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Orientation of the chromophores in the reaction center of *Rhodopseudomonas viridis*. Comparison of low-temperature linear dichroism spectra with a model derived from X-ray crystallography *

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Linear dichroism (LD) and absorption (A) spectra of reaction centers from *Rhodopseudomonas viridis* included in the native chromatophores or reconstituted in planar aggregates have been recorded at 10 K. The samples were oriented in squeezed polyacrylamide gels and the primary donor P was in the reduced or (chemically) oxidized state. The LD spectra of reaction centers in these two states are in favor of a dimeric model of P in which excitonic coupling between the two non-parallel Q_Y transitions leads to a main transition at 990 nm (parallel to the membrane plane) and another one of smaller oscillator strength at 850 nm (tilted at approx. 60° out of the membrane plane). These assignments are in close agreement with the ones proposed in a previous LD study at room temperature (Paillotin, G., Verméglio, A. and Breton, J. (1979) Biochim. Biophys. Acta 545, 249–264). The main Q_X excitonic component of P has a broad absorption peaking at 620 nm and it corresponds to dipoles exhibiting the same orientation as those responsible for the 850 nm transition. On the basis of the present LD study and of CD data of chemically oxidized-minus-reduced reaction centers, we proposed that the minor Q_X excitonic component of P is oriented close to the membrane plane and absorbs around 660 nm. The two monomeric bacteriochlorophylls exhibit a positive LD for both their Q_Y transitions (unresolved at 834 nm) and their Q_X transitions (resolved at 600 and 607 nm), indicating that the planes of these molecules are only slightly tilted out of the membrane plane. The two bacteriopheophytins exhibit strong negative LD with identical LD/A values for their Q_Y transitions (resolved at 790 and 805 nm) and small positive LD for their Q_X transitions (resolved at 534 and 544 nm), demonstrating that these two molecules are strongly tilted out of the membrane plane with each of the Q_Y transitions tilted at approx. 50° out of that plane. A comparison of these LD data with the structural model derived from X-ray crystallography (Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398) clearly suggests that a good agreement exists between the results of the two techniques under the following conditions: (i) the C-2 symmetry axis of the reaction center runs along the membrane normal; (ii) excitonic coupling is present only in the primary donor special pair; and (iii) the direction of the optical transitions of the monomeric bacteriochlorophylls and of the bacteriopheophytins is not significantly perturbed by the interactions among the pigments. In addition, a carotenoid is detected in

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Abbreviations: LD, linear dichroism; CD, circular dichroism; P, primary electron donor; B, 'accessory' bacteriochlorophyll; BPh, bacteriopheophytin.

the isolated reaction center with an orientation rather perpendicular to the C-2 symmetry axis. Finally, a comparison of these data with similar ones obtained on the bacteriochlorophyll *a*-containing reaction center of *Rhodopseudomonas sphaeroides* 241 points towards a geometrical arrangement of the chromophores which is indistinguishable from the one observed in the reaction center of *Rps. viridis*.

Introduction

The reaction centers from photosynthetic bacteria are highly organized transmembrane pigment-protein complexes which perform the primary charge separation and stabilization steps with a remarkable efficiency. In the case of purple bacteria they can be isolated in a functional state and contain four bacteriochlorophyll molecules, two bacteriopheophytins and one iron-quinone complex which are anchored to two polypeptides called L and M. In most of the bacterial species the pigments are of the *a*-type, while in *Rhodopseudomonas viridis* they are of the *b*-type. The molecular organization of the pigments in these reaction centers has been investigated by various spectroscopic techniques such as resonance Raman, electron spin resonance, absorption, circular dichroism (CD) and linear dichroism (LD). This last technique allows for a description of the orientation of the transition moments of the various pigments with respect to a macroscopic axis in the reaction center, for example the normal to the plane of the photosynthetic membrane in which the reaction center is embedded. In the related technique of photodichroism, one measures the relative angles between pairs of transition moments. There have been several LD and photodichroism studies [1–9] on reaction centers of purple bacteria which have led to specific models for the organization of the pigments. For a review of the work prior to 1981 see Ref. 1. In the case of the reaction center of *Rps. viridis* two quite different models have been proposed: the first one, described by Shuvalov and Asadov [8], is based upon CD and photodichroism of isolated reaction centers, while the second one proposed by Verméglio, Paillotin and Breton [1,3,9,10] is based upon LD of oriented *Rps. viridis* cells [9] and photodichroism of isolated reaction centers [1,3]. Regarding the relative geometry of the Q_Y transition dipoles of the primary electron donor (P), these two models are quite mutually exclusive so that other spectroscopic observations [11–13] have been utilized to favour one or the other of these models.

The reaction center from *Rps. viridis* has been crystallized [14] and an X-ray diffraction study at 0.3 nm resolution has recently led to a 3-D model indicating the general organization of the pigments within this chlorophyll-protein complex [15]. Although only qualitatively, this model allows the orientation of the *X* and *Y* axes along which the optical transitions are mainly polarized to be observed for each of the six pigments. This has prompted us to reinvestigate the low temperature LD spectra of reaction centers (either in the isolated state or still included in the native chromatophore membrane) with P in the reduced or oxidized states. It is thus possible to extend significantly our previous model of the reaction center of *Rps. viridis* [1,9], to draw analogies with the case of bacteriochlorophyll *a*-containing reaction centers, and to compare critically the models derived from the polarized optical spectroscopy investigations versus the one obtained from the X-ray crystallography study.

Materials and Methods

Reaction centers were prepared according to Refs. 16 and 17 from chromatophores isolated by French-press treatment. In some cases further purification as well as exchange of the lauryldimethylamine *N*-oxide detergent for sodium cholate were performed by high-performance liquid chromatography [18]. Reaction centers were stored at $-90°C$ after which treatment they form small planar aggregates which could be pelleted in 30 min by centrifugation at $20\,000 \times g$. The reaction centers or the chromatophores (purified by centrifugation on a sucrose density gradient) were included in polyacrylamide gels containing 60% glycerol and oriented by uniaxial squeezing of the

gel [19,20]. Chemical oxidation of the reaction centers was achieved by incubating the gels with 20 mM potassium ferricyanide in 60% glycerol at 4°C.

The samples were cooled in a cryostat operating with a temperature-regulated flow of helium gas (SMC, France). LD spectra were recorded on a previously described apparatus [21] equipped with a S1 phototube (R316, Hamamatsu, Japan) and a 850 W tungsten-halogen lamp. Absorption (A) spectra were recorded on the same instrument and with the same beam geometry as used for the LD spectra. They were obtained by recording the intensity of the transmitted light (I_0, intensity of blank gel with no sample; I, intensity of gel containing the sample) as a function of wavelength in a Tractor 1710B (Tracor Northern, U.S.A.) equipped with a photometric unit, and calculating $\log I_0/I$. The concentration of the sample was adjusted to give optical densities of 0.4–0.6 at approx. 830 nm at low temperature. For reaction centers at 10 K the absorption and LD spectra were arbitrarily normalized to 1 at the 830 nm maximum and the LD/A spectrum was calculated. The LD set-up was optimized to measure precisely small LD and absorption signals on the same instrument and with the same beam geometry in the spectral range 450–1000 nm. Due to the low-light level above 980–1000 nm, some non-linearity in the response of the set-up was observed so that some caution should be exercized in interpreting the precise shape of the absorption (and to a lesser extent the LD) spectra above 980–1000 nm. Furthermore, while LD and absorption spectra can be obtained precisely, the exact position of the baseline in these spectra is much less accurately known. This introduces uncertainties in the LD/A values observed in the regions of low absorbance.

CD spectra were recorded at room temperature on a Mark V instrument (Jobin-Yvon, France) using 0.5 cm optical cells.

Results and Discussion

Orientation of the reaction center

The absorption, LD and LD/A spectra at 10 K of aggregated reaction centers from *Rps. viridis* oriented in polyacrylamide gels are presented in Fig. 1. The large absorption at 990 nm clearly shows that P is in the reduced state. With our conventions a positive LD is associated with transition moments oriented close (less than 35°) to the plane of largest cross-section of the aggregate. At such a low temperature a good resolution of the various spectral components is observed as shown by the close correspondence between the absorption (Fig. 1a) and LD (Fig. 1b) peaks and also by the relative simplicity of the LD/A spectrum (Fig. 1c). After incubating the gels for 24 h with ferricyanide, the absorption spectrum (Fig. 2a) shows complete oxidation of P. Due to some swelling of the gel during the incubation, the LD spectrum (Fig. 2b) is of a lesser quality than the one presented in Fig. 1b and comparison with several other spectra of oxidized reaction centers indicates some downward drift of the baseline. In the spectra shown in Fig. 1a and 2a, a relatively large 685 nm band which corresponds to a breakdown product of bacteriochlorophyll-*b* [13] is visible. Other samples with significantly less 685 nm contribution did not orient quite as well in the gel. However, the signs and relative magnitudes of the various LD bands were the same for all samples.

The antenna Q_Y transitions of *Rps. viridis* absorb maximally above 1000 nm and the reaction

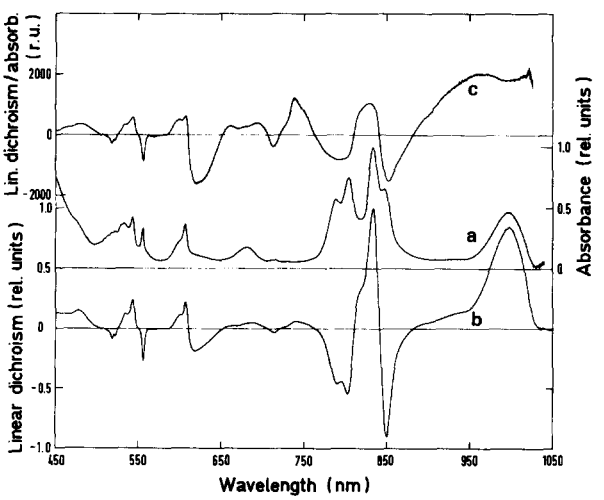

Fig. 1. (a) Absorption (A); (b) linear dichroism (LD); and (c) LD/A spectra at 10 K of reaction centers from *Rps. viridis* oriented in polyacrylamide gel with the primary donor (P) in the reduced state. Above 980–000 nm the spectra are slightly distorted. See text for details.

center bands around 830 nm can already be distinguished in the room temperature LD spectra [9]. This is also the case for isolated chromatophores at 10 K (Fig. 3), where the improved spectral resolution clearly allows the bands seen in the LD spectra of aggregated reaction centers (Fig. 1b) to be recognized. The sample of oriented chromatophores used for these spectra had been incubated for 6 h with ferricyanide so that a mixture of P and P^+ states was present. Unoxidized and fully oxidized samples led to absorption and LD spectra similar in the 830 nm spectral range to the ones represented in Figs. 1 and 2, respectively (data not shown). The spectra of Fig. 3 also show the Q_X transition of the antenna at approx. 600 nm with its large negative LD [9] and the orientation of some reduced *c*-558 cytochrome (negative LD at 556 nm) as well as of the Q_X transitions of the BPh molecules (positive dichroism around 540 nm). The identical orientation which is observed for various transition moments with opposite dichroism when comparing the LD specta of reaction centers included either in the chromatophore membrane (Fig. 3b) or in the aggregates (Fig. 1b) clearly demonstrates that in the latter case the transmembrane axis of the reaction center is perpendicular to the plane of the aggregate. Accordingly, in the following discussion we will always describe the orientation of the chromophores with respect to the membrane plane irrespective of the presence or absence of a natural membrane environment.

Deisenhofer et al. [15] have proposed that the C-2 symmetry axis evident from their X-ray crystallography data runs along the membrane normal. This proposal was based upon (i) the transmembrane character of the charge separation and (ii) the orientation of the chromophores as described in Ref. 9. In the following discussion we will further stress that the orientation of the pigments as seen by LD and by X-ray spectroscopy is strongly in favour of this position of the symmetry axis. However, we can also add another observation in favour of this assignment based upon the orientation of the α-helices clearly demonstrated by both the X-ray analysis [15] and the polarized infared spectroscopy data. In the case of the reaction center from *Rps. sphaeroides* as well as of its LM subunit [22] and more generally of many of the hydrophobic chlorophyll-protein complexes [23] a large fraction (about 50%) of the protein secondary structures are α-helices oriented on the average at less than 30° from the membrane normal. A transmembrane orientation of the α-helices has also been predicted from the primary structures of the L and M subunits [24]. It has been recently shown by polarized infrared spectroscopy that a similar geometry of the α-helices was also present in the reaction center of *Rps. viridis* [25] thus further strengthening the positioning of the C-2 symmetry axis perpendicular to the membrane plane.

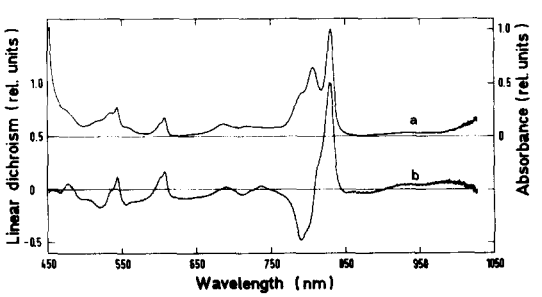

Fig. 2. (a) Absorption and (b) linear dichroism spectra at 10 K of reaction centers from *Rps. viridis* oriented in polyacrylamide gel with the primary donor chemically oxidized (state P^+).

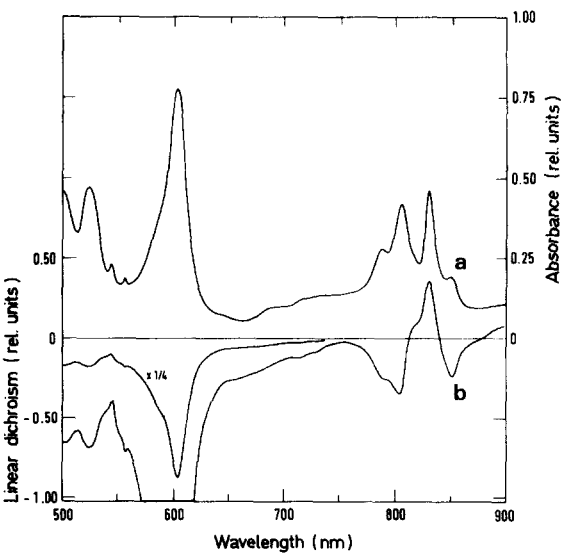

Fig. 3. (a) Absorption and (b) linear dichroism spectra at 10 K of chromatophores from *Rps. viridis* oriented in polyacrylamide gel with a fraction (about 50%) of the reaction centers in the oxidized state.

Structure and orientation of the primary donor P

Upon oxidation of the reaction center, the bleaching of the 990 nm absorption band is accompanied by a bleaching of the 850 nm band and of the broad shoulder at 610–650 nm (Figs. 1a and 2a). Similar bleachings are also observed in the corresponding LD spectra (Figs. 1b and 2b). The positive LD of the 990 nm band and negative LD of the 850 and 620 nm bands are in good agreement with the previous results of the LD study of the photooxidation of P in oriented cells of *Rps. viridis* at room temperature [9]. The LD/A spectrum indicates that the 850 and 620 nm components of P are tilted at approx. 60° out of the membrane plane (assuming the 990 nm transition is essentially parallel to the membrane as demonstrated in Ref. 9). The 620 nm component seen in the P spectra depicted in Fig. 1 is very broad and difference spectra calculated for oxidized-minus-reduced samples (data not shown) indicate that it also extends significantly towards shorter wavelengths. By analogy with results previously obtained on bacteriochlorophyll *a*-containing reaction centers [26,27], we have attempted to locate a lower-energy exciton state associated with the Q_X transition of P. The LD/A spectrum (Fig. 1c) gives an indication that a small transition with a positive LD is present around 660 nm. That this transition could be ascribed to the second exciton state of the Q_X transition of P is confirmed by the CD spectra (Fig. 4) which demonstrate that at room temperature an S-shaped signal with lobes at 620 nm (positive CD) and 670 nm (negative CD) disappears upon oxidation of P.

Upon chemical oxidation of P, a 4 nm blue shift of the 834 nm band is observed together with the bleaching of the 990 and 850 nm bands. It has been proposed that the monomer in P^+ absorbs at 805–810 nm [28] with its Q_Y transition moment oriented at about 20° with respect to the membrane plane [9]. The significant reduction of the negative LD signal at 805 nm (Fig. 1b) observed upon oxidation of the reaction center (Fig. 2b) fully supports this model. However, the amplitude of this absorption band of the monomer is very small compared to the integrated intensity of the 990 and 850 nm transitions. This effect is even more pronounced in the Q_X region where Fig. 1b and 2b give no evidence for a monomer band appearing with a negative LD. These observations suggest that the bacteriochlorophyll molecules in P^+ are still coupled electronically.

Orientation of the bacteriopheophytin (BPh) and monomeric bacteriochlorophyll (B) molecules

At 10 K in reduced reaction centers the BPh absorption bands (Fig. 1a) are resolved both in the Q_Y (at 789 and 805 nm) and in the Q_X regions (at 544 and 534 nm). These bands are only slightly affected upon oxidation of *P* (Fig. 2a). The corre-

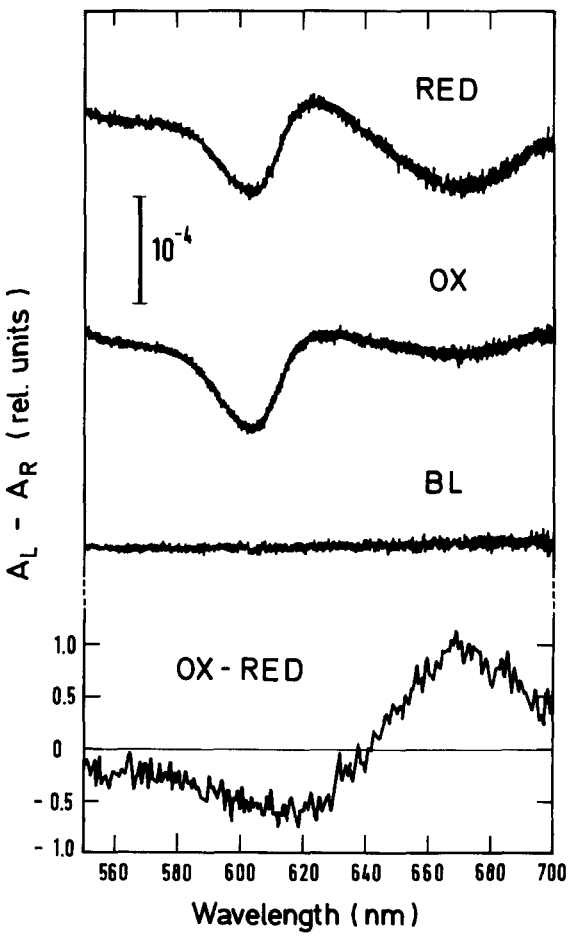

Fig. 4. Circular dichroism spectra at 20°C of reaction centers from *Rps. viridis* with the primary donor reduced (RED) or partially (75%) oxidized (OX). The reaction centers (in a 0.5 cm cuvette) had an absorbance at 830 nm of 0.9 absorbance units. Base line (BL) with 10 mM Tris buffer alone. The (OX-RED) spectrum was obtained by subtracting the spectrum of the reduced reaction centers from the spectrum of the oxidized reaction centers.

sponding peaks of the LD spectra (Fig. 1b) are located at 790 and 803 nm and at 544 and 534 nm. The LD/A spectrum (Fig. 1a) shows a broad featureless band around 800 nm which indicates that the two Q_Y transitions are making the same angle with the membrane plane and are tilted at approx. 50° out of that plane. A different situation is observed for the two Q_X transitions (both tilted at approx. 20° out of the membrane plane) with the 544 nm transition being slightly closer to the membrane plane than the one at 534 nm. It must be further noticed that the 805 and 544 nm transitions are associated with the BPh molecule which undergoes reduction upon illumination of prereduced reaction centers at low temperature [3,29,30].

Both the absorption and LD spectra (Fig. 1a and 1b) show a single band around 834 nm indicating that the Q_Y transitions of the two B molecules are not resolved. The LD/A spectrum (Fig. 1c) indicates that the average of these two transitions is oriented at approx. 20° from the membrane plane as previously reported [9]. On the other hand two bands can be observed at 607 and at approx. 600 nm in the absorption and LD spectra in the Q_X region. These two bands, although of different amplitudes and widths, correspond to two transitions which have an almost identical tilt of approx. 20° with respect to the membrane.

Comparison with the X-ray data

Although only a 3-D picture of the arrangement of the pigments in the reaction center without the atomic coordinates has been reported [15], stereoscopic inspection of the model allows for a qualitative estimation of the tilt angle of the *X* and *Y* axes of each of the six molecules with respect to a plane perpendicular to the C-2 symmetry axis (which represents the plane of the membrane). It is rather clear for example that the plane of each of the two monomeric B molecules is tilted at about 25–30° away from the plane of the membrane implying that the LD of each of the individual transitions should be positive. This situation is observed for the two Q_X transitions at 600 and 607 nm as well as for the average of the two Q_Y transitions unresolved at 834 nm (Fig. 1b). We note that the transmembrane orientation of the C-2 symmetry axis discussed above implies that transition moments belonging to symmetrically arranged molecules which are not in excitonic interactions should exhibit identical LD/A. This is verified for the Q_X of the two B molecules.

The geometry of the two BPh molecules is also rather clear in the model derived from the crystallographic data and shows that the Y axes of the two molecules are tilted out of the membrane plane (although not perpendicular to it), while the *X* axes are much closer to the membrane plane. This geometry is in excellent agreement with the LD data presented in Fig. 1 which, in addition, indicate that the tilt angles of the Q_Y transition of the two BPh molecules are identical. The LD/A spectrum (Fig. 1c) also indicates that there is a small difference in the tilt angle of the two Q_X transitions and that accordingly either the C-2 symmetry of the *X* directions is slightly less well observed than for the *Y* directions or the two Q_X transitions are experiencing different interactions.

As deduced from the X-ray study the planes of the two bacteriochlorophylls constituting the special pair are both rather perpendicular to the membrane plane. They are tilted at approx. 15° from each other and the distance between the two overlapping rings I is approx. 0.3 nm [15]. The *X* directions are located side-by-side and rather perpendicular to the membrane plane, while one of the two *Y* directions is tilted slightly above and the other slightly below this plane. The angle between the two Y directions is close to 35° (Michel, H. and Deisenhofer, J., personal communication). The oscillator strength and orientation of the transitions of P responsible for the bands at 990 and 850 nm and at 620 and 660 nm observed in Fig. 1 can thus be at least qualitatively rationalized according to the theory of excitonic coupling in a symmetric dimer [31] as the vectors sum and difference of the transition moments of the monomers seen in the model derived from the X-ray study.

Comparison of the reaction center model [15] and of the LD data presented here indicates that a good correlation can be found between the geometrical arrangement of the *X* and *Y* axes of the six pigment molecules as seen by X-ray and the orientation of the various transition moments giving rise to the different absorption bands as seen by LD under the following conditions: (i) the C-2 symmetry axis is located along the membrane nor-

mal, (ii) excitonic coupling exists within the special pair with non-parallel Q_Y transition moments and (iii) the electronic interactions which may occur among the four other molecules do not appreciably perturb the direction of their optical transitions. Although we estimate that the present agreement of the results of the two techniques is reasonably good, a more quantitative comparison between the X-ray data and the LD results should give interesting information regarding the interactions among the various pigments [32] and between the pigments and the protein [33]. In addition this comparison will constitute a unique calibration for the LD and photodichroism techniques by allowing the determination of such important parameters as the angle between the Q_X and Q_Y transitions within each molecule which for isolated bacteriochlorophyll *a* in solution has been shown to be 74° [34] and not 90° as usually assumed.

Nevertheless, with the help of the structural model presently available and of the LD data described here we can tentatively assign each resolved band in the spectra to one of the twelve transition moments of the six molecules. These results are presented in Fig. 5 and they generally agree with the previous assignments made in [1,3,9,28–30,35]. In addition to the tentative assignment of the 660 nm band discussed above, we have also ascribed the 600 and 607 nm bands to the Q_X transition of each of the two B molecules. This assignment is based upon the observation that only for the 607 nm transition an electrochromic blue shift is clearly observed upon 100 K photoreduction of the BPh molecule absorbing at 805 and 544 nm [30]. Picosecond spectroscopy has demonstrated that this PBh molecule was also the one undergoing transient reduction during the electron transfer from P to Q_A [36] and X-ray data have suggested (Michel, H. and Deisenhofer, J., personal communication) its association with the L polypeptide. We have accordingly assigned to B_L (the B molecule associated with the L polypeptide) the transition at 607 nm which is the most perturbed by photoreduction of BPh. This possibility of assigning the absorption bands resolved in the 10 K spectra to all but two (B_{YL}, B_{YM}) of the twelve transition moments of the six chlorophylls will probably be useful when the molecular

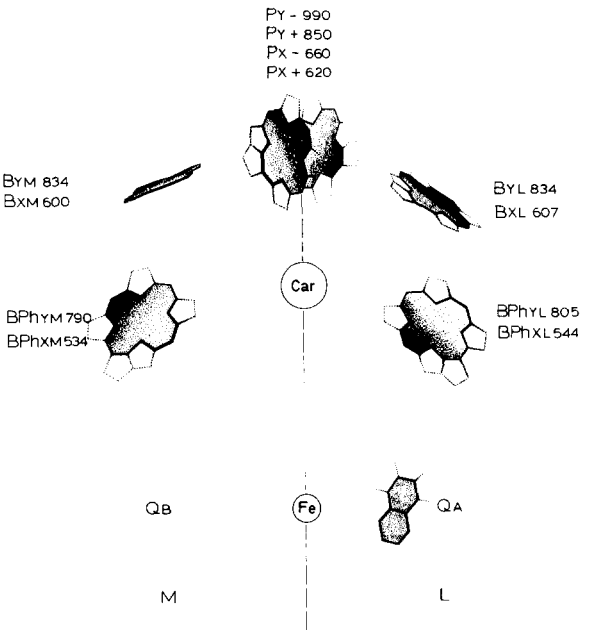

Fig. 5. Wavelength assignment (nm) of the optical transitions of the pigments in the reaction center of *Rps. viridis* (at 10 K). P, B and BPh refer to the primary donor, the monomer bacteriochlorophylls and the bacteriopheophytins, respectively; X and Y are the axes of polarization of the transitions, and L and M refer to the polypeptide chains to which are associated the pigments (the L chain represents the photosynthetic active branch of the electron pathway from P to the primary quinone Q_A). The tentative location of the carotenoid (Car) molecule in the structure is also depicted. Note that the two B_Y bransitions which give rise to the 834 nm absorption band are not spectrally resolved. See text for details.

coordinates obtained from the X-ray study will be released. Not only will it be possible to compare the tilt angles obtained by the two techniques, but also to compare the angles between pairs of transition moments obtained by the X-ray study to the ones that can be obtained very precisely by photodichroism on the states P^+ [1,3,4,8] or BPh_L^- [30] upon excitation within a pure absorption band.

Comparison with previously proposed models

Two models have been proposed to explain the LD and CD spectroscopy of *Rps. viridis* reaction centers. In the model of Shuvalov and Asadov [8] the directions of the Q_Y transitions of the two molecules involved in P are strictly parallel giving only one transition, while in our model [1,3,9,10] they are at approx. 35° from each other, giving rise to a second exciton band in addition to the

990 nm transition. This model constituted an extension of a model previously proposed by Verméglio et al. for the reaction center of *Rps. sphaeroides* and derived from LD [37] and photodichroism studies [38]. On the basis of its orientation at a large angle from the 990 nm transition [9], the 850 nm transition of *Rps. viridis* was ascribed to the higher energy exciton component of P, an assignment also proposed earlier to rationalize (i) picosecond absorption changes accompanying the formation of P* [36] and (ii) low-temperature absorption spectra [28,35]. The ratio of the areas under the 990 and 850 nm bands (approx. 10) was utilized to estimate [31] the 35° angle between the *Y* directions of the monomers [9]. Furthermore, the orientation of the 990 and 620 nm transition dipoles at less than 10° and approx. 60° from the membrane plane, respectively, and the small tilt (20°) of the Q_Y transition dipole of the monomer band appearing in P^+, led us to propose that the two molecules constituting the special pair are both almost perpendicular to the membrane plane with their Q_Y transitions tilted at approx. $+17°$ and $-17°$ with respect to the membrane plane [1,9]. The assignment of the 850 nm transition to P_{Y+} (Fig. 5) has been subsequently strengthened by photoselection experiments [1,3], but also criticized [39,40], and rejected mainly on the grounds that no large bleaching was observed at this wavelength in the spectra of ^3P, the triplet state of P [11,12]. The model of Shuvalov was thus preferred by these authors. In this model [8] the 850 nm transition is ascribed to the Q_Y transition of one of the two monomeric B molecules interacting both with the Q_Y transition (at 834 nm) of the other B molecule and with the 990 nm transition of P. This type of assignment for the 850 nm band has also been later favored by Kirmaier et al. [4] and Seftor and Thornber [13].

As described in the preceding section, the model derived from the X-ray data seems to reflect rather closely the organization of the pigments proposed in Ref. 9. Relevant to the 850 nm transition we note that its strongly negative LD (Fig. 1b), indicating an orientation at approx. 60° out of the membrane, cannot be easily reconciled with the assignment propounded by Shuvalov and Asadov [8] to one of the two B molecules which, as seen by X-ray and LD, have both their plane tilted at a small angle to the membrane. On the other hand an application of the first-order exciton coupling theory [31] to the two molecules assigned to P in the model derived from X-ray leads to four new transitions which have qualitatively the orientations and oscillator strengths observed in the present study for the 990, 850, 620 and possibly the 660 nm transitions. When a more quantitative comparison of our LD results to the X-ray data will become available, it will be possible to further refine our simple model. For example our data show that both the 850 and the 620 nm transitions are not strictly parallel to the C-2 symmetry axis. This could be due to such effects as an asymmetric environment of P [48], some coupling between B and P, overlap of bands or non perfect linear polarization of the transitions.

Regarding the features of the ^3P spectra which have led to the rejection [11,12] of our model it should be stressed that the oscillator strength of the 850 nm component is only about 10% of that of the 990 nm component. Furthermore, the nature of the electronic interactions among the two bacteriochlorophylls of the special pair in the state ^3P or in P^+ can be sufficiently different so that the amplitude and wavelength position of the first excited singlet of the remaining monomer can be very dissimilar. Electrochromic shifts will affect more strongly the pigments in the state P^+ than in the state ^3P. Finally, Scherz and Parson [32] have recently proposed that intensity borrowing could occur among all the six interacting pigments. In these conditions a redistribution of oscillator strength among all the pigments can be expected whenever the electrostatic interactions are locally modified. Such a mechanism could lead to large differences between the P^+-minus-P and the ^3P-minus-P spectra. All these factors make a direct comparison of the two types of spectra, depicted in Fig. 6, rather complicated. However, we suggest, as an alternative explanation to the red-shift of the 850 nm band proposed by Den Blanken and Hoff [12], that the monomer in ^3P absorbs at lower energy and has a larger oscillator strength than in the state P^+. In this case the peak observed around 870 nm in the ^3P spectrum corresponds to the appearance of the long wavelength wing of the monomer absorption band, and the trough at 850 nm to the bleaching of the 850 nm component of P

superimposed to the appearance of this monomer. Under these conditions the data from ^{3}P-minus-P spectra [11,12] can be reconciled with our model of the special pair. We further notice that the polarization values at 870 and 850 nm recently obtained on ^{3}P [41] are compatible with the interpretation proposed above.

Orientation of other components

The high potential cytochrome *c*-558 absorbs at 556 nm at 10 K and exhibits a large negative LD signal (Fig. 1). This result is consistent with the LD data previously obtained on chromatophores at room temperature [42] indicating an orientation preferentially perpendicular to the membrane of this cytochrome.

At 475–480 nm a shoulder is discernible in the absorption spectra of the reaction centers used both in this study (Fig. 1a) and also for preparing the crystals [5]. The presence of about one carotenoid molecule per reaction center has been reported [35], and it is thus reasonable to assign the 475 nm optical transition to this species. The positive LD signal associated with it (Fig. 1b) indicates that the carotenoid is oriented rather parallel to the membrane as it has also been observed in the case of reaction centers isolated from *Rps. sphaeroides* G1C oriented in electric field [43] and *Rps. sphaeroides* 241 oriented in polyacrylamide gels (Fig. 7). This carotenoid has not been located in the X-ray study, probably because, like for the secondary quinone Q_B [44], the conditions which are required for the crystallization of the reaction centers, lead to a partial loss of these molecules. However, utilizing several spectroscopic observations on the carotenoid we can already speculate on the location of this molecule in the reaction center model. On the basis of the remarkable similarity of the geometries of bacteriochlorophyll *a*- and bacteriochlorophyll *b*-containing reaction centers which will be discussed in the next section, we will assume that the orientation of the carotenoid relative to the long-wavelength transition of the special pair (P_Y-) is the same for both types of reaction centers. In the case of *Rps. sphaeroides* 241 this angle has been estimated to be approx. 75° by photoselection [38] and magnetophotoselection [45]. Accordingly, the carotenoid molecule has its transition moment

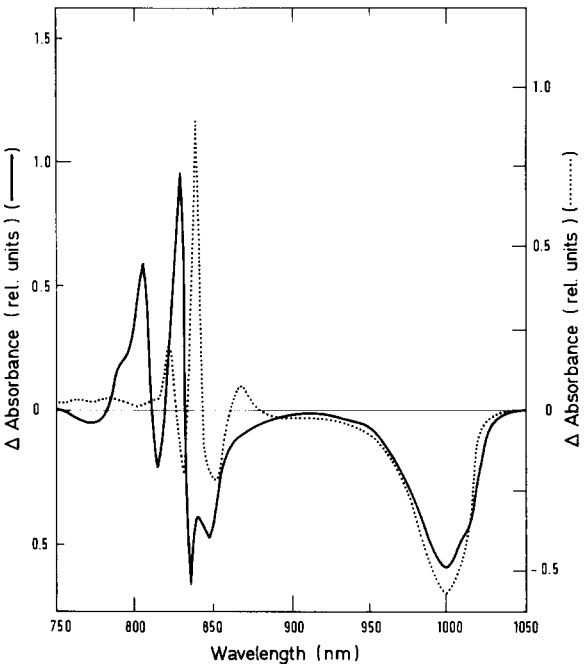

Fig. 6. Comparison between the low-temperature difference spectra of reaction centers from *Rps. viridis* with the primary donor (P) in the photooxidized (P^+) or triplet (^{3}P) states. ———, P^+-minus-P spectrum at 4.2 K redrawn from Breton, J. and Verméglio, A. (Ref. 1); ·····, ^{3}P-minus-P spectrum at 1.2 K redrawn from Den Blanken, H.J. and Hoff, A.J. (Ref. 12).

rather perpendicular to both the C-2 symmetry axis and the 990 nm transition. Furthermore, it must be located quite close to the special pair in order to meet the requirements for the transfer of triplet character from ^{3}P to the carotenoid [46] which implies orbital overlap between the two partners. Finally, in order to take into account the extractability of the carotenoid [35] as well as the ease of reconstitution [47] which bear analogies with the case of the Q_B molecule, we will tentatively locate this carotenoid within the inner pocket between the L and M subunits [15]. In order to maintain the remarkable C-2 symmetry of the system it is further proposed to locate this carotenoid on the symmetry axis as shown in Fig. 5.

Comparison with bacteriochlorophyll a-containing reaction centers

There is much evidence pointing toward a very similar geometry of the special pair in all the reaction centers from purple bacteria. For example

an arrangement similar to the one of *Rps. viridis* has been predicted for the reaction center of *Rsp. rubrum* [48,49]. The oxidized-minus-reduced CD difference spectra of various reaction center preparations have similar S-shaped features with conserved sign both in the Q_Y [50,51] and in the Q_X regions (Refs. 50 and 51 and this Fig. 4). The orientation of each of the transitions relative to the membrane plane is also similar [1]. This can be appreciated when the spectra shown in Fig. 1 for *Rps. viridis* are compared to the corresponding ones (Fig. 7) obtained for the reaction center of *Rps. sphaeroides* 241, although the position (and thus the overlap) of the various absorption bands are fairly different in the two types of reaction centers. This similarity of orientation is also observed for the reaction center of *Rps. sphaeroides* R-26 (data not shown) and extends to the case of the oxidized reaction centers (Breton, J., in preparation). Besides LD and CD data, the results of photodichroism studies on the two types of reaction centers also point towards identical values for the relative angles between pairs of transition moments [1,3,4,7,30,38,52–54]. The results from these various studies (see also Ref. 55) all point toward the view that the six chlorophylls and the carotenoid adopt very similar geometrical orientations in the two types of reaction centers. The small differences which are observed in the relative positions of the absorption bands among different strains [7] or species [4,13] appear to us more probably related to subtle changes in the electronic interactions of the pigments with amino acid residues [33] as well as with one another leading to slight modifications of the intensity borrowing [32] and charge-transfer character [3,11,38,56,57] of the electronic transitions rather than to any large difference in the geometrical organization of the chromophores. Further work in progress on the structure of the reaction centers of *Rps. sphaeroides* as seen by low temperature LD and by X-ray crystallography should allow for a test of this interpretation.

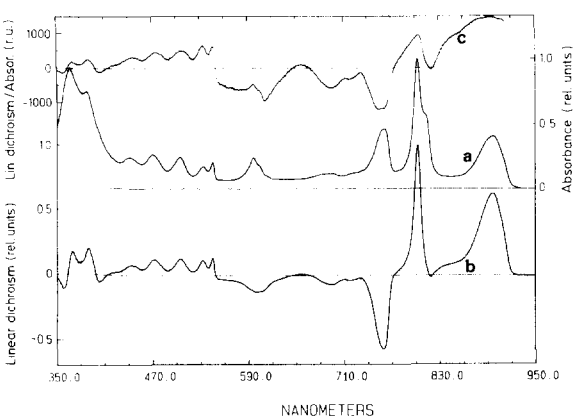

Fig. 7. (a) Absorption (A); (b) linear dichroism (LD); and (c) LD/A spectra at 6 K of reaction centers from *Rps. sphaeroides* 241 oriented in polyacrylamide gel with the primary donor in the reduced state. Note the pronounced feature at 813 nm with a negative LD and also the structure around 590–600 nm in the LD/A spectrum.

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