

# SPECTROSCOPIC DETERMINANTS IN THE REACTION CENTER OF *RHODOPSEUDOMONAS VIRIDIS*

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**ABSTRACT** Assignments are proposed for the long wavelength absorption bands observed in the reaction center of *Rhodopseudomonas viridis*. The assignments are based on a theoretical treatment in which quantum mechanical calculations are first carried out on the individual chromophores of the reaction center. The energies and wave functions that are obtained are then introduced into an exciton-type perturbation treatment in which extensive configuration interaction is carried out between the excited states of the four bacteriochlorophylls and two bacteriopheophytins of the reaction center. Calculated values for absorption maxima, transition moments, linear dichroism, and rotational strength are compared with experiments in an attempt to distinguish among different assignments. The calculations alone do not lead to unambiguous assignments; indeed it is difficult to account for the reaction center spectra without introducing assumptions as to the effects of the protein on the energy levels of the individual molecules. Even if these effects are treated as free parameters, the experimental spectra still provide useful constraints that restrict the models that are possible. The major result of this work is that the weak 850-nm absorption band is due, primarily, to the higher energy exciton state of the bacteriochlorophyll special pair. Accounting for the 960-nm absorption band of the low energy exciton state of the special pair requires either that a large spectroscopic effect of the protein be introduced, or possibly, that charge transfer states play a major spectroscopic role. The difference in spectra seen in the formation of oxidized or triplet state reaction centers can be understood in terms of a combination of electrochromic effects and modified exciton interactions.

## INTRODUCTION

The problem of accounting for the absorption spectra of bacterial photosynthetic reaction centers has been a subject of considerable interest for some time (see Parson, 1982 and Pearlstein, 1987 for reviews). The reaction centers contain six chromophores, four bacteriochlorophylls (BChl), and two bacteriopheophytins (BPh), whose near infrared visible absorption bands are shifted to longer wavelengths than the corresponding bands of the isolated chromophores in solution. The source of these shifts, and of the splitting that is also observed in vivo, have been attributed in the past to both BChl-BChl and protein-BChl interactions. The recent publication of the x-ray analysis of crystals of the reaction center of *Rhodopseudomonas viridis* (Deisenhofer et al., 1984, 1985) now makes it possible to elucidate the nature of the spectroscopic determinants in the reaction center in detailed structural terms.

The geometry of the reaction center chromophores has been discussed extensively by Deisenhofer et al. (1984, 1985). The chromophores are associated with either the L or M protein subunits and are arranged about a pseudo twofold axis of symmetry. After the notation of Deisenhofer et al. (1985) the BChl-b molecules are designated BC<sub>LP</sub>, BC<sub>LM</sub>, BC<sub>LA</sub>, and BC<sub>MA</sub>, where L and M denote the

protein subunit and P and A denote special pair and accessory, respectively. The BPh-b molecules are denoted BP<sub>L</sub> and BP<sub>M</sub>. The special pair is made up of two BChl-b molecules which overlap closely in space and which, after excitation of the 960-nm absorption band, transfers an electron to the BPh-b molecule of the L subunit, BP<sub>M</sub>. Why the transfer of an electron proceeds in only one direction and how the rates of the electron transfer reactions between the reaction center components are controlled are fascinating questions whose answers will require a detailed analysis of the both protein-BChl and BChl-BChl interactions.

In this paper we attempt to assign the absorption spectrum of the *viridis* reaction center to the various electronic states of its constituent chromophores. The assignments are guided by quantum mechanical calculations of transition energies, oscillator strengths, and rotational strengths. While the calculations alone do not permit an unambiguous assignment of the reaction center absorption bands, they do provide important constraints on the interpretation of available spectroscopic measurements in terms of specific models. Before discussing the calculations it is of value to summarize the relevant experimental data and to consider previous assignments that have been made.

The long wavelength absorption spectrum of the *viridis* reaction center at 5°K has been reproduced in Fig. 1. (The data were kindly provided by Dr. J. Breton, see Breton [1985].) The longest wavelength transition centered at ~990 nm (this is the 960-nm room temperature band) corresponds to the lower energy component of a pair of exciton states ( $\nu^+$  and  $\nu^-$ ) which are produced by the splitting of the  $Q_y$  bands of the special pair BChl-bs. There is good experimental evidence that the 990-nm band has some charge transfer character as well (Boxer et al., 1986; Meech et al., 1986). The bands at 790 and 805 nm are due to the two BPh molecules. The intense band at 830 nm, the shoulder at 850 nm (and any postulated weak transition that does not give rise to a distinct peak) must be due to the two accessory BChl-b molecules,  $BC_{LA}$  and  $BC_{MA}$ , as well as to the higher energy exciton component,  $\nu^+$  of the special pair.

Much of the uncertainty concerning band assignments has centered around a few key questions (some of which are presented in the stimulating discussions of Pearlstein [1987] and Parson [1982]). Clearly, the most interesting question concerns the factors that shift the BChl-b absorption band from ~790 nm *in vitro* to 960 nm in the *viridis* reaction center (corresponding to ~2,300  $cm^{-1}$ ). In the past few years this shift has been attributed all or in part to: BChl-BChl exciton interactions (Scherz and Parson,

1985), an environmental shift due to an electric field produced by the protein (Davis et al., 1981; Eccles and Honig, 1983), exciton interactions coupled with a protein-induced shift (Knapp et al., 1985), and exciton interactions plus charge transfer states coupled with a protein-induced shift (Parson et al., 1985). Since all of these factors are likely to play at least some role, the problem becomes one of determining the individual contributions to the total 2,300  $cm^{-1}$  shift.

It is obvious from the reaction center structure that exciton interactions are responsible for at least part of the shift. The size of the effect can be calculated theoretically and a number in the range of 500–1,000  $cm^{-1}$  is predicted (see below). If the remainder of the shift is due solely to protein-induced perturbations, these must account for an additional 1,300–1,800  $cm^{-1}$ . Knapp et al. (1985), in an attempt to fit the spectrum parametrically, assumed a 900- $cm^{-1}$  exciton shift and an additional protein-induced perturbation that shifted the zero order level of the special pair to ~890 nm. The upper exciton level,  $\nu^+$ , was placed at 811 nm. Parson et al. (1985) used an exciton interaction of ~750  $cm^{-1}$  (as estimated from their Fig. 2) and a protein-induced shift from 790 to 830 nm (600  $cm^{-1}$ ). The remainder of the shift to 960 nm could be obtained under certain conditions if charge transfer states were included in the calculations. This result is consistent with the experimental evidence for some charge transfer character in the 960-nm band. However, the spectroscopic importance of possible charge transfer contributions to the 960-nm absorption band is still unclear.

It is likely that it will be difficult to account for the large spectral shift that produces the 960-nm absorption band based on calculations alone. For example, the zero order energies of the charge transfer states are difficult to calculate reliably even *in vacuo* and the susceptibility of these energies to protein-induced shifts is even less certain. However, if the higher energy transitions can be assigned, and in particular if the energy of the  $\nu^+$  state can be determined, these will provide strong constraints on any model that is proposed. The location of  $\nu^+$  has been discussed extensively in the past. The most recent assignments are of Breton (1985) who place it at 850 nm and of Knapp et al. (1985) and Parson et al. (1985) who place it near 800 nm.

The 850-nm assignment (Breton, 1985) was based on linear dichroism measurements and, primarily, the disappearance of the 850-nm band in the state  $P^+$  in which the special pair is oxidized. The other absorption changes seen in going from the initial state, P, to  $P^+$  are the disappearance of the 960-nm band and small (3 nm) blue shifts in the 833-nm band and the two BPh-b bands. The major objection to the 850-nm assignment to  $\nu^+$  is the shape of the difference spectrum between P and the triplet state of the reaction center (Shuvalov and Parson, 1981; Parson, 1982). There is a decrease in intensity ~850 nm and an increase between 830 and 840 nm. These changes were

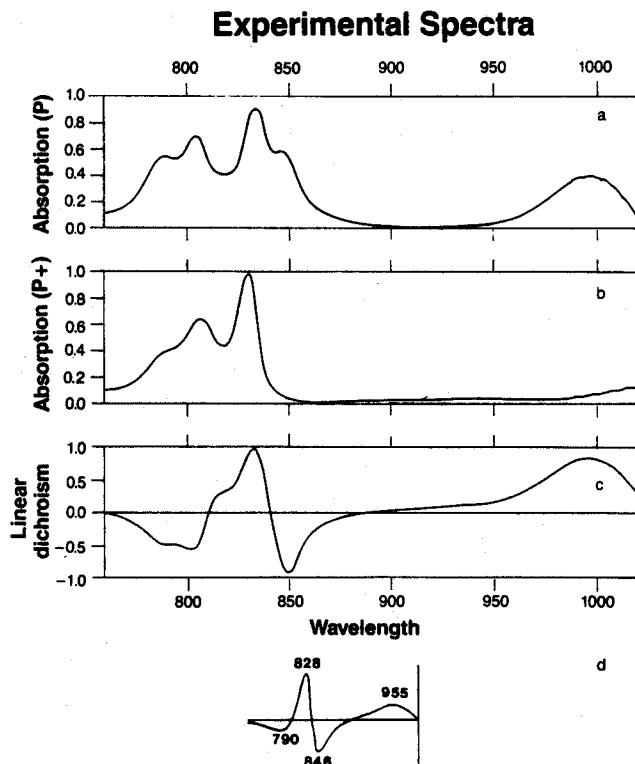


FIGURE 1 Experimental spectra. The data for *a-c* provided by J. Breton (private communication). See also Breton (1985). (*a*) Absorption spectrum of P at 77°K. (*b*) Absorption spectrum of  $P^+$  at 77°K. (*c*) Linear dichroism spectrum of P at 77°K. (*d*) Circular dichroism spectrum of P at 298°K. Redrawn from Philipson and Sauer, 1973.

interpreted in terms of a blue shift of the 850-nm absorption band, which is assigned to an accessory BChl-b. Knapp et al. (1985) also assign the 850-nm band to an accessory BChl-b (but with a strong admixture of special pair intensity).

The analysis presented in this work concludes, in agreement with Breton (1985), that the 850-nm band is due primarily to  $\nu^+$ . Objections that have been raised to this assignment are considered in the discussion. If the 960-nm absorption is due primarily to exciton and protein-induced interactions, our results imply that the protein shifts the zero order energy of the  $Q_y$  state of the special pair BChls to  $\sim 900$  nm,  $\sim 1,500$   $\text{cm}^{-1}$ . This conclusion would have to be modified if the charge transfer states play an important spectroscopic role.

## METHODS

The reliability of semiempirical molecular orbital methods for the calculation of spectral properties of chlorophylls and other porphyrin based compounds has been established (see e.g., Zerner, 1981). The CNDO/S and INDO/S methods have been used to calculate the spectral shifts to due perturbations of the molecule (Eccles and Honig, 1983; Hanson et al., 1987).

In the present case we are interested in spectral shifts due to neighboring chlorophylls and pheophytins. The most direct approach would be to apply the CNDO/S method to the complete set of molecules of interest. Such an approach would quickly become prohibitively time consuming for several chlorophyll sized molecules even if the calculations were feasible. A more important constraint lies in the fact that Hartree-Fock methods are known to be unstable for systems consisting of multiple regions with little bonding interaction between them. This is because the highest filled molecular orbitals are so nearly degenerate that a change in occupation number is sufficient to cause a reordering of the energies and thus induce a change in occupation numbers in the next iteration. The electron density then oscillates in each iteration and convergence is difficult to obtain. While methods do exist to enhance convergence, for a system with several mutually nonbonded regions (i.e., the six reaction center chromophores) the process would be extremely difficult.

We have chosen instead to perform the calculation separately for each molecule in the reaction center and then use a perturbation approach to find the properties of the collective system. In the first step, a CNDO/S calculation including 130–150 singly excited configurations is carried out for each molecule in the reaction center (Eccles and Honig, 1983). For the  $n$ th excited state in molecule  $N$ , the wave function will be

$$\Phi_N^n = \sum_i A_{N_i}^n \phi_{N_i}^i \quad (1)$$

where the  $\phi_{N_i}^i$  are the singly excited configurations obtained from moving an electron from a filled to an unfilled molecular orbital in molecule  $N$ .

The treatment at this point employs a standard exciton approach. The ground state wave function of the entire system is just

$$\Psi_0 = \prod_N \phi_N^0 \quad (2)$$

where the product is over all the molecules included in the calculation and  $\phi_N^0$  are just the ground state configurations for each molecule. The excited state wave functions  $\Psi^e$  of the system are constructed from product wave functions,  $\psi_N^e$ , in which only a single molecule,  $N$ , has been excited and all other molecules,  $M$ , are in their ground electronic states. Thus,

$$\psi_N^e = \Phi_N^n \cdot \prod_M \phi_M^0 \quad (3)$$

Finally,

$$\Psi^e = \sum_N \sum_n B_{Nn}^e \psi_N^e \quad (4)$$

The energy matrix is formed with rows and columns indexed by a transition,  $n$ , and the molecule,  $N$ , on which the transition takes place. Charge transfer states and doubly excited configurations within a single molecule are excluded from the expansion. The diagonal elements of the matrix are just the calculated transition energies for the individual molecules (the ground state energy is simply subtracted from all state energies). The off-diagonal elements are zero if the row and column refer to the same molecule. If not they simply correspond to the interaction between the individual transition moments of the different electronic states. These may be calculated with the dipole approximation or using transition monopoles located on each atom. Unless stated otherwise, the transition monopole method was employed.

The calculation of the transition moments,  $\mu^e$ , and rotational strengths,  $R^e$ , of the combined system is straightforward. For each product function in the basis set, since only one molecule is excited, the transition moment is just that of a particular state in the isolated molecule,  $\mu_N^e$ . The total transition moment is simply the vector sum of the transition moments of each state weighted by the coefficient of the function in the eigenvector. Similarly, the rotational strengths for a particular transition are obtained from the weighted sum of all pairs of terms of the form,  $r^{nm} \cdot \mu_N^e \cdot \mu_M^e$ , where the  $\mu$ 's are the transition moments for states  $n$  and  $m$  on molecules  $N$  and  $M$ , respectively.

As stated above, this is a standard exciton treatment. The only novelty is that the basis set is an extensive configuration interaction expansion for the isolated monomers. A program has been written which uses the wave functions and energies of the monomers as input and which diagonalizes the interaction matrix for a system consisting of 2–6 chromophores with a variable (defined as program input) number of excited states per molecule. Environmental effects on the individual chromophores can be incorporated by fixing the diagonal elements before diagonalization. The dielectric constant of the medium enters through the off-diagonal elements. In general, the dielectric constant was set to 2 for all interactions to account for the electronic polarizability of the medium. Interactions between electronic states of the special pair chlorophylls were generally assumed to have a dielectric constant of 1 although this term in any case was used as a parameter.

## RESULTS

### Individual Chromophores

Calculations were carried out on each of the reaction center chromophores (coordinates kindly supplied by Drs. Dieneshofer and Michel). The BChl-b atoms shown in Fig. 1 of Eccles and Honig (1983) were included in the calculations. Hydrogen atoms were added to the crystal coordinates in standard geometry. For the BChl's, calculated absorption maxima ranged between 722 and 746 nm for the  $Q_y$  state and between 581 and 597 nm for  $Q_x$  state. The calculated absorption maxima of the BPh molecules are 711 and 744 nm. The calculated values correspond reasonably well with the experimental values (Davis et al., 1979) although the  $Q_y$  bands are a bit too high in energy. Since these zero order transition energies will be treated as parameters in the calculations, the deviations from experimental values are irrelevant for this work. The dipole strengths calculated for each of the BChl-b molecules is  $\sim 75$   $D^2$  while the BPh molecules have a dipole strength of  $\sim 50$   $D^2$ . These values are  $\sim 50\%$  larger than experimen-

tal ones, a standard consequence of using the transition dipole approximation in molecular orbital theory. The sensitivity of our results to this parameter will be discussed below.

The small differences between the transition energies of the four BChl molecules is due to the fact that their coordinates are different in the crystal structure. Until the coordinates are further refined, it will not be clear whether these differences are important spectroscopically.

### The Interaction Matrix

Table I contains the interaction matrix for the  $Q_y$  transition calculated within the transition-dipole and transition-monopole approximations. There are a number of interesting features to Table I. Perhaps the most striking is that for certain geometries, the point dipole approximation breaks down, even for molecules that are fairly far apart. In some cases it works quite well but in general it seems clear that the dipole approximation cannot be used for reaction center chlorophylls and pheophytins.

The most severe error introduced by the point dipole approximation is for the interaction between the special pair BChls. Here, the exciton interaction is overestimated by a factor of 3. It is worth pointing out in this regard that the transition-dipole approximation overestimates the oscillator strength of the  $Q_y$  transition by ~50%. Scaling down the  $1,523 \text{ cm}^{-1}$  matrix element appropriately leads to a value of  $\sim 1,000 \text{ cm}^{-1}$ , close to that used by Knapp et al. (1985). However, given the major errors inherent in the point-dipole approximation, neither value can be taken as meaningful.

On the other hand, it is not clear to what extent the interaction matrix, even calculated with the monopole approximation, can be used with confidence. There are simply insufficient experimental data to confirm or reject the accuracy of the numbers that are obtained. For this reason, the numbers in Table I will be used as a guide in interpreting experimental data and will not be taken literally.

In most of the calculations reported below, all matrix elements except for those involving the special pair, were divided by 2 to account for the dielectric screening effect of electronic polarizability. The matrix element ( $V_y^{23}$ ) coupling the two  $Q_y$  transitions of the special pair BChls was

treated as a parameter and allowed to vary so as to optimize the fit with the observed spectra.

### The Effect of Higher Excited States

All of the calculations reported in this work were carried out using 1, 2, and 10 excited states per molecule. Although the final results are reported for 10 states, we found only small differences from calculations which used only the  $Q_y$  state for each molecule. For this reason, the description of the  $Q_x$  region of the spectrum has little effect on the results obtained for the  $Q_y$  region, which is the focus of this work. As mentioned in the Introduction, we did not take into account the effects of charge transfer states which would add an additional level of complexity and uncertainty in our theoretical treatment.

### Protein Induced Shifts

The absence of high-resolution x-ray coordinates of the protein makes it impossible to calculate possible spectral shifts induced by protein-chromophore interactions. (This could in principle be done by treating each charged and polar atom in the protein as a point charge, and calculating the pairwise interactions between the protein and chromophore atoms as described by Eccles and Honig [1983].) However, it is clear from the spectrum that the protein must have some effect in shifting the absorption maxima of the individual molecules to longer wavelengths than their absorption in solution. Knapp et al. (1985) assumed that the two special pair BChls were shifted to 885 nm, the accessory BChls to 824 nm, and the BPhs to 791 nm for  $BPh_M$  and 810 nm for  $BPh_L$ . Parson et al. (1985) assumed a protein-induced shift of all BChls to 830 nm. Thus all studies reported to date have used the effect of the protein as a parameter. Parson et al. (1985) assumed a smaller effect than Knapp et al. (1985) because they attribute much of the shift of the 960-nm band to charge transfer interactions.

This work also treats the proteins-induced shift as a parameter. Since we are testing different models, the magnitude of the effect varies somewhat (see Table II). However, in most of our calculations the absorption maximum of the special pair (in the absence of exciton splitting) is  $\sim 900 \text{ nm}$ . The two exciton states,  $\nu^+$  and  $\nu^-$ , are split in an approximately symmetric fashion around this value.

### Fitting Models to Spectra

Three types of models were tested. Model A (Fig. 2) assumes that the 850-nm band can be identified as  $\nu^+$ . To obtain the 960-nm absorption maximum,  $V_y^{23}$  was increased to  $650 \text{ cm}^{-1}$ . The accessory BChls were placed at 827 nm.

Models B and C (Figs. 3 and 4) place  $\nu^+$  at  $\sim 810 \text{ nm}$ . In keeping with the suggestion of Knapp et al., the diagonal element of the special pair is set at 885 nm and  $V_y^{23}$  is increased to  $900 \text{ cm}^{-1}$ . Thus, model B is quite similar to

TABLE I  
TRANSITION MATRIX FOR THE  $Q_y$  STATE. EXCITON  
INTERACTION ELEMENTS\* IN  $\text{cm}^{-1}$

	$BC_{MA}$	$BC_{MP}$	$BC_{LP}$	$BC_{LA}$	$BP_M$
$BC_{MP}$	-48 (-11)				
$BC_{LP}$	-243 (-264)	521 (1523)			
$BC_{LA}$	48 (52)	-288 (-298)	-40 (3)		
$BP_M$	209 (250)	-11 (-22)	46 (49)	-16 (-19)	
$BP_L$	-18 (-19)	59 (94)	-7 (-14)	244 (496)	9 (2)

\*Numbers are calculated with the monopole or (dipole) approximation.

TABLE II  
SPECTROSCOPIC PROPERTIES OF THE SIX LOWEST ENERGY TRANSITIONS IN THE *VIRIDIS* REACTION CENTER  
PREDICTED BY DIFFERENT MODELS

		States					
		1	2	3	4	5	6
Model A*	$\lambda$	963	855	830	822	802	799
	$f$	0.78	0.24	0.61	0.13	0.23	0.25
	$R$	5.3	-1.2	2.1	-1.1	-1.5	-1.85
	$\phi$	85	5	76	14	35	35
Monomer	BC <sub>MP</sub> , BC <sub>LP</sub>	BC <sub>MP</sub> , BC <sub>LP</sub>	BC <sub>MA</sub> , BC <sub>LA</sub>	BC <sub>MA</sub> , BC <sub>LA</sub>	BP <sub>L</sub>	BP <sub>M</sub>	
Model B <sup>†</sup>	$\lambda$	972	836	828	814	802	788
	$f$	0.80	0.36	0.61	0.03	0.22	0.25
	$R$	5.3	-2.0	2.2	-4.1	-1.5	-1.9
	$\phi$	85	4	82	20	35	35
Monomer	BC <sub>MP</sub> , BC <sub>LP</sub>	all BChl	BC <sub>MA</sub> , BC <sub>LA</sub>	all BChl	BP <sub>M</sub>	BP <sub>L</sub>	
Model C <sup>‡</sup>	$\lambda$	972	853	832	817	803	788
	$f$	0.81	0.44	0.42	0.06	0.27	0.25
	$R$	5.4	-4.2	.71	1.6	-1.5	-1.9
	$\phi$	85	42	62	88	29	35
Monomer	BC <sub>MP</sub> , BC <sub>LP</sub>	BC <sub>LA</sub>	all BChl	all BChl	BP <sub>L</sub>	BP <sub>M</sub>	

Results include absorption maxima ( $\lambda$ , in nm), oscillator strengths ( $f$ ), rotational strengths ( $R$ , in Debye-Bohr magnetons), angle made by the transition moment with the two-fold crystal axis ( $\phi$ , in degrees), and the monomers making the largest contribution to the transition.

\*Diagonal elements (nm): BC<sub>MA</sub> (823), BC<sub>MP</sub> (895), BC<sub>LP</sub> (895), BC<sub>LA</sub> (823), BP<sub>L</sub> (786), BP<sub>M</sub> (802).  $V_y^{23} = 650 \text{ cm}^{-1}$ , all other matrix elements as in Table I.

†Diagonal elements (nm): BC<sub>MA</sub> (820), BC<sub>MP</sub> (882), BC<sub>LP</sub> (882), BC<sub>LA</sub> (820), BP<sub>L</sub> (786), BP<sub>M</sub> (802).  $V_y^{23} = 900 \text{ cm}^{-1}$ , all other matrix elements as in Table I.

‡Diagonal elements (nm): BC<sub>MA</sub> (823), BC<sub>MP</sub> (895), BC<sub>LP</sub> (895), BC<sub>LA</sub> (846), BP<sub>L</sub> (786), BP<sub>M</sub> (802).  $V_y^{23} = 900 \text{ cm}^{-1}$ , all other matrix elements as in Table I.

that of Knapp et al. (1985) except that the monopole approximation matrix elements of Table I are used. This model does not produce a band located at 850 nm. Rather, the longest wavelength transition (other than  $\nu^-$ ) is located at 836 nm (838 in the paper of Knapp et al. [1985]). In an attempt to force this model to place a transition at 850 nm, BChl<sub>M</sub> was assumed to be shifted by the protein to this wavelength. This corresponds to model C. The particulars of each model are described in the caption to Table II.

Table II and Figs. 2-4 describe the absorption spectra obtained from the different models. Fig 1 contains the experimental spectra at low temperatures. We have used Gaussians to describe each of the absorption bands. Since the low temperature spectra are well resolved we have tried to reproduce these results rather than the room temperature spectra which do not distinguish as well among models. Consequently, all bands, with the exception of  $\nu^-$ , were assigned a halfwidth of  $225 \text{ cm}^{-1}$  corresponding approximately to that observed at 5°K (Breton, 1985).  $\nu^-$  was assigned a halfwidth of  $450 \text{ cm}^{-1}$ .

It appears from Fig. 1 that the absorption spectrum of model A is quite similar to that observed experimentally. The overall shape of model B appears satisfactory, however, there is no intensity at 850 nm. The band at 835 nm, which Knapp et al. (1985) assume produces the shoulder at 850 nm is quite intense and broadens the 830-nm band. The relatively large dipole strength calculated for this transition is due to the fact that the accessory BChls are

not strongly coupled and thus each molecule produces an absorption band with the approximate intensity of one BChl. This behavior is also evident in Table II of Knapp et al. where the 830- and 838-nm bands have approximately equal intensity. If one forces model B to produce intensity at 850 nm thus yielding model C the absorption maximum is of course reproduced, but, as is evident from Fig. 2, the discrepancy with the experimental intensities becomes more severe. There seems no way around this problem. Thus, based on the absorption spectra alone model A, which assumes that  $\nu^+$  is located at 850 nm, would appear to be correct.

The same basic conclusion arises from analysis of the LD and CD spectra. Model A does a good job in reproducing the experimental spectra while, superficially, the spectrum produced by model B is also adequate. However, model B does not produce the required intensity near 850 nm. When the unperturbed transition energies are modified so as to produce intensity at 850 nm, the shape of the spectrum no longer resembles the experimental results.

### Electrochromic Shifts

It has been postulated that some of the changes seen in the difference spectrum between P and P<sup>+</sup> are due to electrochromic shifts in the absorption of the accessory chlorophylls arising from the positive charge on the special pair (see e.g., Parson, 1982; Hanson et al., 1987). To introduce these effects into our assignment scheme, we calculated the

### Model A

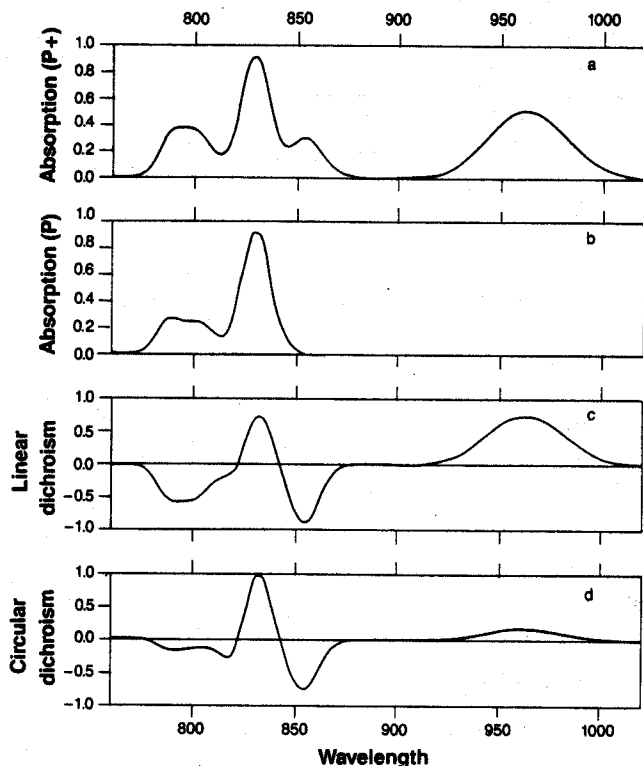


FIGURE 2 Spectra calculated from model A. Spectra plotted assuming Gaussian bands with maxima shown in Table II. All bandwidths taken as  $200 \text{ cm}^{-1}$  to conform with  $77^\circ \text{ K}$  spectra. (a) Absorption spectrum of P. (b) Absorption spectrum of  $P^+$ . (c) Linear dichroism spectrum of P. (d) Linear dichroism spectrum of  $P^+$ .

spectral shifts induced in the accessory Bchls induced by two charges of magnitude  $+0.5$  placed at the center of each special pair BChl. Assuming a dielectric constant of 1 the shift is calculated to be  $782 \text{ cm}^{-1}$ . However, the effective dielectric constant is likely to be  $\sim 4$ , or larger if there is considerable local relaxation of the protein around the special pair cation (Honig et al., 1986; Gilson and Honig, 1986). The predicted shift of the 833-nm band is to 820 nm for a dielectric constant of 4 and 828 nm for a dielectric constant of 10. Thus, our calculations support the suggestion that the small blue shift around 830 nm, as seen in oxidized reaction centers, is due to an electrochromic effect.

### DISCUSSION

Our primary goal in this work has been to determine the extent to which a well-defined model with a limited set of assumptions could account for the spectroscopic properties of the reaction center of *Rhodospseudomonas viridis*. In particular we have tested the hypothesis that the absorption, CD and LD spectra of the reaction center can be explained entirely in terms of exciton interactions between the various BChls and BPhs whose coordinates are defined from the x-ray structure. It is apparent that this type of

### Model B

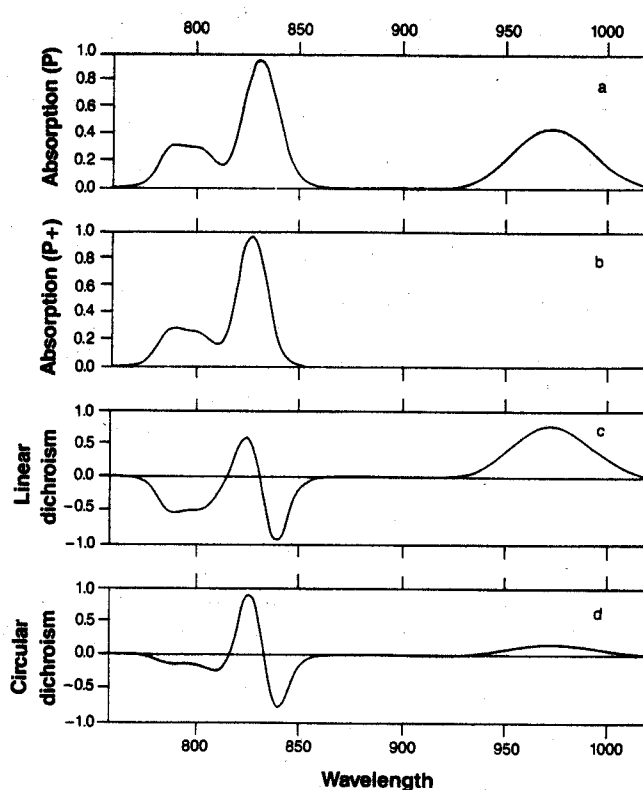


FIGURE 3 Spectra calculated from model B. See caption of Fig. 2.

### Model C

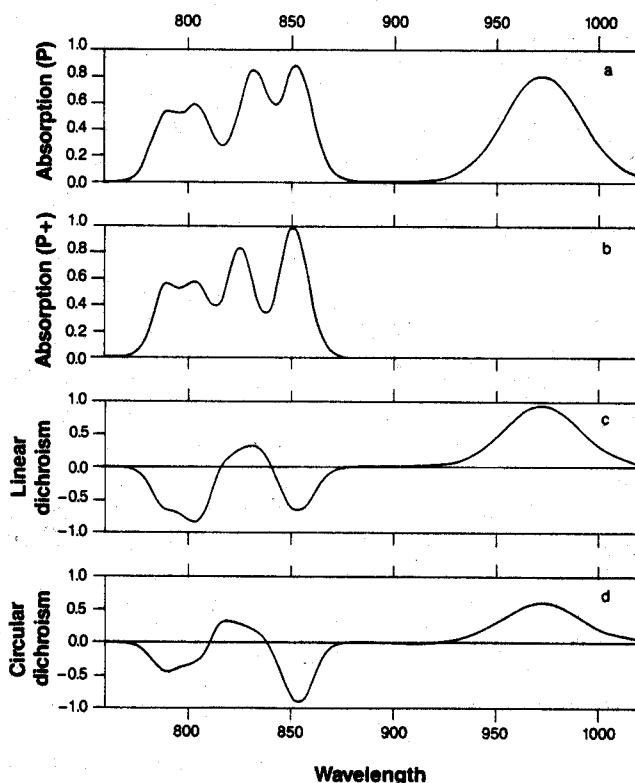


FIGURE 4 Spectra calculated from model C. See caption of Fig. 2.

model requires the additional assumption that the protein shifts the zero order energy levels of the individual chromophores, presumably through an electrostatic effect. The major factor which has not been considered is the possible contribution of charge-transfer states which have been suggested to play an important spectroscopic role (Parson et al., 1985). The benefit of limiting the analysis to two effects, exciton interactions, and protein-induced shifts, is that the consequences of this restricted model can be fully explored. The extent to which charge transfer interactions might alter the conclusions of this work will be discussed below.

A number of our conclusions are purely methodological. Thus we have found that the dipole approximation cannot be used to calculate interactions between reaction center components and that there is little intensity borrowing between the  $Q_y$  and higher excited states. However, the central conclusion of this work concerns the location of the higher energy exciton state,  $\nu^+$ . As discussed in the Introduction, arguments have been presented, which place this state alternatively at either 810 or 850 nm. Since there is distinct intensity in the absorption, CD and LD spectra at 850 nm, placing  $\nu^+$  near 810 nm has required that the 850-nm intensity be attributed in large part to the accessory BChls. To test this possibility, we introduced two models (B and C) which place  $\nu^+$  near 810 nm. Since  $\nu^-$  is near 960 nm, these models require a rather large exciton interaction matrix element between the special pair BChls of  $\sim 1,000 \text{ cm}^{-1}$ . The models are thus quite similar to that of Knapp et al. (1985) who introduced similar assumptions.

As discussed above we were unable to obtain satisfactory fits to the experimental low temperature spectra using models which place  $\nu^+$  near 810 nm. Model B produces spectra similar in shape to the experimental spectra but there is missing intensity at 850 nm in the low temperature absorption and LD spectra of the P state of the reaction center. The problem is less severe when comparing with room temperature spectra (see e.g., Knapp et al., [1985]; Parson et al., 1985) since in this case the broad absorption bands can obscure weak transitions and displace peaks from their intrinsic transition energies. However, the narrow low temperature spectra clearly indicate the presence of a transition near 850 nm and this cannot be accounted for by a transition at 838 nm as assumed by Knapp et al. (1985).

Model C is an attempt to add intensity at 850 nm by shifting the zero order transition of one of the accessory BChls to this wavelength. It is clear from Fig. 4 that under this assumption, the overall shape of the various spectra is not reproduced. Most seriously, there is now too much absorption intensity at 850 nm in P but, in addition, the CD and LD spectra no longer have the proper form. This result is independent of any uncertainties in our calculations and can be understood on the basis of simple considerations. Since the accessory BChls are not strongly

coupled, each, if separated energetically (i.e., one near 830 nm and one near 850 nm), carries the intensity of an isolated BChlb. Thus, one would predict approximately equal intensity at 830 and 850, which is just the result produced by model C, but which is contrary to experiment. Our calculations suggest then, that it is difficult if not impossible to account for the spectra in the region 830–850 nm if  $\nu^+$  is placed below the accessory BChlb  $Q_y$  states, i.e., near 810 nm.

In contrast, model A, which places  $\nu^+$  near 850 nm, cannot be ruled out on the basis of the low temperature spectra. Model A assumes a protein induced shift of the special pair and accessory BChlb transition energies to near 900 and 830 nm, respectively, and  $\sim 700 \text{ cm}^{-1}$  exciton matrix element in the special pair (close to the 550-nm value calculated with the transition monopole approximation). With these assumptions, the calculated spectra agree quite well with experiment. Improved agreement might be obtained by varying the line shapes, zero order transition energies, matrix elements, etc. but extensive parameter fitting would not be in the spirit of this work.

A possible objection to the placement of  $\nu^+$  at 850 nm is that the absence of this state in  $P^+$  leads to the prediction of a small red shift near 830 nm in the  $P^+$ -P difference spectrum. (This is because the weak exciton interaction between the 850- and 830-nm bands is absent in  $P^+$  allowing the 830-nm band to shift to longer wavelengths). In fact, a small blue shift is observed (Breton, 1985). However, the electrochromic effect on the accessory bands due to the charge on the special pair in  $P^+$  can produce a sizable blue shift (see above) and provides a natural explanation of the  $P^+$  spectra. In contrast, one would predict a red shift in the  $^3P$ -P difference spectra since in this case no charge resides on the special pair. The experimentally observed  $^3P$ -P difference spectra (Shuvalov and Parson, 1981) can, in fact, be interpreted in these terms. There is a decrease in intensity at 850 nm and an increase between 830 and 840 nm. Shuvalov and Parson (1981) have interpreted these changes as due to a blue shift of the 850-nm band, which they assign to an accessory BChlb. The results of this work suggest that the disappearance of the 850-nm band is due to the disappearance of  $\nu^+$  while the new intensity between 830 and 840 nm is due to a red shift of the accessory BChlb bands located  $\sim 830 \text{ nm}$  in P.

It is worth emphasizing that the success of model A in accounting for the experimental spectra does not prove that the model is correct. The model assumes large protein induced shifts of the zero order transition energies of the special pair  $Q_y$  states and there is still insufficient structural information available to test whether this assumption is reasonable.

Another source of uncertainty is that the possible spectroscopic role of CT states have not been considered in this work. Recently, Parson et al. (1985) have presented a model in which CT states located above  $\nu^-$  interact

strongly with  $\nu^-$  and shift it to longer wavelengths. These workers place  $\nu^+$  near 800 nm, which is unlikely given the calculations presented here. However this discrepancy does not affect the validity of their suggestion that CT states are responsible for a large fraction of the red shift of  $\nu^-$  to  $\sim 960$  nm. A large contribution from CT states would imply a smaller spectroscopic role for the protein in directly influencing the  $Q_y$  energy levels of the special pair. It may be difficult to resolve this issue experimentally because the evidence for some CT character in  $\nu^-$  does not measure the extent of the mixing between  $Q_y$  and CT states nor its spectroscopic consequences. In any case, the conclusions of this work suggest that any successful model will have to attribute a large fraction of the intensity at 850 nm to  $\nu^+$  rather than to accessory BChl transitions.

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## REFERENCES

- Boxer, S., T. Middendorf, and D. Lockhart. 1986. Reversible photochemical holeburning in *R. viridis* reaction centers. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 200:237-241.
- Breton, J. 1985. Orientation of the chromophores in the reaction center of *rhodospseudomonas viridis*. *Biochim. Biophys. Acta.* 801:235-245.
- Davis, M., A. Forman, L. Hanson, J. Thornber, and J. Fajer. 1979. Anion and cation radicals of bacteriochlorophyll and bacteriopheophytin b. *J. Phys. Chem.* 83:3325-3332.
- Davis, R. C., S. L. Ditson, A. F. Fentiman, and R. M. Pearlstein. 1981. Reversible wavelength shifts of chlorophyll induced by a point charge. *J. Am. Chem. Soc.* 102:6823-6826.
- Deisenhofer, J., O. Epp, K. Miki, R. Huber, and H. Michel. 1984. X-ray structure analysis of a membrane protein complex. *J. Mol. Biol.* 180:385-398.
- Deisenhofer, J., O. Epp, K. Miki, R. Huber, and H. Michel. 1985. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodospseudomonas viridis* at 3A resolution. *Nature (Lond.)* 318:618-623.
- Gilson, M., and B. Honig. 1986. The dielectric constant of a folded protein. *Biopolymers.* 25:2097-2119.
- Eccles, J., and B. Honig. 1983. Charged amino acids as spectroscopic determinants for chlorophyll *in-vivo*. *Proc. Natl. Acad. Sci. USA.* 80:4959-4962.
- Hanson, L., J. Fajer, M. Thompson, and M. Zerner. 1988. Electrochromic effects of charge separation in bacterial photosynthesis. *J. Am. Chem. Soc.* (In press).
- Honig, B., W. Hubbell, and R. Flewelling. 1986. Electrostatic interactions in membranes and proteins. *Annu. Rev. Biophys. Bioeng.* 15:163-193.
- Knapp, E., S. Fischer, W. Zinth, M. Sander, W. Kaiser, J. Deisenhofer, and H. Michel. 1985. Analysis of optical spectra from single crystals of *Rhodospseudomonas viridis* reaction centers. *Proc. Natl. Acad. Sci. USA.* 82:8463-8467.
- Meech, S., A. Hoff, and D. Wiersma. 1986. Role of charge transfer states in bacterial photosynthesis. *Proc. Natl. Acad. Sci. USA.* 83:9464-9468.
- Parson, W. W. 1982. Photosynthetic bacterial reaction centers. *Annu. Rev. Biophys. Bioeng.* 11:57-80.
- Parson, W., A. Scherz, and A. Warshel. 1985. Calculations of spectroscopic properties of bacterial reaction centers. In *Antennas and Reaction Centers of Photosynthetic Bacteria*. M. E. Michel-Bayerle, editor. Springer-Verlag GmbH and Co. KG, Heidelberg, Berlin. 122-130.
- Pearlstein, R. M. 1987. Structure and exciton effects in photosynthesis. In *Photosynthesis*. J. Ames, editor. Elsevier North-Holland Biomedical Press, Amsterdam. 299-317.
- Philipson, K., and K. Sauer. 1973. Comparative study of the circular dichroism spectra of reaction centers from several photosynthetic bacteria. *Biochemistry.* 12:535-539.
- Scherz, A., and W. Parson. 1985. Exciton interactions in dimers of bacteriochlorophyll and related molecules. *Biochim. Biophys. Acta.* 766:666-678.
- Shuvalov, V., and W. W. Parson. 1981. Triplet states of monomeric bacteriochlorophyll *in vitro* and of bacteriochlorophyll dimers in antenna and reaction center complexes. *Biochim. Biophys. Acta.* 638:50-59.
- Zerner, M. 1981. Approximate quantum mechanical methods for large molecules. *Ann. NY Acad. Sci.* 367:35-55.