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PHOTODICHROIC STUDIES OF THE PHOTOREACTION CENTER FROM *RHODOSPIRILLUM RUBRUM*

I. ATTRIBUTION OF P_{870} TO TWO NON PARALLEL DIPOLES

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SUMMARY

A randomly oriented sample of photoreaction center prepared from *Rhodospirillum rubrum* was excited at 77 °K by an actinic linearly polarized light of 870 nm. Under such conditions, only those chromophores with components of their absorption dipoles oriented parallel to the polarization of the actinic light are bleached. The change in absorbance at 900 nm of this photoselected sample was observed while varying the angle of polarization of a weak measuring light. The polarization of the absorbance change was thus evaluated as 0.25.

This value is interpreted to mean that P_{870} is attributable to two absorption dipoles forming an angle included between 35.75° and 90°. Comparison with the p value of 0.5 obtained on a similar preparation by polarization of fluorescence (Ebrey, T. G. and Clayton, R. K. (1969) Photochem. Photobiol. 10, 109–117) leads to the conclusion that either these two dipoles emit fluorescence without being coupled by singlet-singlet energy transfer or that only one of them is a fluorescence emitter in the absence of reversible singlet-singlet energy transfer.

INTRODUCTION

Recent studies have shown that the photochemical reaction center isolated from certain *Rhodospirillaceae* contains four molecules of bacteriochlorophyll and two of bacteriopheophytin per $P^{+}{}_{870}$ [1, 2]. Moreover, the preparations from wild type organisms also contain one molecule of a carotenoid [2, 3].

The absorption bands at 870, 800 and 600 nm can be attributed to bacteriochlorophyll. As evidenced by circular dichroism spectroscopy of the 600 and 800 nm bands, the bacteriochlorophyll molecules are not coplanar [4, 5]. The characteristic effect of the oxidation of P_{870} on these spectra was taken to indicate that the four molecules interact with one another [4, 5]. Circular dichroism at 77 °K shows that the 870 nm band is made of at least two components [5]. EPR and ENDOR spectroscopy indicate that the free electron on the $P^{+}{}_{870}$ radical is shared between two bacteriochlorophyll molecules [6–9].

Two main hypotheses for the structure of the bacteriochlorophyll aggregate may be proposed (see for example, ref. 10) : (i) one aggregate of four molecules is responsible for the optical transitions at 870 nm, 800 nm and 600 nm; (ii) this aggregate may be divided into two subsets (of two bacteriochlorophyll molecules each), one of which is responsible for the 800 nm band and the other for the 870 nm band.

Polarization of fluorescence measurements show that fluorescence emission at 920 nm is maximally polarized ($p = 0.5$) with 870 nm excitation [11]. Independently of the two models outlined above, this observation may have three different interpretations: (i) there may be only one absorption dipole responsible for the 870 nm band, but if there are several they must be either (ii) rigidly oriented in parallel fashion or (iii) without reversible singlet energy transfer between them.

In order to try to sort out these various possibilities, we performed photodichroic experiments at 77 °K [12, 13]. The rationale was that if interpretations (i) or (ii) were correct, the polarization observed by fluorescence or by photodichroism should be the same. Conversely, interpretation (iii) would hold if different results were obtained by the two methods.

MATERIALS AND METHODS

Photoreaction center was isolated from wild type *Rhodospirillum rubrum* (ATCC no. 11170) by the method of Noël et al. [14]. The preparations were stored at 4 °C in 25 mM Tris · HCl (pH 8.0) buffer containing 0.03 % dodecyldimethylamine *N*-oxide.

All measurements were performed at 77 °K in an Air Products Corporation Joule-Thomson cryostat cooled by nitrogen gas. The vacuum shroud of the instrument was fitted with three optically flat windows so as to permit illumination of the sample with actinic light while taking its spectrum. The sample was dispersed in 25 mM Tris · HCl (pH 8.0)/0.05 % dodecyldimethylamine *N*-oxide containing 50 % glycerol. The sample cuvette was 3 mm^2 in section.

Absorbance change was measured with a Cary 14 R spectrophotometer modified so as to permit illumination of the sample at 90° to the measuring light beam. Cross illumination was provided by a 650 W tungsten-halogen lamp. A polarizer (Polaroid HN7) and a Baird Atomic interference filter (870 or 900 nm maximum transmission with a half-bandwidth of 15 or 10 nm respectively) were interposed between this light source and the sample. Another identical polarizer placed between the sample and the phototube could be slowly rotated (1/6 rev./min) by a small synchronous motor. An interference filter with maximum transmission at 900 or 870 nm (as above) was placed in front of the phototube in the sample compartment to cut off any scattered light from the exciting beam. Compensating neutral density filters were placed in front of the phototube in the reference compartment. Scattered actinic light could not have contributed significantly to the absorbance change measurements since, in the absence of any photoreaction center, no absorbance change could be detected with the actinic light intensities used in these experiments. A diagram of the experimental set up is given on Fig. 1.

The photodichroic experiment was performed in the following manner. The absorbance at 900 nm of a photoreaction center preparation was recorded as the polarizer was continuously rotated up to 360° in the absence of any actinic light. This

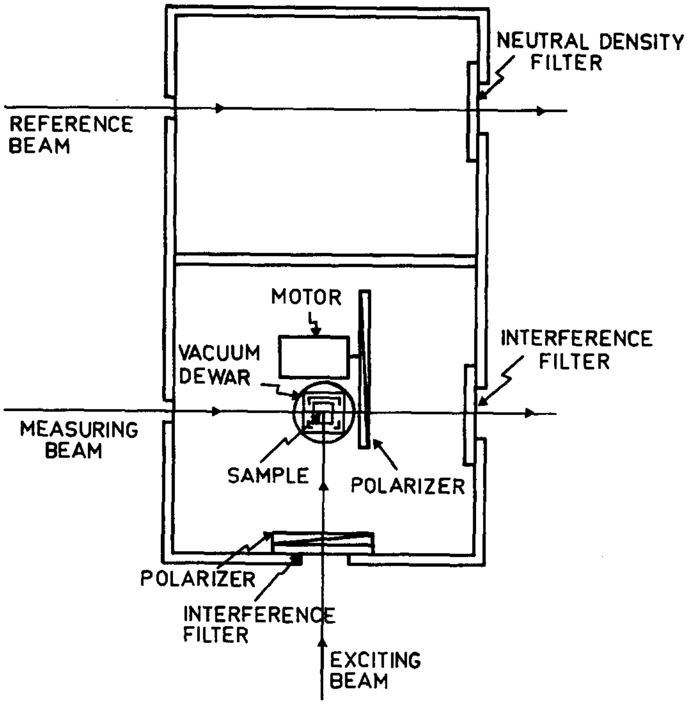

Fig. 1. Schematic diagram of the modifications to the sample and reference chambers of the Cary 14 R spectrophotometer (top view).

recording was used as a baseline for the light-induced absorbance change to compensate for instrumental artifacts. The sample was then excited with continuous 870 nm light of fixed polarization. The absorbance was again recorded as a function of the angle of polarization of the measuring light beam. The absorbance change at each angle of polarization was calculated by subtracting the two curves obtained with and without actinic illumination.

The intensity of the actinic light was adjusted so that the value of $\Delta A/A$ was never greater than 0.2. The reason is that the intensity of the photoselecting light must be low enough not to cause a large deviation from the randomness of the photoselected species (see Appendix I), but high enough to cause easily measurable variations in the absorbance changes.

RESULTS

Fig. 2 shows the near infrared absorption spectrum of the photoreaction center at 77 °K in both its reduced (solid line) and photooxidized forms (dotted line). As observed previously with other photoreaction center preparations [18], the absorption maximum of P_{870} is shifted from 868 nm at room temperature to 893 nm.

A prerequisite for the validity of photoinduced dichroism measurements is that depolarization due to scattering of both the measuring light and the actinic light is kept to a minimum. Depolarization of the measuring light was checked by comparing the

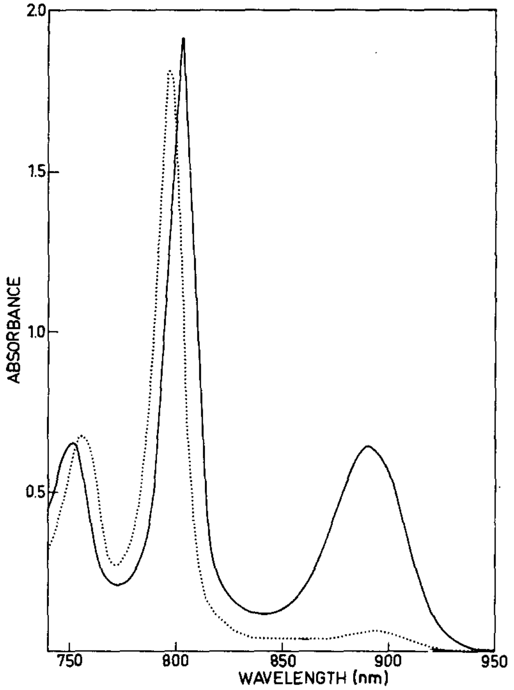

Fig. 2. Absorption spectrum of photoreaction center at 77 °K in 25 mM Tris · HCl (pH 8.0)/50 % glycerol. —, reduced form; ······, oxidized form; spectrum taken under bright undispersed light (I.R.2 mode of Cary 14 R spectrophotometer). From C. Vadeboncoeur and G. Gingras, unpublished.

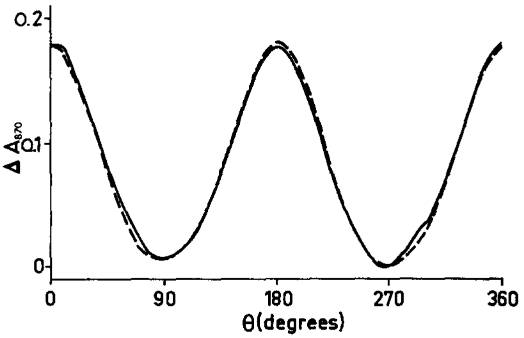

Fig. 3. Change in absorbance at 870 nm as a function of the angle of polarization θ of the transmitted measuring beam. The solid line is obtained at room temperature without the sample cuvette in the vacuum shroud. The dotted line is obtained at 77 °K with photoreaction center in the cuvette mounted inside the shroud. The absorbance in the two cases is not the same and is arbitrarily set at zero for $\theta = 270°$.

intrinsic polarization of the system including the vacuum shroud, but without the sample and the polarization of the system with the sample cuvette containing photoreaction center frozen to an optically clear glass at 77 °K. The intrinsic polarization is shown by the solid line in Fig. 3. It is due to experimental factors such as polarization of the measuring light and dichroism of the mirrors, interference filters and windows including the photocathode of the photomultiplier tube. The dotted line in Fig. 3 shows the data for the system plus photoreaction center at 77 °K in the dark. The two curves have been made to coincide for comparison purposes at a polarization angle of 270°. Their almost perfect superimposability means that the cuvette and the glassy sample at 77 °K introduce very little depolarization of the measuring light. Very similar results were obtained whether the measuring beam was set at 870 nm (Fig. 3) or at 900 nm (not shown).

Another possible source of artifacts would be depolarization of the actinic beam. This was checked as follows. When the actinic light is polarized in the XY plane (see fig. 4) no photodichroism should be observed either in the XY plane or in the YZ plane of analysis for an isotropic sample. Therefore, in this case, the absorbance change should remain constant when the monitoring angle of polarization is varied. However, scattering of the actinic beam (XY plane) would produce partial polarization in the YZ plane resulting in a greater absorbance change in that plane than in the XY plane. Experimentally, no photoinduced dichroism could be observed in this case (square dots in fig. 5). This is in complete agreement with the theoretical expectations (fig. 5; curve a, solid line) for an isotropic sample. Hence, we can safely conclude that our measurements are free from significant artifactual depolarization of either the measuring light or of the actinic light.

Excitation light that is polarized in the XZ plane (Fig. 4) should cause photoselection when viewed along the Y axis since molecules with absorption dipoles with components oriented in the XZ plane will be preferentially bleached. With the actinic light polarized in the XZ plane, let $\Delta A_{\parallel}$ be the change in absorbance when the measuring light is polarized in the YZ plane and $\Delta A_{\perp}$ be the change in absorbance when

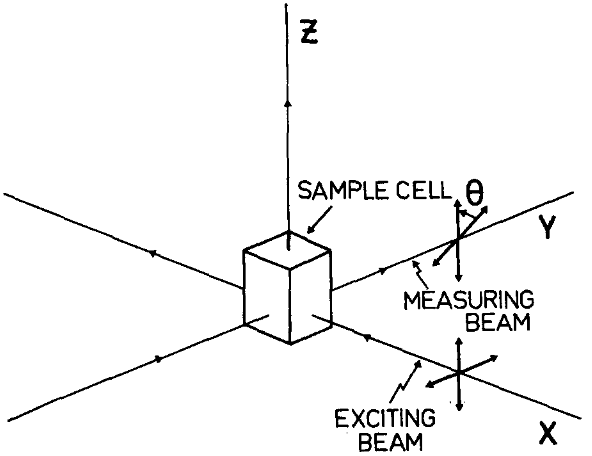

Fig. 4. Orientation of experimental apparatus. The direction of the measuring light beam is along the Y axis. The direction of the actinic light is along the X axis.

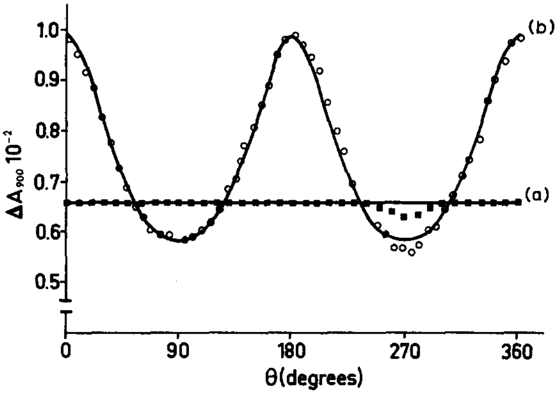

Fig. 5. Change in absorbance at 900 nm (ΔA_{900}) of a photoreaction center preparation at 77 °K as a function of the angle of polarization θ of the transmitted light of the measuring beam. Wavelength of the actinic light beam, 870 nm. Curve (a), experimental data (■) obtained with the actinic beam polarized parallel to the direction of the measuring light. Curve (b), experimental data (○) obtained with the actinic beam polarized perpendicular to the direction of the measuring light beam. ■ and ○, experimental data; —, theoretical predictions.

the measuring light is polarized in the XY plane. Any measuring light which is polarized at an angle θ' with respect to the YZ plane can be split into two components; one polarized in the YZ plane and the other in the XY plane. The change in absorbance of the component which is polarized in the YZ plane (ΔA_z) will be equal to $\Delta A_{||}$ multiplied by the probability that the component is polarized in the YZ plane.

$$\Delta A_z = \Delta A_{||} \cdot \cos\theta' \qquad \text{(1a)}$$

Similarly

$$\Delta A_x = \Delta A_{\perp} \cdot \sin\theta' \qquad \text{(1b)}$$

where ΔA_x is the change in absorbance of the component that is polarized in the XY plane. Combining the two Eqns. 1a and 1b, one can show that

$$\frac{(\Delta A_z)^2}{(\Delta A_{||})^2} + \frac{(\Delta A_x)^2}{(\Delta A_{\perp})^2} = 1 \qquad \text{(2)}$$

Hence in a randomly oriented population of linear oscillators, the change in absorbance created by light polarized in the XZ plane is an elliptical function of the polarization angle of the measuring light. Changing Eqn. 2 to a polar equation:

$$(\Delta A)^2 = \frac{(\Delta A_{||})^2 \cdot (\Delta A_{\perp})^2}{(\Delta A_{||})^2 \cdot \sin^2\theta + (\Delta A_{\perp})^2 \cdot \cos^2\theta} \qquad \text{(3)}$$

Eqn. 3 then shows how the change in absorbance, ΔA, is related to the polarization angle θ of the transmitted measuring light. The theoretical plot using Eqn. 3 is shown by the oscillating smooth line in Fig. 5 (curve b). The circles are the experimental results and they agree well with theory.

Table I shows the results of three independent experiments done with 870 nm

TABLE I

POLARIZATION p AS MEASURED BY CHANGE IN ABSORBANCE

$\Delta A_{\parallel}$ and $\Delta A_{\perp}$ are the changes in absorbance of a photoreaction center preparation at 77 °K when the polarizations of the actinic and measuring light are parallel and perpendicular to each other respectively. The absorbance at 900 and 870 nm is approx. 0.57 and 0.35, respectively, in all cases.

Actinic light (nm)	Measuring light (nm)	$\Delta A_{\parallel}$	$\Delta A_{\perp}$	p
870	900	0.099	0.058	0.261
870	900	0.077	0.046	0.252
870	900	0.092	0.055	0.252
900	870	0.070	0.0375	0.302

actinic light and 900 nm measuring light. The polarization p of the absorbance change is defined in the usual way [12] as

$$p = \frac{\Delta A_{\parallel} - \Delta A_{\perp}}{\Delta A_{\parallel} + \Delta A_{\perp}} \tag{4}$$

The average value of p for three experiments is 0.255. The reverse experiment was done by exciting with 900 nm light and measuring with 870 nm light. In this case, the p value (0.302) is higher. This difference is due to the fact that correction for the polarization of the oxidized photoreaction center has not been made. Making the correction, as outlined in Appendix II, the polarization of the reduced photoreaction center is found to be between 0.28 and 0.22. This ambiguity is due to the fact that the polarization of the oxidized photoreaction center is not known.

DISCUSSION

In the absence of any rotational motion or of energy transfer to nonparallel dipoles, the polarization p_0 observed either by emission or by absorption in a population of randomly oriented dipoles has a maximal theoretical value of 0.5. In the experiments reported here, all the measurements were performed at 77 °K in a 50 % glycerol/water glass. Under comparable conditions, phosphorescence polarization measurements of molecules such as carbazole or indole indicate the absence of any rotational motion within the seconds range [16, 17]. Since the lifetime of charge recombination in the photoreaction center is much shorter ($\tau_{\frac{1}{2}} \approx 20$ ms), the bacteriochlorophyll molecules are undoubtedly rigidly fixed within this time interval. However, since in our experiments photoselection was not performed on a perfectly random sample due to the finite amount of oriented $P^{+}{}_{870}$ present in the steady state, p_0 should be lower than 0.5. But with the intensity of polarized actinic light used here, p_0 should not be lower than 0.461 (see Appendix I). The p value of the reduced photoreaction center found to be between 0.28 and 0.22 is significantly lower than this theoretical number. The implication is that at least two non-parallel dipoles contribute to the 893 nm (77 °K) absorption band and participate in the photochemical reaction.

Further evidence that P_{870} is not due to a single component comes from its resolution into two positive maxima by circular dichroism spectroscopy at 77 °K [5].

The sign of these maxima is characteristic of monomeric bacteriochlorophyll in solution [4]. Moreover, linear dichroism of oriented preparations of photoreaction center show a decrease from 855 to 920 nm which indicates that P_{870} is due to more than one non-parallel dipole with different absorption maxima [15].

The polarization found from fluorescence emission by Ebrey and Clayton [11] is paradoxically much higher ($p = 0.5$) than our median value of 0.25 obtained by photodichroism. Considered in itself, the observation of Ebrey and Clayton may fit five general cases: P_{870} could be attributed (1) to a single absorbing and emitting dipole, (2) to several absorbing and emitting dipoles which are parallel to each other or (3) which are not parallel to each other and not coupled by singlet-singlet energy transfer, and (4) to the set of absorbing and emitting dipoles in the three above cases plus a subset of absorbing but non-emitting dipoles which are parallel, or (5) which are not parallel and with no singlet energy transfer from the non-emitting dipoles to the emitting one. Cases (1), (2) and (4) may be eliminated by our photodichroism experiment since they would lead to a high photodichroic value. We are left, therefore, with the two remaining cases (3) and (5). Hence, the low p value measured by photodichroism and the high p value measured by polarization of fluorescence imply together that P_{870} comprises at least two nonparallel dipoles with no reversible singlet-singlet energy transfer between the dipoles.

The fact that the dipoles of P_{870} are not coupled by reversible singlet-singlet energy transfer and that each one of the P_{800} and P_{870} are attributable to at least two transition dipoles, imposes severe constraints on the model that can be proposed for the aggregation state of the four bacteriochlorophyll molecules. The following models can be ruled out: (i) a strongly interacting tetramer, (ii) two strongly interacting dimers, one giving rise to P_{800} and the other one to P_{870}, and (iii) one strongly interacting trimer giving rise to P_{870} and to part of P_{800}, the other part of P_{800} being completed by a monomer. The model in which there are two strongly interacting dimers, each giving rise to part of P_{800} and part of P_{870}, could be ruled out from polarization of fluorescence data. If such dimers existed, P_{800} would be oriented perpendicular to P_{870} and the polarization of fluorescence at 800 nm would be -0.33 instead of $+0.3$ [11].

The only two models that would appear to satisfy the observations are (1) a strongly interacting trimer as the one proposed by Sauer et al. [4] giving rise by exciton splitting to P_{800} and to part of P_{870}, the other part of P_{870} being due to monomeric bacteriochlorophyll and (2) a strongly interacting dimer or two monomers giving rise to P_{800} and two monomers giving rise to P_{870}.

The first model has difficulty in explaining why the trimer and monomer have the same bathochromic shift as the temperature is lowered such that even at 77 °K the 893 nm absorption band is not resolved into two components [18]. The second model is simpler and appears to be favored by the weight of the presently available evidence. It attributes P_{800} to a subset of two bacteriochlorophyll molecules; P_{870} would then constitute another subset of two molecules with non-parallel transition dipoles and interconnected by charge transfer but not by reversible singlet-singlet energy transfer. Other evidence that P_{800} and P_{870} represent two different subsets of bacteriochlorophyll molecules is the slight difference between the quantum yields of P_{870} photooxidation [19] or fluorescence [20] when either P_{800} or P_{870} is excited, the fluorescence emission from P_{800} at 77 °K [5] and also the marked difference in the extent of the bathochromic shifts of the two absorption bands upon lowering the temperature [5, 18].

Both models are consistent with evidence from electron paramagnetic resonance [6, 7] and electron nuclear double resonance spectroscopy [8, 9] which indicates that, in the oxidized photoreaction center, the unpaired electron spreads over two bacteriochlorophyll molecules. However, the second model would seem to account more simply for these observations since it assumes that the two bacteriochlorophyll molecules that are oxidized are more similar to each other than to those of the other subset.

P_{870} is composed of two non-parallel dipoles: the resultant dipole of a trimer and the dipole of a monomer in one case, the dipoles of two monomeric bacteriochlorophyll molecules in the other case. The measured p value, then, is due to one dipole which absorbs the actinic light and has, therefore, polarization p_0. The other dipole has polarization p_α the value of which depends on the angle α between the two dipoles. The observed polarization is related to p_0 and p_α by Weber's Summation Law [21]:

$$\left(\frac{1}{p}-\frac{1}{3}\right)^{-1} = f\cdot\left(\frac{1}{p_0}-\frac{1}{3}\right)^{-1} + (1-f)\cdot\left(\frac{1}{p_\alpha}-\frac{1}{3}\right)^{-1} \tag{5}$$

where f is the fraction of the total change in absorbance due to the dipole with polarization p_0. Polarization p_α is related to p_0 and angle α [22] by:

$$\frac{1}{p_\alpha}-\frac{1}{3} = \left(\frac{1}{p_0}-\frac{1}{3}\right)\cdot\frac{2}{3\cos^2\alpha-1} \tag{6}$$

Combining eqns. 5 and 6:

$$\left(\frac{1}{p}-\frac{1}{3}\right)^{-1} = \left(\frac{1}{p_0}-\frac{1}{3}\right)^{-1}\left[f+(1-f)\cdot\frac{(3\cos^2\alpha-1)}{2}\right] \tag{7}$$

Using eqn. 7, Fig. 6 shows how the angle α would vary with f for $p = 0.25$ and $p_0 = 0.461$. According to this curve, the angle α between the two dipoles is at some angle

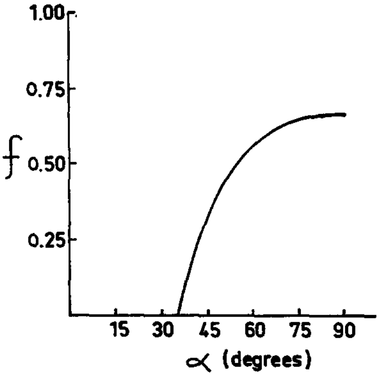

Fig. 6. Fraction (f) of the total change in absorbance due to the dipole which absorbs the polarized light as a function of the angle α between the two dipoles.

between 35.75° and 90°. The exact angle of orientation awaits an exact determination of the fractional change in absorbance due to each of the two absorption dipoles in P_{870}.

Despite this imprecision, and whether or not the two dipoles of P_{870} can be identified with two molecules of bacteriochlorophyll, an interesting conclusion can be drawn from this work. This is that if the two dipoles are fluorescence emitters they cannot be coupled by singlet-singlet energy transfer. If only one of the dipoles is fluorescent, they may also be non-coupled. However, one cannot exclude the possibility of a one way singlet-singlet transfer from the fluorescent to the non-fluorescent dipole.

APPENDIX I

The interpretation of the photodichroism experiment is based on the assumption of an isotropic sample. However, photochemistry by polarized light is a source of anisotropy. If the rate of creation of anisotropy is greater than the rate of relaxation to the isotropic state, then, in the steady state, photoselection is performed on a non-random sample. The difference in the p value thus obtained from that of an isotropic sample will increase with the intensity of the actinic light. Let $\Delta A_{||}$ be the change in absorbance seen by a measuring light polarized parallel to the YZ plane (Fig. 4) with and without an actinic light also polarized parallel to the YZ plane. Then,

$$\Delta A_{||} = KP^{+}(\theta) \cdot \cos^2\theta \tag{8}$$

where if $P^0(\theta)$ is the total concentration of P_{870} with absorption dipole oriented at angle θ to the Z axis, $P^{+}(\theta)$ is the concentration of $P^{+}{}_{870}$ with absorption dipole at the same angle θ, $\mathrm{Cos}^2\,\theta$ is the probability that the P_{870} absorption dipole oriented at angle θ will absorb light that is polarized to the Z axis, and K is the proportionality constant. In steady state conditions, the rate of production of $P^{+}(\theta)$ by a polarized actinic light of intensity $I_{||}$ is equal to the rate of decay of $P^{+}(\theta)$. Assuming that $P^{+}(\theta)$ has no rotational motion within its lifetime,

$$K_b P^{+}(\theta) = [P^0(\theta) - P^{+}(\theta)]\gamma I_{||} \cdot \mathrm{Cos}^2\theta \tag{9}$$

where K_b is the rate constant for the reduction of $P^{+}(\theta)$ and γ is the proportionality constant. Combining Eqns. 8 and 9, one can show that

$$\Delta A_{||} = KP^0(\theta) \cdot \frac{\cos^4\theta}{a+\cos^2\theta} \tag{10a}$$

$$\text{where } a = \frac{K_b}{\gamma I_{||}} \tag{10b}$$

If the absorption oscillators are distributed with random orientation so that their number making an angle θ with the Z axis is proportional to $\sin\theta$ and constant over all azimuth angle ϕ about the Z axis; $P^0(\theta) = P\sin\theta$ where P is a constant, $\Delta A_{||}$ for all angles θ becomes

$$\Delta A_{||} = KP\int_0^{2\pi}\int_0^{\pi/2} \frac{\cos^4\theta}{a+\cos^2\theta} \cdot \sin\theta\,\mathrm{d}\theta\,\mathrm{d}\phi \tag{11}$$

Since $\Delta A_{\parallel}$ approaches the total absorbance A as a approaches zero, we can write the ratio

$$\frac{\Delta A_{\parallel}}{A} = \frac{\displaystyle\int_0^{\pi/2} \frac{\cos^4\theta}{a+\cos^2\theta} \cdot \sin\theta\,\mathrm{d}\theta}{\displaystyle\int_0^{\pi/2} \cos^2\theta \cdot \sin\theta\,\mathrm{d}\theta} \tag{12}$$

Similarly $\Delta A_{\perp}$, the change in absorbance when the measuring light is polarized perpendicularly to the polarization of the actinic light, can be shown to be

$$\Delta A_{\perp} = KP \int_0^{2\pi} \int_0^{\pi/2} \frac{\sin^2\theta \cdot \cos^2\theta}{a+\cos^2\theta} \cdot \sin\theta\,\mathrm{d}\theta \cdot \cos^2\varphi\,\mathrm{d}\varphi \tag{13}$$

Letting $t = \cos^2\theta$ and using Eqns. 4, 11 and 13, the polarization p is equal to

$$p = \frac{3\displaystyle\int_0^1 \frac{t^4}{a+t^2}\,\mathrm{d}t - \int_0^1 \frac{t^2}{a+t^2}\,\mathrm{d}t}{\displaystyle\int_0^1 \frac{t^2}{a+t^2}\,\mathrm{d}t + \int_0^1 \frac{t^2}{a+t^2}\,\mathrm{d}t} \tag{14}$$

From Eqns. 12 and 14, we can evaluate the decrease of p as a function of $\Delta A_{\parallel}/A$. This is shown in Fig. 7. At low actinic light intensities such that $\Delta A_{\parallel}/A$ is small, p is close to the theoretical limit of 0.5, but as the intensity of the actinic light increases, the deviation from 0.5 also increases until at very high intensities, all the P_{870} becomes oxidized and the polarization is zero. The maximum value of $\Delta A_{\parallel}/A$ of 0.2 used in this experiment corresponds to a p value of 0.461. Therefore, the intensity of the actinic light used in this experiment causes an 8 % deviation from the theoretical p value of 0.5.

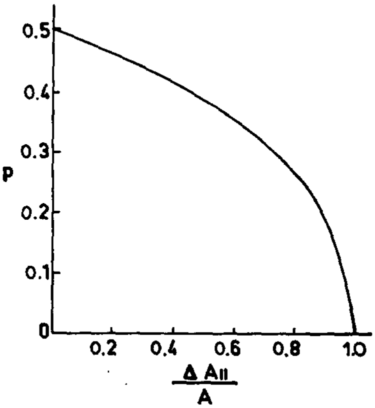

Fig. 7. Theoretical plot of the distortion of the polarization value p introduced by photochemically induced anisotropy. The actinic and measuring light beams are assumed to be polarized parallel to each other.

APPENDIX II

In order to obtain $p_{\rm red}$ the polarization due only to the reduced photoreaction center, one must correct for $p_{\rm ox}$ the polarization of the oxidized photoreaction center. The measured value $\Delta A_{||}$ is equal to

$$\Delta A_{||} = \Delta A_{||,\rm red} - \Delta A_{||,\rm ox} \tag{15}$$

where $\Delta A_{||,\rm red}$ is the absorbance change due only to the reduced species and $\Delta A_{||,\rm ox}$ is due to the oxidized species. Similarly

$$\Delta A_{\perp} = \Delta A_{\perp,\rm red} - \Delta A_{\perp,\rm ox} \tag{16}$$

Substituting these two equations into Eqn. 4, one obtains

$$p_{\rm red} = p(1-q)+p_{\rm ox}q \tag{17a}$$

$$\text{where } q = \frac{\Delta A_{||,\rm ox}+\Delta A_{\perp,\rm ox}}{\Delta A_{||,\rm red}+\Delta A_{\perp,\rm red}} \tag{17b}$$

q is then approximately equal to the absorbance of $P^+{}_{870}$ divided by the absorbance of P_{870}. From Fig. 2, q is equal to 0.105 at 900 nm and 0.117 at 870 nm. For the case in which the exciting light is 900 nm and the measuring light is 870 nm, using Eqn. 17 with q equal to 0.117 and p equal to 0.302 (Table I) curve (a) of Fig. 8 shows how $p_{\rm red}$ should vary with $p_{\rm ox}$. Similarly, using Eqn. 17 with q equal to 0.105 and p equal to 0.255 (average of three p values for 900 nm measuring light in Table I), curve (b) of Fig. 8 shows how $p_{\rm red}$ changes with $p_{\rm ox}$ in the reverse case in which the exciting light is 870 nm and the measuring light is 900 nm. Since there is no difference between exciting at 900 nm and measuring at 870 nm with exciting at 870 nm and measuring at 900 nm, $p_{\rm red}$ should have the same value in both cases. Examining curves (a) and (b) in Fig. 8, in order for $p_{\rm red}$ to have the same value in both cases, $p_{\rm red}$ must not be greater than 0.28, the maximum $p_{\rm red}$ for curve (b). Also it must not be less than 0.22, the minimum $p_{\rm red}$ for curve (a). Since we have no knowledge of $p_{\rm ox}$ for the two cases, $p_{\rm red}$ can have any value between 0.28 and 0.22. The exact value will depend on the $p_{\rm ox}$ at 870 nm and at 900 nm.

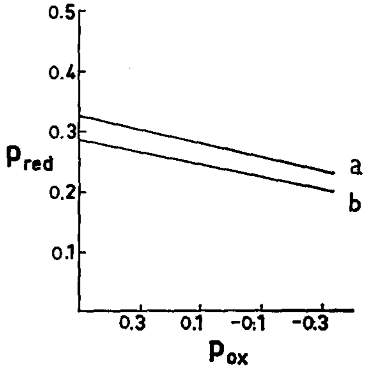

Fig. 8. Polarization of P_{870} ($p_{\rm red}$) as a function of the polarization of $P^+{}_{870}$ ($p_{\rm ox}$). Top curve (a) is for measurement with 900 nm actinic light and 870 nm measuring light. Bottom curve (b) is for measurement with 870 nm actinic light and 900 nnm measuring light.

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