The Asymmetry of P^+ in Bacterial Reaction Centers Revealed by Circular Dichroism Spectroscopy†

John M. Olson,*,‡,§ Mette Miller,‡ and Jan D'Olieslager‖

Institute of Biochemistry, Odense University, DK-5230 Odense M, Denmark, and Katholieke Universiteit Leuven, Departement Scheikunde, B-3001 Heverlee-Leuven, Belgium

Received June 7, 1995; Revised Manuscript Received September 19, 1995⊗

ABSTRACT: The circular dichroism anisotropy, $(A_L - A_R)/A$, has been measured for the far-red absorption band of P^+ in reaction centers of two purple bacteria (*Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*) and one green sulfur bacterium (*Chlorobium tepidum*). The anisotropy values for $P960^+$ (*Rps. viridis*) at 1310 nm was found to be $+(13 \pm 2) \times 10^{-4}$. The corresponding value for $P870^+$ (*Rb. sphaeroides*) at 1250 nm was $+(11 \pm 1) \times 10^{-4}$, but for $P840^+$ (*C. tepidum*) at 1160 nm the value was negative: $-(27 \pm 2) \times 10^{-4}$. These results show that the configuration of the special pair in P840 is significantly different from the configuration in P870 and P960.

The precise orientation of the two bacteriochlorophyll (BChl)[1] *b* molecules constituting the special pair (P960) is known for the reaction center (RC) of the purple nonsulfur bacterium, *Rhodopseudomonas (Rps.) viridis* (Deisenhofer et al., 1984), and similar information has been found for the two BChl *a* molecules making up the special pair (P870) in another purple nonsulfur bacterium, *Rhodobacter (Rb.) sphaeroides* (Allen et al., 1987), but little is known about the orientation of the BChl *a* molecules making up P840 in the RC of the green sulfur bacteria. There is good evidence that P840 is made up of two BChl *a* molecules (Swarthoff et al., 1981b; Wasielewski et al., 1982; Nitschke et al., 1990). These BChl *a* molecules are penta-coordinated, and the C_2 acetyl and C_9 keto carbonyls are free of hydrogen bonding (Feiler et al., 1995). The oxidized form, $P840^-$, has a weak absorption band at 1160 nm (Olson et al., 1992) similar to the weak absorption bands of $P870^+$ (1250 nm) and $P960^+$ (1300 nm) in *Rb. sphaeroides* and *Rps. viridis*, respectively (Reed, 1969; Olson et al., 1985).

The first indication of asymmetry of P^+ in a reaction center (RC) came when the circular dichroism (CD) anisotropy of the 1300-nm band of $P960^+$ in *Rps. viridis* was shown to be $+(10 \pm 3) \times 10^{-4}$ (Olson et al., 1985). Evidence for a major difference in the asymmetry of $P840^+$ (1160 nm) was discovered in the green sulfur bacterium *Chlorobium (C.) tepidum* where the CD anisotropy was found to be negative (Olson et al., 1992).

Parson et al. (1992) analyzed the transition at 1250 or 1300 nm in purple nonsulfur bacteria by means of a semiempirical molecular orbital treatment. Four basis states were constructed and combined to give four eigenstates. Transitions from the lowest state to each of the higher levels gave three absorption bands. The middle transition corresponded to the experimentally observed band at 1250 or 1300 nm. Although the transitions are forbidden in single BChl *a* molecules, they are allowed in the dimer. More recently Reimers and Hush (1995) have proposed that the absorption band at 1250 or 1300 nm is due to a triplet-coupled absorption occurring on the neutral B_M of the special pair. It is a transition localized on one of the BChl monomers, but whose intensity derives entirely from the inter-BChl coupling. The Reimers−Hush approach gives reasonable predictions for the dipole moments of the 1250- and 1300-nm bands, but the corresponding CD anisotropy values have not been calculated (J. Reimers, private communication).

Mattioli et al. (1991) showed by near-infrared Fourier transform resonance Raman spectroscopy that the unpaired electron in $P870^+$ does not share a molecular orbital common to the two components of the dimer on the time scale of ca. 10^{-13} s. The magnitude of the frequency shift of a keto carbonyl of neutral P870 from 1691 to 1717 cm^{-1} upon $P870^+$ formation strongly suggested that B_L in $P870^+$ carries nearly the full +1 charge.

Davis et al. (1993) further showed by modulation analysis of electron spin echo signals of ^{14}N chlorophyll centers that in $P870^+$ and $P960^+$ the unpaired electron is more localized in one half of the BChl dimer with a ratio of 2:1. Lendzian et al. (1993) further showed by ENDOR and TRIPLE resonance studies that in $P870^+$ the unpaired valence electron is unequally distributed over the two BChl *a* molecules; it occupies B_L about two times as much as B_M. This is attributed to the difference in energies of the highest filled molecular π-orbitals of the monomeric halves, B_L and B_M, which is caused by differences in the environments of the two chlorophylls.

In contrast to the situation in $P870^+$ Rigby et al. (1994) have shown by ENDOR and Special TRIPLE spectroscopies that there is a highly symmetrical distribution of electron

† This research was supported by the Danish Natural Science Research Council (SNF) and the Belgian government (programmatie van het Wetenschapsbeleid).

* Author to whom correspondence should be addressed.

‡ Odense University.

§ Present address: Department of Biochemistry and Molecular Biology, Lederle Graduate Research Center, University of Massachusetts, Amherst, MA 01003, USA.

‖ Katholieke Universiteit Leuven.

⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1995.

[1] Abbreviations: BChl, bacteriochlorophyll; CD, circular dichroism; CD anisotropy, $(A_L - A_R)/A$; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; FMO, Fenna-Matthews-Olson; RC, reaction center; P, primary electron donor; LDAO, lauryldimethylamine oxide; TRIPLE, electron nuclear nuclear triple resonance.

0006-2960/95/0434-15230$09.00/0

spin density on the two BChl *a* molecules of $P840^+$ in membranes of *C. limicola*. This conclusion has been confirmed by Feiler et al. (1995) using near-infrared Fourier transform Raman spectroscopy.

Intuitively it seems as though there should be a connection between the distribution of the unpaired electron in P^+ and the CD anisotropy (asymmetry) of P^+. One purpose of this paper is to investigate this possible connection. The other purpose is to demonstrate the difference in configuration between P^+ in two purple nonsulfur bacteria (*Rps. viridis* and *Rb. sphaeroides*) and P^+ in a green sulfur bacterium (*C. tepidum*).

EXPERIMENTAL PROCEDURES

Preparation of Reaction Centers. RCs from *Rps. viridis* were prepared with lauryldimethylamine oxide (LDAO) as described by Thornber et al. (1981) and Olson et al. (1985). The RCs in 0.1% Nonidet P-40, 150 mM NaCl, and 50 mM Tris-Cl (pH 8.0) were concentrated to $A_{960} \sim 8$ with an Amicon ultrafiltration cell (25 000 kDa cutoff) and then dialyzed against 99.7 % D_2O. RC (P960 reduced) concentrations were estimated from $\epsilon_{960} = 100\ mM^{-1}$ (Olson et al., 1985).

Rhodobacter sphaeroides strain R26.1 (kindly provided by R. A. Niederman of Rutgers University) was grown at 28 °C on succinate medium (Schenck et al., 1982). The cells were harvested by centrifugation and stored at −50 °C until use. Crude RC complexes were prepared with LDAO according to a procedure worked out by Peter Gast of Leiden University and similar to that used for RCs from *Rps. viridis*. The final value of A_{855} was ca. 90 for a lightpath of 1 cm, and the RC concentration was estimated to be 90 μM from the magnitude of the photochemical bleaching ($\Delta\epsilon = 20\ mM^{-1}$) at 600 nm.

FMO−RC complexes from *C. tepidum* were prepared with lauryl maltoside as described by Feiler et al. (1992) and modified by Miller et al. (1994). During purification on the Q-Sepharose column, the FMO−RC complex was eluted with 50 mM Tris-HCl (pH 8.0) containing 0.05 % lauryl maltoside and varying amounts of NaCl. Between 50 and 200 mM NaCl a broad band was eluted. The composition of the FMO−RC complex was not uniform over this band, and it was divided into two fractions: *A* (50−100 mM NaCl) and *B* (150−200 mM NaCl). Both fractions were photochemically active, and both were examined in our study as samples *A* and *B*. The FMO−RC complexes were concentrated to an absorbance A_{810} of ca. 30 (1 cm) and dialyzed against D_2O. The RC concentration was estimated to be ca. 8 μM from the photochemical bleaching at 840 nm ($\Delta\epsilon = 100\ mM^{-1}$).

Methods. Absorption spectra were recorded on an AVIV Spectrophotometer Model 17DS UV-VIS-IR, and CD spectra were recorded on an AVIV Circular Dichroism Spectrophotometer Model 41MCD. The CD spectrophotometer is a single-beam instrument with a long optical path and several lenses. Sample and reference have to be measured sequentially and are very sensitive to temperature changes. CD spectra were recorded in 1- or 2-nm steps with 1 s averaging at each wavelength. Spectral bandwidth was 6 nm. Samples (1.5−2.0 mL) were chemically oxidized with microliter aliquots of 1.0 M potassium ferricyanide and chemically reduced with 1.0 M sodium ascorbate. The temperature was between 20 and 25 °C.

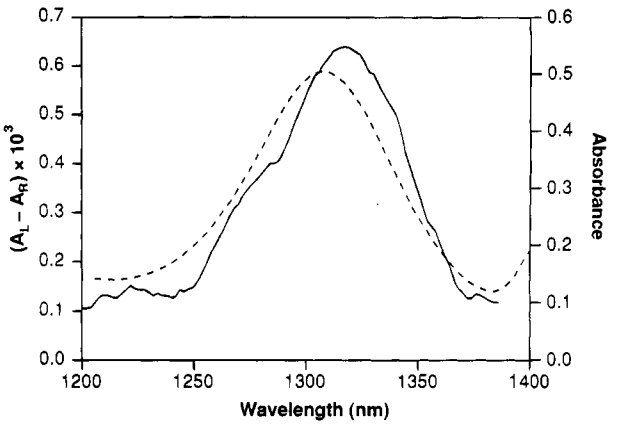

FIGURE 1: Absorption (dashed curve) and CD (solid curve) difference spectra for the 1310-nm band of $P960^+$ in the RC of *Rps. viridis*. [RC] ≈ 30 μM. The difference spectra are ferricyanide oxidized-minus-no additions (see text for details). The CD difference spectrum has been smoothed as described under Experimental Procedures.

The software provided with these instruments permits an increased sensitivity by smoothing the spectra using a polynomial smoothing (Savitsky & Golay, 1964). The order of the polynomial and the number of data points included in the window can be independently selected for each data set. The smoothing operation was performed on each of the CD spectra shown using a 3rd-order polynomial and a multipoint window. The size of the window for each data set was chosen to minimize the residuals. For each spectrum the residual series was checked for randomness (Bennett & Franklin, 1967).

RESULTS

RCs from Rps. viridis. The original preparation contained P960 in the reduced state as judged by the absence of an absorption band at 1310 nm. Absolute absorption and CD spectra of the RCs in the reduced and oxidized state were recorded between 1200 and 1400 nm. Fully oxidized P960 (maximal absorbance at 1310 nm) was obtained by addition of 5 μL of 1 M potassium ferricyanide to a 2.0-mL sample. Fully reduced P960 was regenerated by the addition of 5 μL of 1 M sodium ascorbate.

Oxidized-minus-reduced difference spectra were obtained by subtracting the absolute spectrum (absorbance or CD) of the sample before any additions from the absolute spectrum (absorbance or CD) of the sample in the presence of 5 μL ferricyanide. The two difference spectra in Figure 1 peak at 1310 nm and 1320 nm, respectively, and have about the same shape within the limits of error. The CD anisotropy, $(A_L - A_R)/A$, can be calculated directly to be $+(13 \pm 2) \times 10^{-4}$ at 1310 nm. The main source of error is the uncertainty in estimating the "baseline" of the CD band.

Crude RC Complexes from Rb. sphaeroides. These complexes contain antenna chlorophyll in addition to the RC. The ratio of A_{857}/A_{802} was about 2.0, which is about 4 times that for pure RCs. Nevertheless, these crude RC complexes were suitable for absorbance and CD measurements in the near-infrared where $P870^+$ has its 1250-nm absorption band. Therefore, absolute absorption and CD spectra were recorded from 1150 to 1350 nm.

A 2-mL sample of crude RC complexes was titrated with 1-μL aliquots of ferricyanide up to 8 μL. This was sufficient

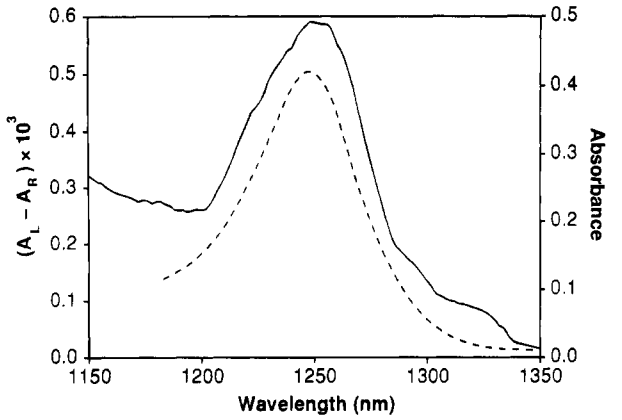

FIGURE 2: Absorption (dashed curve) and CD (solid curve) difference spectra for the 1250-nm band of $P870^+$ in the crude RC complex of *Rb. sphaeroides*. [RC] $\approx$ 25 μM. The difference spectra are ferricyanide oxidized-minus-ascorbate reduced (see text details). Other conditions as in Figure 1.

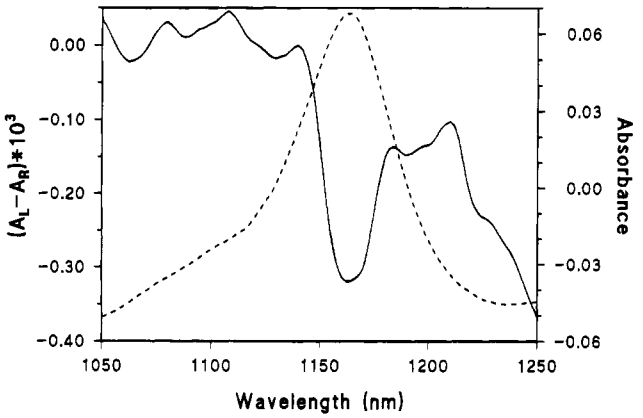

FIGURE 3: Absorption (dashed curve) and CD (solid curve) difference spectra for the 1160-nm band of $P840^+$ in the FMO–RC complex (sample *A*) of *C. tepidum*. [RC] $\approx$ 7 μM. Other conditions as in Figure 2.

to give almost complete oxidation of P870, and absolute absorbance and CD spectra were recorded. Addition of 5 μL of 1 M ascorbate restored P870 to the fully reduced state. Oxidized-minus-reduced difference spectra were obtained by subtracting the absolute spectrum (absorbance or CD) of the sample in the presence of ascorbate from the absolute spectrum (absorbance or CD) of the sample in the presence of 8 μL of ferricyanide. The two difference spectra in Figure 2 both peak at 1250 nm and have about the same shape. The CD anisotropy can be seen to be $+(11 \pm 1) \times 10^{-4}$ which is about the same as the anisotropy for RCs from *Rps. viridis*.

FMO–RC Complexes from C. tepidum. The RC concentrations in samples *A* and *B* (1.5 mL) were substantially less than the RC concentrations in the samples from *Rps. viridis* and *Rb. sphaeroides*. This meant that the signal-to-noise ratio in the CD spectra was substantially less than in the CD spectra obtained from the earlier samples. Initial absolute absorption and CD spectra were recorded from 1050 to 1250 nm. For sample *A*, subsequent spectra were recorded after each of 4 additions of 0.5 μL of ferricyanide and a final addition of 2 μL of ascorbate. The oxidized-minus-reduced difference spectra shown in Figure 3 were obtained by subtracting the absolute spectra (2 μL of ascorbate) from the absolute spectra (2 μL of ferricyanide). For sample *B* spectra were recorded after the addition of 1 μL of ferricyanide and again after addition of 1 μL of ascorbate. The difference spectra for sample *B* shown in Figure 4 correspond to those for sample *A* in Figure 3. Taken together, these two figures show that the CD band is negative at 1160 nm and that the anisotropy is $-(27 \pm 2) \times 10^{-4}$. Because of the poor resolution of the CD spectra, it is impossible to say whether the shape of the CD band is the same as that of the absorption band.

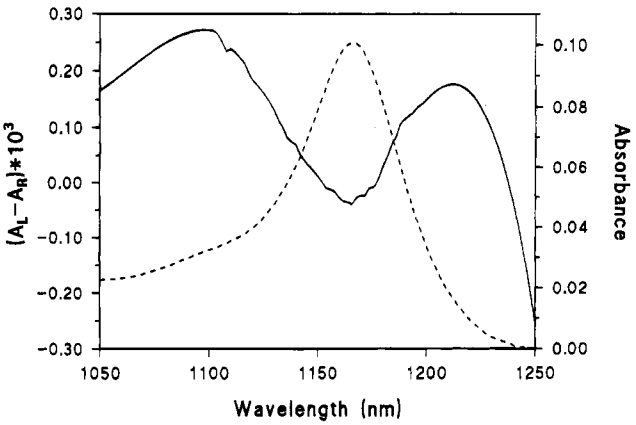

FIGURE 4: Same as Figure 3 except that sample *A* has been replaced by sample *B*. [RC] $\approx$ 6 μM. See text for details.

Table 1: Summary of CD Anisotropy Values

bacterium	P/P$^+$	λ (nm)	anisotropy $\times 10^4$	reference
Rps. viridis	P960	955	+33	Philipson and Sauer (1973)
	$P960^+$	1300	$+(10 \pm 3)$	Olson et al. (1985)
		1310	$+(13 \pm 2)$	present work
Rb. sphaeroides R26	P870	865	+24	Sauer et al. (1968)
R26.1	$P870^+$	1250	$+(11 \pm 1)$	present work
C. limicola	P840	842	+30	Olson et al. (1973)
C. tepidum	$P840^+$	1160	−(5 or 8) (corrected)	Olson et al. (1992)
			$-(27 \pm 2)$	present work

In principle, the difference spectra for samples *A* and *B* should be the same, but Figures 3 and 4 are sufficiently different to merit some comment. First the CD difference spectra are much smaller than any of the absolute spectra. Secondly small temperature changes in the apparatus between additions of ferricyanide or ascorbate may have introduced artifacts in the absolute spectra—artifacts not canceled out by subtraction of the absolute spectra. The CD difference spectra shown in Figures 3 an 4 both show a distressing dip in the baseline at 1250 nm. This dip is thought to be an instrumental artifact perhaps associated with inadequate temperature control, but the exact cause is not known. [Previous work (Olson et al., 1973) indicates that the CD difference spectrum for $P840^+$ is close to zero at 1250 nm.]

These results are summarized in Table 1.

DISCUSSION

Comparison with Previous Results. The anisotropy value of $+(13 \pm 2) \times 10^{-4}$ found for $P960^+$ is in good agreement with the value of $+(10 \pm 3) \times 10^{-4}$ determined previously (Olson et al., 1985), but the value of $-(27 \pm 2) \times 10^{-4}$ found for $P840^+$ is about 40 times the values of $-(5$ or $8) \times 10^{-5}$ determined previously (Olson et al., 1992). (The

value of -5×10^{-5} was found for the light-induced oxidation of P840, while the value of -8×10^{-5} was found for the chemical oxidation of P840.) If one compares the experiment on $P840^+$ in 1992 to the present work, one finds that the FMO−RC complexes were prepared in the same way from *C. tepidum*. In the 1992 experiment the chemically oxidized-minus reduced difference spectra gave a ΔA of ca. 0.15 and a $\Delta(A_L - A_R)$ of ca. -8×10^{-6} at 1160 nm. In the present work the difference spectra (Figure 4) give a ΔA of 0.11 and a $\Delta(A_L - A_R)$ of -2.9×10^{-4}.

Reexamination of the calibration procedure used for the 1992 experiment revealed an error in the calculation of $A_L - A_R$ at 660 nm for the sample of chlorophyll *a* in diethyl ether ($A = 1.06$) used for the calibration $A_L - A_R = (A/\epsilon) \times (\epsilon_L - \epsilon_R)$. From Houssier and Sauer (1970), $\epsilon = 86.3 \times 10^3$ M^{-1} cm^{-1} and $\epsilon_L - \epsilon_R = -13.8$ M^{-1} cm^{-1}. Thus $A_L - A_R = -1.7 \times 10^{-4}$ (the erroneous value used in the 1992 experiment was -1.7×10^{-5}). This means that the corrected values for anisotropy based on the 1992 work are $-(5$ or $8) \times 10^{-4}$. The value based on the present work is still about 4 times larger than the corrected 1992 values. We cannot explain the remaining differences.

Comparison of P960 and P870. The X-ray crystal structure determinations of the RCs of *Rps. viridis* and *Rb. sphaeroides* show that the configurations of the two BChl molecules in P960 and P870 are very similar, but not identical. In the first place P960 is composed of two BChl *b* molecules, while P870 is composed of two BChl *a* molecules. Although the center-to-center distance between B_L and B_M is almost the same in the two species, the interaction energy is larger in P960 because the two BChls are closer together where they overlap in ring I (Breton et al., 1992).

Ermler et al. (1994) have further pointed out that in P960 (*Rps. viridis*) the ring I acetyl carbonyl oxygens of B_L and B_M are H-bonded to His L168 and Tyr M195, respectively. In P870 (*Rb. sphaeroides*) a similar H-bond is formed between the conserved His L168 and the ring I acetyl of B_L. In contrast the residue at the symmetry-related position on the M-side, Phe M195, cannot form a H-bond to B_M. Thus the symmetry in H-bonding to the ring I acetyl groups in the BChl dimer of P960 is not preserved in P870. No other H-bond to the special pair is found in P870. In P960 Thr L248 forms a H-bond with the keto carbonyl of ring V of B_L, while in P870 L248 is a Met residue that cannot form a H-bond.

Since the CD anisotropy values for $P960^+$ and $P870^+$ are so similar, it appears that the difference in BChl species and the difference in H-bonding pattern are not very important for the CD anisotropy. We tentatively assume that the overall configuration of the special pair is responsible for the sign and magnitude of the anisotropy. The magnitude $[(11-13) \times 10^{-4}]$ is large compared to the values for the Q_y band of monomeric chlorophylls [BChl *a* or *b* in ether ($\leq 0.6 \times 10^{-4}$), BChl *a* in CCl_4 + pyridine (2.0×10^{-4}) (Olson et al., 1985), or chlorophyll *a* in ether (-1.6×10^{-4}) (Houssier & Sauer, 1970)], but is of the same order of magnitude as that found for the Q_y band of P in bacterial reaction centers ($20-30 \times 10^{-4}$; see Table 1). We ascribe the anisotropy to the asymmetry of the special pair with the positive charge (unpaired electron) localized on one BChl only. Since an electron jumps back and forth from one BChl to the other, the anisotropy measured should be the average for the two locations of the hole. We assume that the configuration of the two BChls in the special pair is sufficiently symmetrical that the anisotropy of $B_L{\cdot}B_M^+$ is essentially the same as the anisotropy of $B_L^+{\cdot}B_M$. The positive sign on the anisotropy means that the 1250/1310-nm transition has a large magnetic dipole (helical character) and a preference for left circular polarized light. We suppose the position of the neutral BChl with respect to the positively charged BChl determines the sign and magnitude of the magnetic dipole and the preference for left or right circular polarized light.

Configuration of P840. The RCs of green sulfur bacteria are quite different from the RCs of purple bacteria in many respects. This can be seen from a comparison of the spectral characteristics of the two kinds of RCs. The oxidized-minus-reduced difference spectrum of P840 shows two relatively narrow negative peaks at 830 and 842 nm (Olson et al., 1976), whereas the difference spectrum for P960 or P870 shows a single relatively broad negative peak. The reduced RC-core spectrum for *C. tepidum* at 77 K shows two narrow bands at 833 and 836 nm, a narrow band at 817 nm, and lesser band at 795 nm (Miller et al., 1994), whereas the reduced spectrum for the RC from *Rb. sphaeroides* at 77 K shows a broad band at 890 nm, a band at 802 nm, and a lesser band at 756 nm (Lockhart & Boxer, 1988). Analysis of the light-induced absorption difference spectra of P840 and of RC triplet formation in *Prosthecochloris aestuarii* at 80 K indicates that the bleaching of the P840 band at 836 nm is accompanied by band shifts of BChls (in the RC-core complex) absorbing at 797, 816, and 833 nm. The pigments responsible for these absorption changes, P840 and the BChl *a* absorbing at 833 nm, have about the same orientation as the bulk pigments absorbing in this wavelength region with the Q_y transitions more or less parallel to the membrane and the Q_x transitions more or less perpendicular to the membrane. (Swarthoff et al., 1981a).

In contrast to the RC of purple bacteria which contains L and M protein subunits (31 and 36 kDa), the RC of green sulfur bacteria contains a single large subunit of about 65 kDa (Buettner et al., 1992). The implication is that the RC of green sulfur bacteria is a homodimer with one BChl *a* of P840 bound to each large protein subunit. The configuration of the special pair of BChl molecules in P840 is not known, but the anisotropy data make it clear that the configuration is distinctly different from that in the purple bacteria. The negative sign shows that the 1160-nm transition has a negative magnetic dipole and that the helical character of the transition has the opposite sense to that of the transition in $P960^+$ and $P870^+$.

Relationship between CD Anisotropy and the Distribution of Charge over P^+. It would appear that the CD-anisotropy is independent of the average distribution of charge over P^+. In the case of $P870^+$ in isolated RCs from *Rb. sphaeroides* the anisotropy is $+11 \times 10^{-4}$ and the positive charge distribution is 2:1 in favor of B_L (Lendzian et al., 1993). In *Rhodospirillum (Rs.) rubrum* RCs the charge distribution is 1.6:1 in favor of B_L, and in RCs of *Rb. capsulatus* and *Rs. centenum* the charge is localized mainly on B_L (Rautter et al., 1994). The internal geometry of the two BChls of the special pair was found to be quite similar in all four RCs. In contrast to the results obtained for isolated RCs, the charge distribution of P^+ is identical in chromatophores of all four bacterial species investigated and corresponds to a ratio of

2:1 in favor of B_L. It seems likely that the anisotropy values for $P870^+$ in RCs of all purple bacteria would be about the same because of the strong conservation of structure in the L, M, and H subunits of the RC. The wide variation in distribution of charge over P^+ in isolated RCs from these four purple bacteria suggests that the distribution of charge is highly sensitive to the microenvironment of the RC, whereas the anisotropy is probably not. Finally it should be mentioned that the anisotropy measurements are made on a time scale of 10^{-15} s, while EPR, ENDOR, and TRIPLE resonance measurements are made on a time scale of 10^{-10} s. The optical measurements show the state of P^+ when the charge is localized on one BChl or the other. The microwave measurements show the average state of P^+ on a longer time scale.

In the case of $P840^+$ in the RC from the green sulfur bacterium *C. tepidum* the CD anisotropy is -27×10^{-4}, and the positive charge is equally shared by both BChls of the special pair (Rigby et al., 1994; Feiler et al., 1995). Both phenomena result from the loss of an electron by P840, but beyond that they are largely independent. The anisotropy is the result of the configuration of the special pair and the fact that the positive charge is localized on one BChl or the other during the time scale of the method. On the time scale of microwave spectroscopy, the delocalization of the single charge over both members of the special pair, while depending somewhat on the configuration of the special pair, depends primarily on the microenvironment of each BChl. Since the RC in this case is a homodimer, one might expect the microenvironment of the two BChls to be the same.

Finally, it is interesting to compare the anisotropy values (Table 1) for the bands at 1160, 1250, and 1310 nm in P^+ to the values for the corresponding bands at 842, 865, and 955 nm in P. In the nonoxidized reaction centers all values are positive, but in $P840^+$ the value is negative. This means that in the reaction center of green sulfur bacteria the anisotropy of the major infrared band changes from positive to negative upon oxidation of P840.

ACKNOWLEDGMENT

We thank Robert A. Niederman (Rutgers University) for providing a culture of *Rb. sphaeroides* R26.1, and Dorte Michaelsen for growing the bacteria. We thank Peter Gast (Leiden University) for telling us how to prepare crude RC complexes, and Jack Pedersen for preparing them. We thank Kim Lambertsen Larsen and especially Raymond Cox for help with figure preparation. We thank Jeff Reimers and Tony Mattioli for sending us manuscripts before publication. Finally we thank Jack Aviv (Aviv Associates) for putting J.M.O. and J.D. in touch with each other.

REFERENCES

Allen, J. P., Feher, G., Yeates, T. O., Komiya, H., & Rees, D. C. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5730−5734.

Bennett, C. A., & Franklin, N. L. (1967) *Statistical Analysis in Chemistry and the Chemical Industry*, pp 667−684, John Wiley, New York.

Breton, J., Nabedryk, E., & Parson, W. W. (1992) *Biochemistry 31*, 7503−7510.

Davis, I. H., Heathcote, P., MacLachlan, D. J., & Evans, M. C. W. (1993) *Biochim. Biophys. Acta 1143*, 183−189.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., & Michel, H. (1984) *J. Mol. Biol. 180*: 385−398.

Ermler, U., Michel, H., & Schiffer, M. (1994) *J. Bioenerg. Biomembr. 26*, 5−15.

Feiler, U., Nitschke, W., & Michel, H. (1992) *Biochemistry 31*, 2608−2614.

Feiler, U., Albouy, D., Robert, B., & Mattioli, T. A. (1995) *Biochemistry 34*, 1099−1105.

Houssier, C., & Sauer, K. (1970) *J. Am. Chem. Soc. 92*, 779−791.

Lendzian, F., Huber, M., Isaacson, R. A., Endeward, B., Plato, M., Bonigk, B., Mobius, K., Lubitz, W., & Feher, G. (1993) *Biochim. Biophys. Acta 1183*, 139−160.

Lockhart, D. J., & Boxer, S. G. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 107−111.

Mattioli, T. A., Hoffman, A., Robert, B., Schrader, B., & Lutz, M. (1991) *Biochemistry 30*, 4648−4654.

Miller, M., Cox, R. P., & Olson, J. M. (1994) *Photosynth. Res. 41*, 97−103.

Nitschke, W., Feiler, U., & Rutherford, A. W. (1990) *Biochemistry 29*, 3834−3842.

Olson, J. M., Philipson, K. D., & Sauer, K. (1973) *Biochim. Biophys. Acta 292*, 206−217.

Olson, J. M., Prince, R. C., & Brune, D. C. (1976) *Brookhaven Symp. Biol. 28*, 238−245.

Olson, J. M., Trunk, J., & Sutherland, J. C. (1985) *Biochemistry 24*, 4495−4499.

Olson, J. M., MIller, M., Trunk, J. G., Polewsky, K., & Monteleone, D. (1992) in *Research in Photosynthesis, Vol. 1* (Murata, N., Ed.) pp 401−404, Kluwer, Dordrecht.

Parson, W. W., Nabedryk, E., & Breton, J. (1992) in *The Photosynthetic Bacterial Reaction Center II* (Breton, J., & Vermeglio, A., Eds.) pp 79−88, Plenum, New York.

Rautter, J., Lendzian, F., Lubitz, W., Wang, S., & Allen, J. P. (1994) *Biochemistry 33*, 12077−12084.

Reed, D. W. (1969) *J. Biol. Chem. 244*, 4936−4941.

Reimers, J. R., & Hush, N. S. (1995) *J. Am. Chem. Soc. 117*, 1302−1308.

Rigby, S. E. J., Thapar, R., Evans, M. C. W., & Heathcote, P. (1994) *FEBS Lett. 350*, 24−28.

Sauer, K., Dratz, E. A., & Coyne, L. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61*, 17−24.

Savitsky, A., & Golay, M. J. E. (1964) *Anal. Chem. 36*, 1627−1639.

Schenck, C. C., Blankenship, R. E., & Parson, W. W. (1982) *Biochim. Biophys. Acta 680*, 44−59.

Swarthoff, T., Amesz, J., Kramer, H. J. M., & Rijgersberg, C. P. (1981a) *Isr. J. Chem. 21*, 332−337.

Swarthoff, T., Gast, P., & Hoff, A. J. (1981b) *FEBS Lett. 127*, 83−86.

Thornber, J. P., Seftor, R. E. B., & Cogdell, R. J. (1981) *FEBS Lett. 134*, 235−239.

Wasielewski, M. R., Smith, U. H., & Norris, J. R. (1982) *FEBS Lett. 149*, 138−140.

BI951277S