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CIRCULAR DICHROISM SPECTRA OF ORIENTED PHOTOREACTION CENTER FROM *RHODOSPIRILLUM RUBRUM*

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The circular dichroism spectra of oriented and unoriented photoreaction centers of *Rhodospirillum rubrum* are compared. Orientation is achieved by pressing photoreaction center suspended in polyacrylamide gel. The biphasic bands at 870 and 810 nm and at 630 and 600 nm undergo a rotatory strength decrease when measured in the direction of the pressure, but not when measured in the direction normal to the pressure. Such a decrease in oriented photoreaction center is consistent with the model according to which these bands are dimer exciton bands of the special pair bacteriochlorophyll.

Introduction

Purified photoreaction centers isolated from the purple photosynthetic bacteria *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum* contain four molecules of bacteriochlorophyll (BChl) and two molecules of bacteriopheophytin (BPh) [1,2]. Two of the BChl molecules (the special pair BChl) act as the primary electron donor [3,4] and one of the BChl and BPh molecules may act as the first electron acceptors [5,6]. Energy-transfer studies [7] have shown that there are singlet-singlet interactions between all the chromophore molecules in the photoreaction center. There is, however, speculation as to the strength of this interaction. On the basis of circular dichroism spectroscopy (CD), Sauer et al. [8] have suggested a trimer of interacting bacteriochlorophylls. In this model, the three CD bands at 795, 810 and 870 nm are due to exciton interactions. Vermeglio and Clayton [9], on the basis of linear photodichroism spectroscopy of oriented chromatophores, suggested that only the special pair BChl has strong exciton interaction resulting in absorption and CD bands at 870 and 810 nm. Shuvalov et al. [10], on the basis of photoselection studies in photoreaction center, have suggested that the special pair BChl has strong exciton interaction resulting in an absorption and CD band at 870 nm and a forbidden band at 765 nm. They further suggested that the other two BChl molecules form strong exciton bands at 800 and 810 nm. To decide between the three models, a method must be found to distinguish exciton bands from monomer bands. A method of choice which we have used in this work is oriented circular dichroism spectroscopy.

Mandel and Holzwarth [11] first showed that this technique can provide a stringent test for strong exciton bands because it yields information on the individual diagonal components of the rotatory strength tensor. For example, theoretical calculations show that the rotatory strength of exciton transitions is zero in a cylindrically symmetric system analysed with light parallel to the symmetry axis [12]. Muccio and Cassim [13] have

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin L.

studied the absorption and CD spectra of purple membrane from *Halobacterium halobium*, in which the plane of the membranes is oriented perpendicularly to the incident light beam. They found that the biphasic band in the visible region of the CD spectrum, that is observed in randomly oriented samples, is replaced by a single positive band in oriented preparations. They found these results to be consistent with a cyclic-trimer exciton model of three closely lying chromophores related by a C_3 axis of symmetry. In the present study, we compared the absorption and CD spectra of oriented and unoriented photoreaction center isolated from *Rsp. rubrum*. We feel that our results can best be explained by the model, in which the biphasic CD bands at 870 and 810 nm and at 630 and 600 nm are dimer exciton bands of the special pair BChl.

Materials and Methods

Photoreaction center was isolated from wild-type *Rsp. rubrum* S1 (ATCC No. 11170) by the method of Noël et al. [14] as modified by Vadeboncoeur et al. [15]. For measurement, the photoreaction center was dispersed in 25 mM Tris-HCl (pH 8.0)/0.05% dodecyldimethylamine *N*-oxide/ 50% glycerol. Oriented samples were prepared by suspending photoreaction center into a polyacrylamide gel (10% (w/v) acrylamide; 0.3% (w/v) *N*,*N*′-methylenebisacrylamide) and squeezing the gel to make it stretch so as to align the photoreaction center along the direction of stretching [16]. The sample was polymerized in the dark for 12 h at 4°C in the presence of 0.03% *N*,*N*,*N*′,*N*′-tetramethylenediamine (v/v) and 0.005% ammonium persulfate (w/v). A 1-cm square solid block was obtained. In studies where the pressure was applied to a pair of opposing sides, the gel was placed in a cuvette consisting of two aluminium plates, in which the gel could be squeezed evenly by tightening screws at each corner of the plates. Each plate had an optical window so that the absorption and CD spectra could be taken in the direction of the applied pressure. In studies in which the pressure was applied to two pairs of opposing sides, the gel was placed in another cuvette consisting of two aluminium blocks. The bottom block contained a 0.85 cm groove, into which the sample was placed. Pressure was applied to the sample with the top block by tightening screws evenly at each corner. Each block had an optical window so that the absorption spectra could be taken both parallel and normal to the direction of the applied pressure. Pressure was applied to the sample until the absorption spectrum measured in the direction of the pressure showed no linear dichroism and the CD spectrum measured in the same direction showed little change on rotating the cuvette about the axis of measurement.

Absorption and linear dichroism spectra were recorded with a Cary 14R spectrophotometer. For linear dichroism spectra, the measuring light was polarized by sheet polarizers (Polaroid HR for wavelengths of 810–950 nm, Polaroid HN7 for wavelengths of 650–810 nm and Polaroid HR 2.8 for wavelength of 500–650 nm). The CD spectra were recorded with a Jasco S-20 spectropolarimeter modified to be sensitive in the 800–900 nm range by the use of a Hamamatsu R669 photomultiplier. To check for possible artifact in the CD spectra due to depolarization by the polyacrylamide, the tandem-cell technique was used [17]. CD spectra of photoreaction center were recorded with a sample of polyacrylamide gel in front and then at the back of a solution of photoreaction center. The two spectra were identical, indicating that depolarization due to the polyacrylamide gel is negligible. Possible artifact due to linear dichroism was checked by comparing the CD spectra on rotating the cuvette about an axis defined by the incident beam [13,18]. The CD spectra were the same within 5%.

Results

Orientation of photoreaction center

Abdourakhmanov et al. [16] showed that photoreaction center can be oriented in a polyacrylamide gel by applying pressure to stretch the gel. To study the circular dichroism of any oriented system, care must be taken to ensure that the sample is not linearly dichroic, since this in itself would give rise to CD [19,20]. In the following experiments, in which the photoreaction center was oriented in a block of polyacrylamide gel, the effects of linear dichroism was eliminated in two different ways. The first was by applying pressure

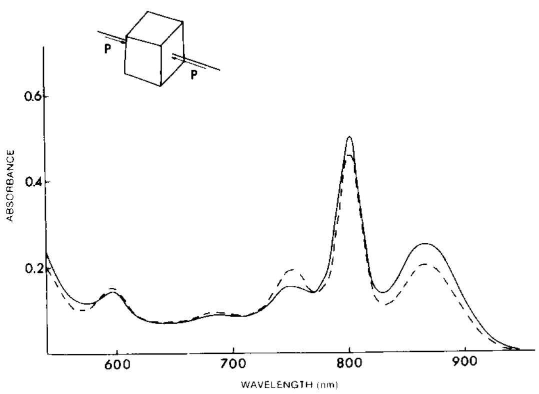

Fig. 1. Absorption spectra of oriented (———) and unoriented (— — —) photoreaction center from *Rsp. rubrum*. Photoreaction center was suspended in 10% polyacrylamide gel. Orientation of photoreaction center was achieved by applying pressure (P) to the gel in the direction of measurement. The spectrum of the unoriented sample was corrected for absorption pathlength so as to normalize the two spectra.

only in the direction of propagation of the measuring-light beam. In this case, the pressure compresses the block and stretches the gel equally in all directions normal to the direction of the pressure. This results in a symmetrical alignment of the photoreaction center about the light-propagation axis. The second was by applying pressure equally in all directions normal to the measuring-light beam. The pressure in this case stretches the gel in the direction of propagation of the measuring light. Because of the equal pressure on all four sides, the photoreaction center will also be aligned symmetrically about the light-propagation axis.

Fig. 1 shows the absorption spectrum of photoreaction center of *Rsp. rubrum* dispersed in 10% polyacrylamide gel. It is identical to the absorption spectra of photoreaction center in solution. No difference was found in the absorption spectra measured with linearly polarized light of orthogonal planes of polarization (not shown), indicating the complete random orientation of the chromophores in the gel. On applying pressure to a pair of opposite sides of the cubic gel so that the thickness decreased from 1 cm to approx. 0.55 cm and the sides expanded from 1 cm to about 1.4 cm, we found that the absorption spectrum of the photoreaction center measured in the direction of the pressure could not be accounted for by a mere decrease in pathlength (Fig. 1). Indeed, the absorbance of the 870 and 800 nm bands increased and the absorbance of the 750 and 600 nm bands decreased with pressure. These changes in absorbance must be due to a reorientation of the transition dipoles with the applied pressure. The absorbance increase of the 870 and 800 nm bands indicates that these transition dipoles are reoriented towards the stretching direction of the gel (normal to the light-beam propagation axis), while the 750 and 600 nm transition dipoles are reoriented towards a direction normal to the stretching direction (parallel to the light-beam propagation axis). On measuring the spectrum with linearly polarized light in the direction of the pressure, we found no linear dichroism at any wavelength, indicating that the transition dipoles are isotropically oriented in all directions normal to the pressure. On measuring the absorption spectrum at right angles to the direction of the pressure (Fig. 2), we found that the bands are highly dichroic indicating a reorientation of the transition dipoles. Fig. 2 shows that the transition dipoles of the 870 and 800 nm bands are oriented in the general direction of stretching, while the transition dipoles of the 750 and 600 nm bands tend to orient perpendicularly to the stretching direction. These observa-

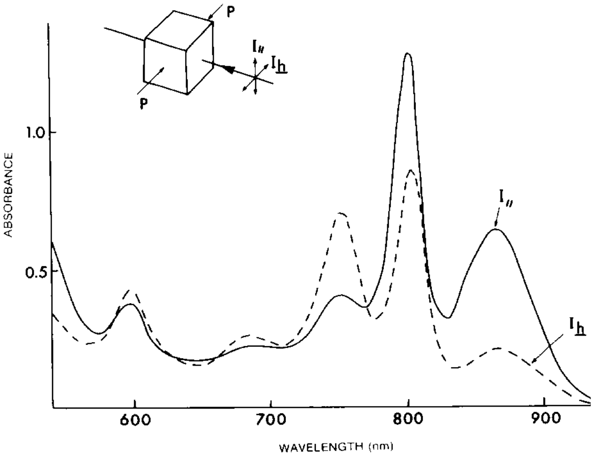

Fig. 2. Absorption spectra of oriented photoreaction center from *Rsp. rubrum* measured with linearly polarized light of electric vector parallel (———) and perpendicular (— — —) to the direction of stretching of the sample. Experimental conditions were the same as in Fig. 1, except that the sample was rotated by 90°.

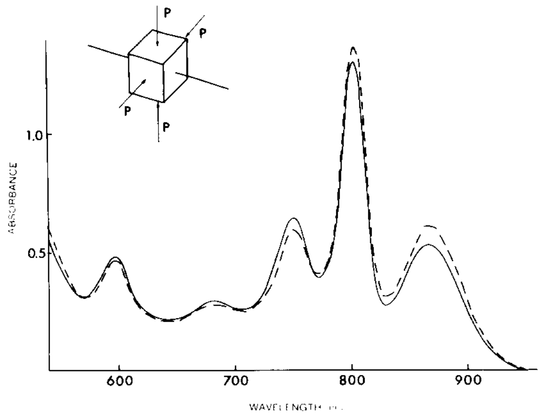

Fig. 3. Absorption spectra of oriented (————) and unoriented (— — —) photoreaction center from *Rsp. rubrum*. Photoreaction center was suspended in 10% polyacrylamide gel. Orientation of the photoreaction center was achieved by applying pressure (P) to the gel in all directions normal to the direction of measurement. The spectrum of unoriented sample was corrected for absorption pathlength so as to normalize the two spectra.

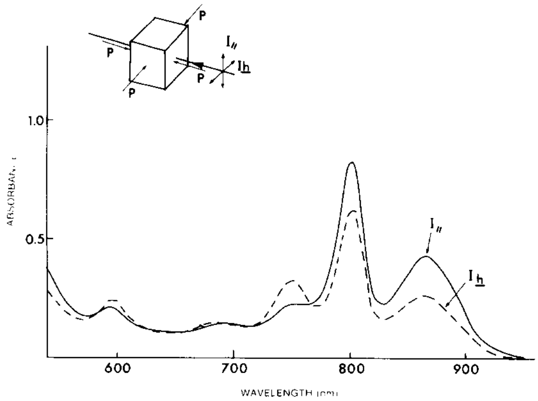

Fig. 4. Absorption spectra of oriented photoreaction center from *Rsp. rubrum* measured with linearly polarized light of electric vector parallel (————) and perpendicular (— — —) to the direction of stretching of the sample. Experimental conditions were the same as in Fig. 3 except that the sample was rotated by 90°.

tions agree with the results shown in Fig. 1. These experiments show that on applying pressure to a pair of opposite sides of the gel, we can orient the photoreaction center. Since the sample does not show any linear dichroism when measured in the direction of the applied pressure, it is well suited to oriented CD work. We will hereafter refer to this system of orientation as alignment A.

Another oriented system suitable for CD measurements can be obtained by applying pressure equally on all four sides of the polyacrylamide block normal to the light propagation axis. We squeezed a block with the following approximate initial dimensions: 1.3 cm in height, 1 cm in width and 1 cm in length to the following final approximate dimension: 0.9 cm in height, 0.85 cm in width and 1.7 cm in length. By comparison with the absorption spectrum of photoreaction center of equal pathlength (Fig. 3), the pressure causes the 870 and 800 nm bands to decrease and the 750 and 600 nm bands to increase. This is due to a reorientation of the 870 and 800 nm transition dipoles towards the light propagation axis and of the 750 and 600 nm transition dipoles at right-angles to the same axis. On measuring the absorption spectrum in a direction normal to the pressure with linearly polarized light, we found no linear dichroism at any wavelength, indicating that the photoreaction center is isotropically oriented around the light propagation axis (not shown). On measuring the absorption spectrum in the direction of the pressure, we found that the absorption bands are highly dichroic, as expected (Fig. 4).

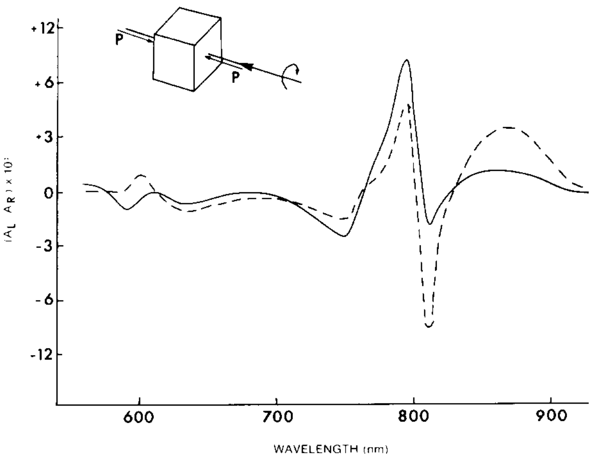

Fig. 5. Circular dichroism spectra of oriented (————) and unoriented (— — —) photoreaction center from *Rsp. rubrum*. The sample used in the measurement was the same as in Fig. 1. Oriented CD spectrum was measured in a direction normal to the direction of stretching of the gel. The unoriented CD spectrum was corrected for optical pathlength so as to normalize the two spectra.

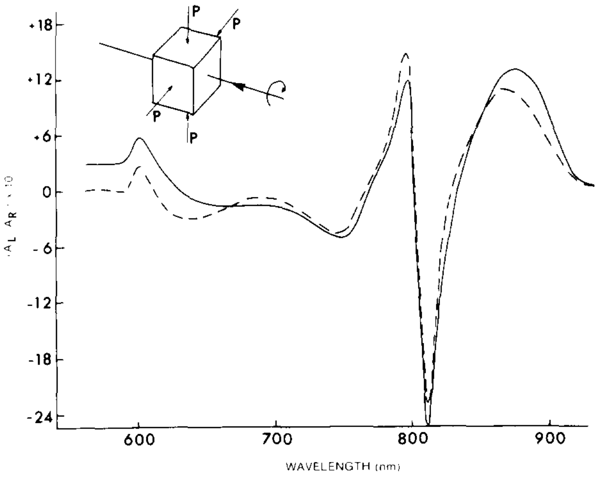

Fig. 6. Circular dichroism spectra of oriented (————) and unoriented (— — —) photoreaction center from *Rsp. rubrum*. The sample used in the measurement was the same as in Fig. 3. Oriented CD spectrum was measured in a direction parallel to the direction of gel stretching. The unoriented CD spectrum was corrected for optical pathlength so as to normalize the two spectra.

This shows that the 870 and 800 nm transition dipoles are oriented in the stretching direction of the gel and the 750 and 600 nm transition dipoles are oriented normal to it. These results are consistent with those of Figs. 1–3 and show that on applying pressure on all four sides of the polyacrylamide gel, we can also orient the photoreaction center. Since in this case the sample does not show any linear dichroism when measured in the stretching direction of the gel, this orientation is also suitable for CD work. We will hereafter refer to this system of orientation as alignment B.

Circular dichroism of oriented photoreaction center

Unoriented photoreaction center from *Rsp. rubrum* embedded in a 10% polyacrylamide gel (Fig. 5) has essentially the same CD spectrum as in solution. However, when the photoreaction center is oriented in alignment A (Fig. 5), its CD spectrum is quite different. This is witnessed by a decrease in the rotary strength of the 870, 810 and 600 nm bands and by an increase in the rotatory strength of the 795 and 750 nm bands. The CD spectrum of the oriented photoreaction center resembles that of an oxidized preparation, except that the oxidized center has peaks at 799 and 820 nm instead of at 795 and 810 nm (not shown). On the other hand, the CD spectrum of photoreaction center oriented in alignment B is not very different from the CD spectrum of an unoriented sample of the same pathlength (Fig. 6). The most notable difference is in the rotatory strength which increases at 870 and 810 nm and decreases at 795 nm.

Discussion

The CD spectra of oriented chromophores, if they are monomeric, can be predicted from their absorption spectra. Since the rotatory strength of a monomer transition depends on its electric transition dipole moment [12], it would be expected to increase (or decrease) if the transition dipoles are aligned, so as to increase (or decrease) the absorption. Comparing the corresponding absorption (Figs. 1 and 3) and CD spectra (Figs. 5 and 6) of oriented photoreaction center, only the 795 nm band behaves in this manner. The other CD bands have a different behavior which cannot be explained simply by the alignment of transition dipoles.

The CD of the 750 nm band of unoriented photoreaction center is smaller than that of oriented photoreaction center in either alignment A (Fig. 5) or in alignment B (Fig. 6). The 750 nm band has been attributed to two BPh monomer bands [21]. In this case, the increase in the rotatory strength of the BPh band in both alignments A and B implies an increase in the total rotatory strength. This increase could be due to the pressure causing the magnetic dipole moment to align closer to the electric dipole moment.

The largest changes in the CD are the decreases of the 870, 810 and 600 nm bands when measured in a direction normal to the stretching direction of the sample gel (alignment A, Fig. 5). Since the absorbance at 870 nm increases under the same conditions (Fig. 1), the decrease in the corresponding CD band cannot be attributed to the alignment of the 870 nm transition dipole. Another possible explanation, a decrease in the total rotatory strength caused by the pressure, must also be ruled out, since the CD of the 870 nm band increases when the sample is stretched in the direction of light propagation (alignment B, Fig. 6).

However, these observations can easily be interpreted on the basis of an exciton model. According to the sum rule [12], for a given orientation axis, Z, the total rotatory strength, R_{ZZ}, of all the exciton transitions, A, must be equal to zero, i.e.:

$$\sum_{A}(R_{ZZ})_{A} = 0 \qquad (1)$$

As shown in Figs. 5 and 6, the 870 and 810 nm bands of the CD spectrum of the photoreaction center appear to obey the sum rule. When the sample is in alignment A, the positive rotatory strength of the 870 nm band and the negative rotatory strength of the 810 nm band decrease together. When the sample is in alignment B, the rotatory strengths of both bands increase. This provides strong support for the interpretation that the 810 and 870 nm bands are exciton bands. Since these same bands are bleached on oxidation, they can be attributed to a Q_Y exciton formed by the special pair that was deduced from EPR and ENDOR spectroscopy [3,4]. Similarly, the 600 and 630 nm bands can be attributed to a Q_X exciton of the special pair.

In principle, some additional information about the geometry of the special pair can be deduced from these experiments. In the strong coupling theory, the rotatory strength R, of each exciton transition, A, can be expressed in terms of its monomer components [13]. For light incident along the Z direction, the rotatory strength, R_{ZZ}, has been shown by Muccio and Cassim [13] to be:

$$(R_{ZZ})_{A} = \frac{2\pi\nu_{A}}{c}\sum_{i=1}^{n}\sum_{j>i}^{n} C_{iA}C_{jA}(Z_{j}-Z_{i}) \times[(\mu_{Y})_{i}(\mu_{X})_{j}-(\mu_{Y})_{j}(\mu_{X})_{i}] \qquad (2)$$

where ν_A is the frequency for the transition, A, c is the speed of light, n is the number of monomers which are strongly coupled, C_{iA} is the ith coefficient of the excitonic wavefunctions, Z_i is the component of the position vector which is in the Z direction for the ith monomer and $(\mu_Y)_i$ is the Y component of the transition dipole moment of the ith monomer. According to Eqn. 2, no exciton bands should be observed by CD when either or both terms $(Z_2 - Z_1)$ and $((\mu_Y)_1(\mu_X)_2 - (\mu_Y)_2(\mu_X)_1)$ are equal to zero. For the first term, this will occur when the position vectors of the two monomers have equal projections in the light propagation vector. The second term will be zero when the electric-dipole transition moments of the monomers have parallel projections in the plane normal to the light-propagation axis. These restrictions serve as a boundary to the possible models that could be proposed for the special pair. An interesting instance where both terms vanish is when the system has cylindrical symmetry. Tinoco has shown in all generality [12] that, in such a system, the CD excitonic transitions will disappear when light is incident along the symmetry axis. This interpretation of our results would attribute a cylindrical symmetry axis – and therefore C_2 symmetry – to the special BChl dimer. C_2 symmetry would imply that the molecular planes of the two BChl molecules are parallel to each other. They could not be coplanar, however, as indicated by the large rotatory strength of the biphasic 870 and 810 nm bands. This interpretation of our data would be in line with a model proposed for the primary electron donor of system I, P-700 [22]. However, it must be kept in mind, as pointed out above, that this interpretation is not unique.

The CD spectra of oriented photoreaction center presented here do not support the two dimer pair exciton model of Shuvalov et al. [10]. This model predicts that one of the dimers would give rise to an allowed transition at 870 nm and to a forbidden transition at 765 nm. In this case, no exciton CD bands should be observed at these wavelengths. In fact, a CD band is indeed observed at 870 nm but none at 765 nm. The other prediction of this model, that the 795 nm and 810 nm CD are coupled exciton bands, would entail that, according to the sum rule, their summed rotatory strengths should be zero for any given orientation. As seen from Fig. 5, this is not verified in alignment A where the sum of the rotatory strengths at these wavelengths is clearly greater than zero.

The trimer model, in the form proposed by Sauer et al. [8] is also difficult to reconcile with the observations reported here: if the CD bands at 795, 810 and 870 nm were due entirely to exciton interaction between BChl molecules, the sum rule would predict that the sum of their rotatory strengths should be zero for any given orientation. This is not verified in alignment A due to the large rotatory strength of the 795 nm band (Fig. 5). If the additional postulate was made that the 795 nm CD band is also largely due to other factors such as a dissymmetric interaction of BChl with its environment or a contribution by bacteriopheophytin, the sum rule would be impossible to apply to such a system without knowing the contribution of the excitonic interaction to the rotatory strength of each of the CD bands. Although such more elaborate models might be consistent with our findings, we prefer the simpler interpretation of a dimer model involving the BChl special pair and giving rise to CD bands at 810 and 870 nm.

Acknowledgement

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