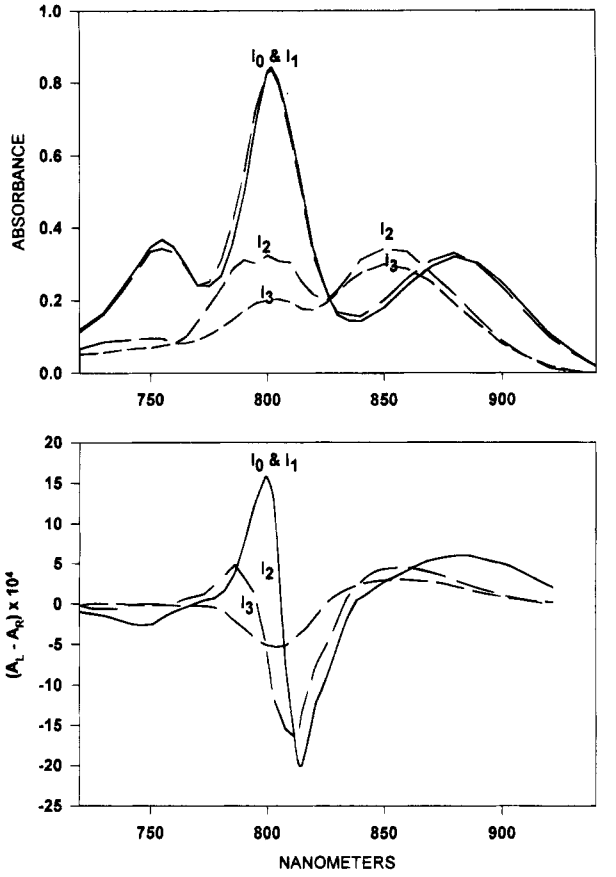

FIGURE 7: Calculated absorption (top) and CD (bottom) spectra of the intermediary states of the *Ectothiorhodospira* sp. RC produced by illumination in the presence of ascorbate. The spectra are labeled I_0 to I_3 to indicate the order of phototrapping.

The absorption and CD spectra of the SP, alone or with one of the non-SP Bchs, can, in theory, be simulated from the arrangement of the bacteriochlorins and the excitonic interactions between their Q_Y dipole moments. To make the comparison, the absorption and CD spectra of these two species must first be extracted from the experimental spectra. The spectral modifications observed after illumination with ascorbate (Figures 3 and 5) are due to the trapping of doubly reduced intermediary states (Mar & Gingras, 1991, 1993). Such spectra are the composite sum of RC's spectra with no phototrapped states (I_0), of some containing only one trapped intermediary state (I_1) and of some containing several intermediary states (I_2, I_3, ...). Each spectrum consists of a statistical distribution of these intermediary states. To find their spectra, we first determined the number of significant components by analyzing the variation of absorption as a function of the time of illumination. Figure 6 shows absorption kinetic curves at 755, 802, and 850 nm and CD kinetic curves at 750, 790, and 810 nm. We found four kinetic components independently of any kinetic model. Our only assumption, to fit the data, was that each of these components was due to an intermediate decaying exponentially into the next (see Appendix). The absorption and CD spectra of each intermediate state at each wavelength were found by matching the calculated and measured kinetic curves (Figure 6). Figure 7 shows the absorption and CD spectra of the intact RC identified as I_0 and of intermediate states I_1, I_2, and I_3 used in these simulations.

The near-infrared spectrum of the first intermediate, I_1, closely resembles that of I_0, since neither of them contains any trapped reduced pigments: I_1 comes from the phototrapping of Q_AH_2 in I_0 (Okamura *et al.*, 1979). I_2, the next intermediary state, not only is devoid of any absorption and CD bands attributable to Bph but also is characterized by a large bleaching of the 800-nm absorption band. I_2 is formed from I_1 by the simultaneous trapping (with a single rate constant) of Φ_AH_2 and Φ_BH_2 and of either B_AH_2 or B_BH_2. The absorption dipoles of I_2 are, therefore, those of the SP plus those of either B_A or B_B. I_3, the third intermediary state, results from the phototrapping of the last non-SP Bch in its doubly reduced state, as evident from the further bleaching of the 800-nm band. The absorption and CD spectra of I_3 thus are those of the special pair. Noticeably, the far-red band of the SP undergoes a 30-nm hypsochromic shift, from 880 to 850 nm, with respect to the original RC. We believe that this blue shift is due to a change in the transition energy of the first excited singlet state of the individual Bch rather than to a change in the interaction energy of the dimer.

The experimental CD spectrum of I_3, corresponding to the SP's, fits well with that calculated from the simple exciton model (Figure 8). The assumptions made in the calculations were that the Q_Y singlet transition energy of the SP's individual Bchs is 12098.7 cm^{-1} and that its dipole strength is 51 D^2. The rotational strength of each CD band was calculated to be 2.3 D magneton from the known structure of the special pair from *Rp. viridis* (Deisenhofer *et al.* 1985).

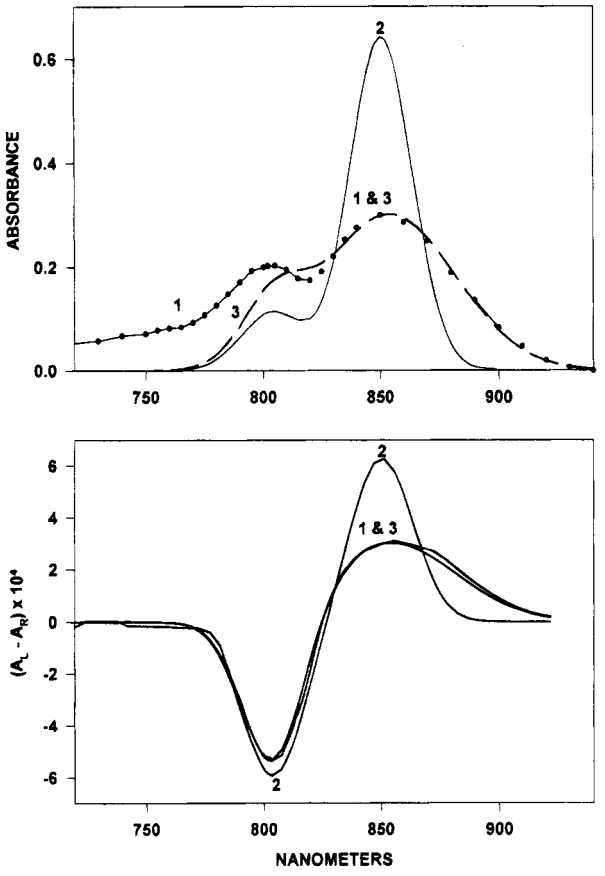

FIGURE 8: Absorption (top) and CD (bottom) spectra of I_3, the special pair: (1) experimental spectrum, (2) calculated spectrum (see text) from the simple exciton model, and (3) the same calculated spectrum but with the lower energy exciton band having a bandwidth of 28 nm.

To better fit the data, the mutual orientation between the Q_Y absorption dipoles of the special pair was taken to be 133°. The angles found for *Rp. viridis* and *Rhodobacter sphaeroides* were 151° and 140° ± 10°, respectively (Knapp et al., 1985; Allen et al., 1987). We found an energy splitting of 678.2 cm^{-1} between the two peaks. Reassuringly, this is twice the calculated interaction energy between the Bchs of the SP. The bands were assumed to be Gaussian, and the bandwidths were assumed to be $1/\sqrt{2}$ of the monomer bandwidth (Hemenger, 1977). The experimental bandwidth of the lower energy band, however, is much greater than predicted for a symmetric exciton (Figure 8). This band broadening may be due to a coupling of the lower energy exciton band to a charge-transfer band of the SP (Parson & Warshel, 1987; Won & Freisner, 1988). Broadening the lower energy band to 28 nm and red-shifting is peak by 3.6 nm, without changing its rotational strength, gives a good fit of the calculated and experimental CD spectra (Figure 8). The same assumptions lead to a good fit between the experimental and calculated absorption spectra of the lower energy exciton band (Figure 8). The dipole strengths of the lower and higher energy exciton bands are calculated to be 86 and 16.3 D^2, respectively. The fit, however, is not as good for the higher energy exciton band. This may be due to the presence of other absorption bands not related to the two exciton bands. These putative absorption bands would have small optical activities and would not be seen in the CD spectrum.

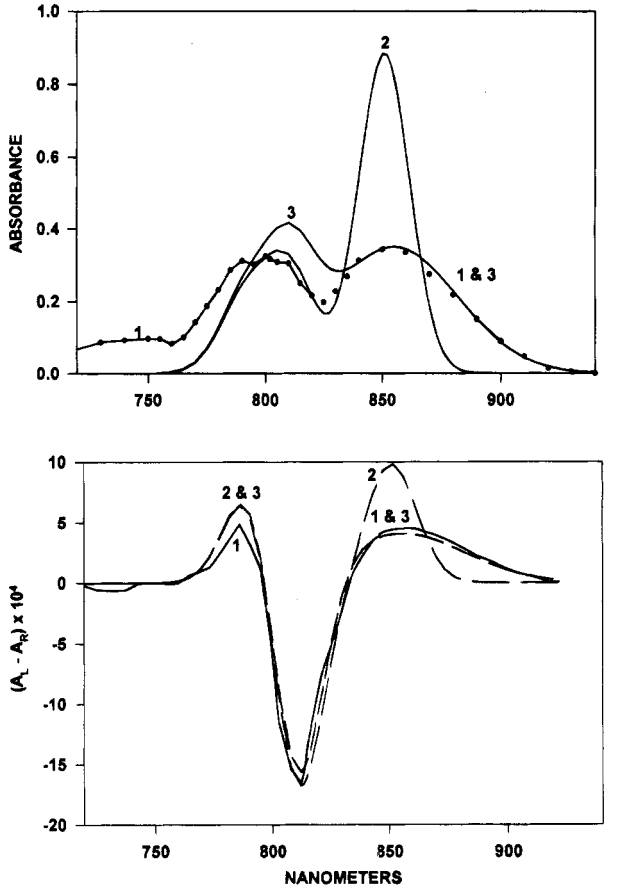

FIGURE 9: Absorption (top) and CD (bottom) spectra of I_2, comprising the special pair with one intact non-special-pair bacteriochorophyll molecule: (1) experimental spectrum, (2) calculated spectrum (see text) from the simple exciton model, and (3) the same calculated spectrum but with the lower energy exciton band having a bandwidth of 27 nm.

The theoretical and experimental CD and absorption spectra of I_2, which consists of the SP and of one non-SP Bch, are shown in Figure 9. I_2 is similar to the RC from *Chloroflexus aurantiacus* (Vasmel *et al.*, 1986), which has three Bchs and three Bphs. The CD spectrum in the *C. aurantiacus* RC is conservative and is in good agreement with the spectrum calculated here by using the simple exciton model (Vasmel *et al.*, 1986; Scherer & Fischer, 1988). The I_2 CD spectrum is also conservative and fits the exciton model (Figure 9). The calculation of the I_2 CD spectrum uses all the parameters previously employed for I_3 and further assumes that the lowest singlet transition energy of monomeric Bch is 12 540 cm^{-1}. Calculation of the rotational strengths of the 791-, 810-, and 851-nm bands yields values of 28.9, −60.6, and 32.5 D magneton, respectively. Diagonalizing the 3 × 3 Hamiltonian shown in Table 1 gives the energy of each peak. The bands were assumed to be Gaussian, and their bandwidths were assumed to be $3^{-1/2}$ of that of the monomer (Hemenger, 1977). Again, exciton theory alone cannot explain the broad bandwidth of the lowest energy transition. As in the case of the SP, this lowest energy exciton band of the trimer may be coupled to a charge-transfer band. By broadening the width of that band to 27 nm without changing its rotational strength and red-shifting its peak by 4.2 nm, the calculated and experimental CD spectra for the trimer fit quite well (Figure 9). The calculated absorption spectrum, however, does not fit as well (Figure 9). We find respective values of 23.9, 33.4, and 96.1

D^2 for the absorption dipoles of the 791-, 810-, and 851-nm bands. Although the fit is quite good for the calculated spectrum of the lowest energy band, it is not as good for the higher energy bands. A better fit could be obtained by moving the two higher energy peaks 5 nm to the blue. However, we have no compelling reasons to assume that the CD and absorption bands do not have the same peaks and widths.

APPENDIX

Simulation of the Spectral Changes as a Function of Light Exposure. The absorbance at wavelength λ, after a light exposure of duration t, is equal to the sum of the absorbance, $A_i(\lambda)$, of each intermediary state, i, multiplied by the probability, $p_i(t)$, of finding the RC in that particular state

$$A(\lambda,t) = \sum p_i(t)A_i(\lambda) \quad (5)$$

We assume that each intermediary state decays exponentially to the next with a rate constant k. The survival probability $p_i(t)$ of a particular intermediary state, i, as a function of the time of illumination should obey the following set of equations:

$$p_0(t) = \exp(-k_1t) \quad (6)$$

$$p_1(t) = \{k_1/(k_2 - k_1)\}\{\exp(-k_1t) - \exp(-k_2t)\} \quad (7)$$

$$p_2(t) = \{k_1k_2/(k_2 - k_1)(k_3 - k_1)(k_2 - k_3)\}\{(k_2 - k_3) \times \exp(k_1t) + (k_3 - k_1)\exp(-k_2t) + (k_1 - k_2)\exp(-k_3t)\} \quad (8)$$

$$p_3(t) = 1 - \{p_0(t) + p_1(t) + p_2(t)\} \quad (9)$$

Numerical values of the survival probabilities, p_i, can be obtained from the rate constant k_i, calculated from the absorption spectra as a function of light exposure (Figure 3). k_2 and k_3 were estimated from a plot of log A_{802} *versus* light exposure. Since some photodestruction follows the bleaching, this must be taken in account by multiplying p_2 and p_3 by $\exp(-k_D)$ where k_D is the photodestruction time constant. A rough estimate of k_D was made from a plot of log A_{850} versus light exposure. We then used eqs 2 to fine-tune the values of the rate constants k_1, k_2, and k_3 and the supposed absorbance values of the intermediary states I_1, I_2, and I_3 such that the calculated absorbance changes with different light exposures fit the measured values at 755, 802, 805, and 880 nm. For phototrapping with ascorbate, the rate constants used are as follows: $k_1 = 0.5$ min^{-1}, $k_2 = 0.042$ min.$^{-1}$, $k_3 = 0.40$ min.$^{-1}$, and $k_D = 0.0015$ min.$^{-1}$. Using these time constants and eqs 1 and 2, the changes in the CD spectra as a function of light exposure (Figure 3) were also simulated. The CD spectra for the intermediary states used are shown in Figure 6.

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