Biochimica et Biophysica Acta, 765 (1984) 125–132
Elsevier

BBA 41478

PHOTOSELECTION STUDIES OF THE P-800 BAND IN THE PHOTOREACTION CENTER OF *RHODOSPIRILLUM RUBRUM*

TED MAR and GABRIEL GINGRAS

Département de Biochimie, Université de Montréal, Montréal, Québec, (Canada)

(Received November 1st, 1983)

Key words: Reaction center; Photoselection; Polarization; Bacteriochlorophyll orientation; (R. rubrum)

Polarization measurements of light-induced absorption changes in photoreaction center prepared from *Rhodospirillum rubrum* indicate that the 870 nm band is most likely due to a single transition dipole. The 800 nm band appears to be formed by transition dipoles with at least three different orientations. In photoreaction center from strain G9, none of the transition dipoles of the 800 nm band appears to form an angle larger than 70° with the 870 nm transition dipole.

Introduction

Purified photochemical reaction centers isolated from *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum* have four molecules of bacteriochlorophyll (BChl) and two of bacteriopheophytin (BPh) per functional unit [1,2]. At room temperature, BChl and BPh give rise to major absorption bands respectively at 600, 800 and 870 nm and at 540 and 760 nm. The relative orientation of the transition moments of these bands has been studied by polarization of fluorescence [3], polarization of light-induced absorbance change [4–6], linear dichroism [7–11] and magnetophotoselection [12,13]. Similar experiments have also been done in the bacterial photoreaction centers of *Rhodopseudomonas viridis* [14–16] and of *Chromatium minutissimum* [17] and in the B-700 particle of green plants [18,19]. Using polarization of light-induced absorbance changes, the relative orientations of the Q_x and Q_y transition moments of the two BPh molecules in *Rps. sphaeroides* have been found [6,11]. The relative orientations of the Q_x and Q_y transition moments of the four BChl molecules have not yet been fully determined due to the complexity of the BChl absorption bands.

A related question is the type of interaction that exists between the BChl molecules. It is generally agreed that the unpaired electron of the oxidized primary donor is shared among two BChl molecules, the so-called special pair [20,21]. But this does not necessarily involve any coupling at the singlet-state level. Information on this point must be provided by optical spectroscopy. Recently, we have found [22] that the circular dichroism spectrum of oriented photoreaction center can best be interpreted by a model first postulated by Vermeglio and Clayton [8] that the special pair BChl forms an exciton dimer and has absorption and circular dichroism bands at 870 and 810 nm. If the exciton bands can be described by the exciton model of Kasha et al. [23], then the two transition dipoles should be orthogonal to each other and the 800 nm band should consist of at least three transitions. In the present work, we attempted to verify the exciton model by a thorough analysis of polarization measurements of the absorbance changes within the 800 and 870 nm bands.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

Materials and Methods

Photoreaction center was isolated from wild type *R. rubrum* S1 (ATCC No. 11170) by the method of Noël et al. [24] as modified by Vadeboncoeur et al. [25]. Photoreaction center from strain G9 (carotenoidless) was isolated by the method of Vadeboncoeur et al. [25]. For measurements, photoreaction center from strain S1 was dispersed in 25 mM Tris-HCl (pH 8.0)/0.05% dodecyldimethyl-amino-*N*-oxide containing 50% glycerol and the photoreaction center from strain G9 was dispersed in 10 mM Tris-HCl (pH 7.5)/0.1% Triton X-100 containing 50% glycerol. The extinction coefficient of the photoreaction center at 125 K was calculated to be 196 $mM^{-1} \cdot cm^{-1}$ at 885 nm from concentrations measured at room temperature using an extinction coefficient of 143 $mM^{-1} \cdot cm^{-1}$ at 868 nm [2].

Photoselection experiments with continuous exciting light were done with a Cary 14R spectrophotometer as described previously [4]. Excitation light was provided by a tungsten lamp (650 W) filtered through a 500 mm monochromator (Bausch and Lomb) with dispersion of 9.9 nm. Both the excitation and measuring light beams were polarized by sheet polarizers (Polaroid HR 2.8, specially selected for high degree of polarization). The polarizers were mounted so that they could be rotated from 0° to 90° around an axis perpendicular to the face of the polarizer. Three narrow slits were placed between the sample chamber and the photomultiplier to cut down the scattered light. By using low intensity actinic light, we can observe the absorbance change at the wavelength of the exciting light.

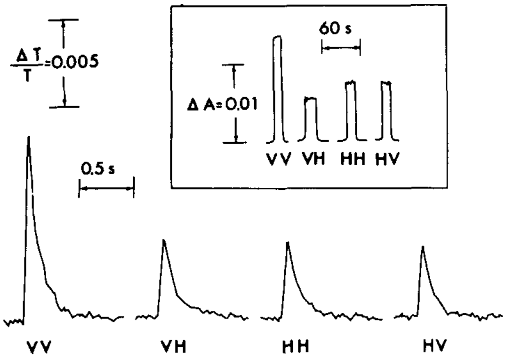

Fig. 1. Kinetics of light-induced absorbance changes of photoreaction center from strain S1 of *R. rubrum* at 125 K. Wavelengths of the exciting and measuring light were 900 nm and 870 nm, respectively. VV denotes that the exciting and measuring light beams are vertically polarized. VH denotes that the exciting and measuring light beams are vertically and horizontally polarized. HH and HV are similarly defined. Kinetics with short excitation time (30 ms) were recorded with a single beam spectrophotometer and averaged 8 times (bottom). The kinetics with long excitation time (15 s) were recorded with a Cary 14R spectrophotometer (inset).

Photoselection experiments with short actinic illumination duration were done as described by Vermeglio et al. [6]. The excitation light was provided by a 450 W xenon lamp filtered through a Baird atomic interference filter. The duration of the actinic illumination (30 ms) was controlled by an electromechanical shutter. All measurements were performed at 125 K in an Air Products cryostat cooled with nitrogen gas.

Polarization, p, is defined as $(\Delta A_v - \Delta A_h)/(\Delta A_v + \Delta A_h)$ where ΔA_v and ΔA_h are changes in absorbance when the polarization of the exciting and measuring beams are parallel and perpendicular to each other, respectively.

Results and Discussion

Orientation of transition moments in the 870 nm band

Fig. 1 shows the absorbance changes at 870 nm induced by polarized 900 nm light in a photoreaction center preparation of *R. rubrum* S1 at 125 K. A polarization value of 0.42 was obtained whether the duration of the excitation light was 30 ms or 15 s (inset). This shows that, under our experimental conditions, the duration of the excitation flash is unimportant and that the determination of p is not being affected by slow relaxation of the oxidized state [6]. All subsequent experiments were done with 15 s light flashes. The high p value is essentially the same as that for the photoreaction center of *Rps. sphaeroides* using short excitation time [6] but disagrees with our previous determination [4]. This is due to the use of improper polarizers in our first study. The polarizer (Polaroid HN7) previously used had a degree of polarization V of 0.953 at 870 nm and 0.694 at 900 nm. V is defined as $(K_1 - K_2)/(K_1 + K_2)$ where K_1 and K_2 are the principal transmittance values. This low degree of polarization and its variation at 870 and

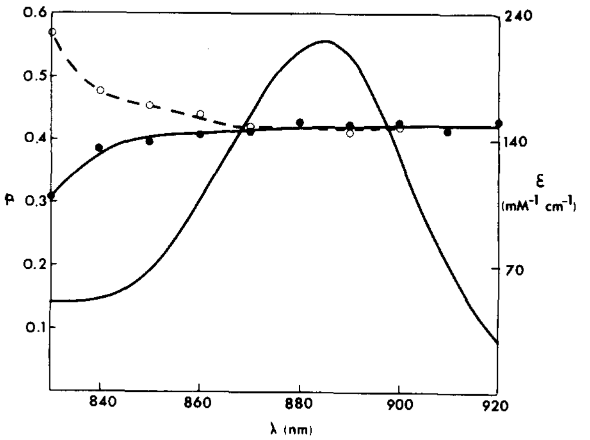

Fig. 2. The measured polarization of the absorbance change of photoreaction center from *R. rubrum* S1 at 125 K. ●——●, polarization of the $\Delta\epsilon_{885}$ as a function of excitation wavelength; ○ – – – – ○ polarization of the $\Delta\epsilon_{830-920}$ with a fixed excitation wavelength of 885 nm. The smooth curve shows the absorption spectrum of the preparation at 125 K.

900 nm account for our report of a low p value and of its difference when exciting at 870 nm and observing at 900 nm and when exciting at 900 nm and observing at 870 nm. The polarizers used in the present measurement were Polaroid HR 2.8 polarizers specially selected by Polaroid for their high V value across the 870 nm band. At 890 nm, V is equal to 0.995.

To check whether the 870 band might be formed of two only slightly resolved components, we measured p at many wavelengths throughout the band. We found a p value of 0.429 independent of the excitation and observation wavelengths (Fig. 2). This high p value and its constancy with wavelength is consistent with the results of fluorescence polarization [3], linear dichroism [8,9] and photoselection experiments [6]. The simplest interpretation is that the 870 nm band is due to a single electronic transition, although the possibility of two parallel transitions cannot be excluded a priori.

The deviation of p from constancy, when exciting the short wavelength side of the 870 nm band, is due to the appearance of at least another band with a unparallel dipole moment. This is most likely a vibrational band. The high value of p when exciting at 890 nm and observing at 830 nm (also reported by Shuvalov et al., [5]) can be explained as follows. At all wavelengths, the absorbance change is the sum of a negative component due to the disappearance of P, the reduced state of the photoreaction center, and a positive component due to the appearance of P$^+$, the oxidized state of the photoreaction center. If the polarization of the two components is different, large polarization values can be measured. At wavelengths where the extinction coefficient of P is much larger than that of P$^+$, this effect can be neglected. But this is not the case at 830 nm where the positive component due to the formation of P$^+$ is large with respect to the negative component due to the disappearance of P.

Orientation of transition moments in the 800 nm band

For an absorption band with a unique orientation, the theoretical polarization value is 0.5 [26] rather than 0.43, our highest value for the 870 nm band. Since, as discussed in Appendix I, this deviation from theory is most likely due to instrumental depolarization artifacts, we applied a correction factor to the measured p values (Eqn. A-4). The purpose of this correction was to allow a more accurate analysis of our polarization data. All subsequent polarization data are reported in corrected form (p_c).

Fig. 3 shows how corrected p varies as a function of excitation wavelength at different observation wavelengths indicated in the figure. The results for photoreaction center from strain S1 and strain G9 are shown in Fig. 3a and b, respectively. These data can be used to calculate the orientation of the absorption dipoles which constitute the 800 and the 870 nm bands. Since several transitions are implicated in the 800 nm absorption band, we will use the more interpretable parameter $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ introduced by Vermeglio et al. [6] for absorbance changes observed parallel or perpendicular to the direction of the excitation. p is related to the ratio of $\Delta A_{\parallel}$ to the total absorbance change, ΔA_{T}, by the equation:

$$\left(\frac{\Delta A_{\parallel}}{\Delta A_T}\right)^{\lambda\lambda'} = \frac{1+3p^{\lambda\lambda'}}{3-p^{\lambda\lambda'}} \qquad (1)$$

where λ and λ′ are the wavelengths of the exciting and the observing light beams, respectively. $(\Delta A_{\parallel}/\Delta A_{\mathrm{T}})^{\lambda\lambda'}$ and $(\Delta A_{\perp}/\Delta A_{\mathrm{T}})^{\lambda\lambda'}$ are related to

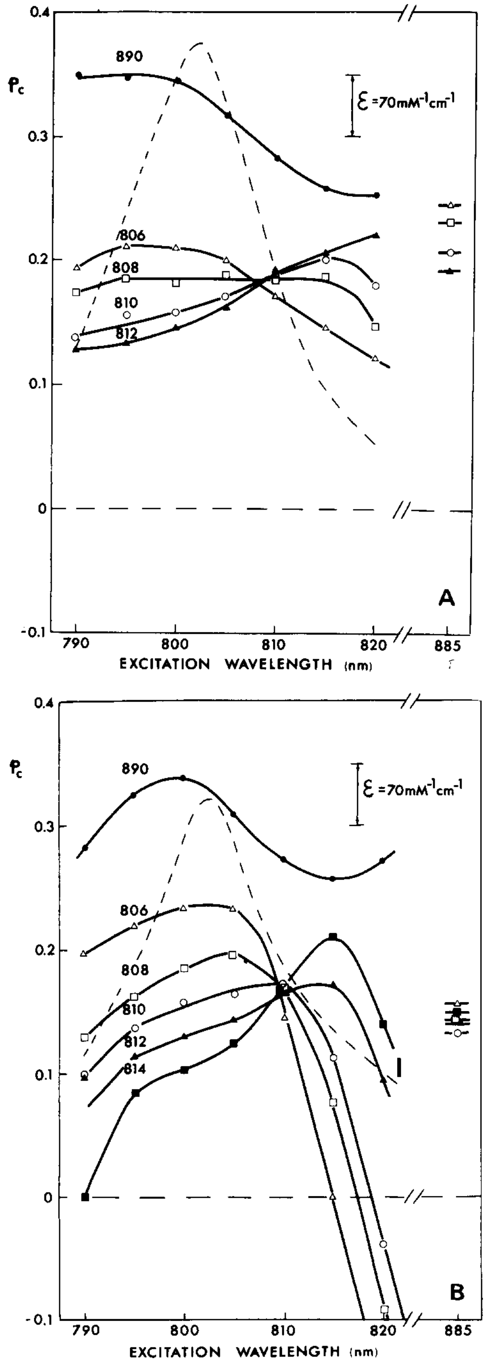

Fig. 3. (a) The corrected polarization of the absorbance change of photoreaction center from *R. rubrum* S1 as a function of the excitation wavelength at various observation wavelengths (●, 890 nm; △, 806 nm; □, 808 nm; ○, 810 nm; ▲, 812 nm). The dashed line shows the absorption spectrum of the preparation. (b). The corrected polarization of the absorbance change of photoreaction center from *R. rubrum* G9 as a function of the excitation wavelength at various observation wavelengths (●, 890 nm; △, 806 nm; □, 808 nm; ○, 810 nm; ▲, 812 nm; ■, 814 nm). The dashed line is the absorption spectrum of the preparation.

the angles α_{ij} between the transition dipoles i and j by the equation (see appendix B):

$$\left(\frac{\Delta A_{\parallel}}{\Delta A_T}\right)^{\lambda\lambda'} = \sum_i^m g_i^{\lambda} \sum_j^n h_j^{\lambda'} \cos^2 \alpha_{ij}$$

$$\left(\frac{\Delta A_{\perp}}{\Delta A_T}\right)^{\lambda\lambda'} = \sum_i^m g_i^{\lambda} \sum_j^n h_j^{\lambda'} \sin^2 \alpha_{ij} \tag{2}$$

where

$$\sum_i^m g_i^{\lambda} = \sum_j^n h_j^{\lambda'} = 1$$

g_i^{λ} is the probability that excitation light of wavelength λ, absorbed by the ith transition dipole, will produce a change in absorbance ΔA at wavelength λ'; $h_j^{\lambda'}$ is the probability that the jth transition dipole will undergo a change in absorbance at wavelength λ'; m and n are the total number of transition dipoles involved at wavelengths λ and λ'. The angles α_{ij} are related to each other by the law of cosines in spherical trigonometry.

To solve Eqn. 2, we must first determine the number of differently oriented transition dipoles within the 800 nm band. In principle, this can be done by writing our experimental data in matrix form and performing a rank analysis of the matrix. The rank of a matrix is a measure of the number of its linearly independent rows or columns. It must be kept in mind, however, that since the rank is a discontinuous function it may be sensitive to experimental error. Rewriting Eqn. 2 in matrix form, we have:

$$\mathbf{A}_{\lambda\lambda'} = \mathbf{G}_{\lambda i} \cdot \mathbf{T}_{ij} \cdot \mathbf{H}_{j\lambda'}$$

where the individual elements of the matrices $\mathbf{A}_{\lambda\lambda'}$, $\mathbf{G}_{\lambda i}$, $\mathbf{T}_{ij}$, $\mathbf{H}_{j\lambda'}$ are $(\Delta A_{\parallel}/\Delta A_{\mathrm{T}})^{\lambda\lambda'}$, g_i^{λ}, $\cos^2 \alpha_{ij}$ and $h_j^{\lambda'}$, respectively. In Table I, we have presented our experimental data in matrix form. For the preparations from both strains S1 and G9, the matrix ($\mathbf{A}_{\lambda\lambda'}$) formed by the polarization data from 790 to 820 nm has a minimum rank of 3. This means that within this wavelength range, at least three different components are detected. However, if we delete the 820 nm excitation data from the matrix, its minimum rank decreases to 2. We assume that there is a small spectral component with a peak near 820 nm. This component could perhaps be a higher-energy vibrational band of the 870 nm transition. For simplicity's sake, the following analysis will consider only the data from 790 to 815 nm and will label the corresponding matrices with $\mathbf{A}'_{\lambda\lambda'}$ ($\mathbf{A}'_{\lambda\lambda'} = \mathbf{G}'_{\lambda i} \cdot \mathbf{T}'_{ij} \cdot \mathbf{H}'_{j\lambda'}$). Since the $\mathbf{A}'_{\lambda\lambda'}$ matrix has a rank of 2 and since the rank of a product matrix cannot exceed the rank of any of its components, it follows that the matrices $\mathbf{G}'_{\lambda i}$, $\mathbf{T}'_{ij}$ and $\mathbf{H}'_{j\lambda'}$ also must have ranks of 2 or higher.

For matrix $\mathbf{T}'_{ij}$ to have a rank of 2 would imply that there are at least two non-parallel transition dipoles within the 790–815 nm range. To check the possibility of only two transition dipoles, we used a computer program built around Eqn. 2 that varied its parameters until a best fit to the data was obtained. The hypothesis of two non-parallel transition dipoles did not provide any fit the the experimental data and was rejected on those grounds. However, the hypothesis of three non-parallel components ($\mathbf{T}'_{ij}$ of rank 3) fit the polarization data for the preparations from both strains S1 and G9. The photoreaction center has four BChl molecules with a maximum number of four main Q_y transition dipoles. Since we already attributed the 870 nm band to one of these, the remaining three are most likely the three non-parallel transition dipoles of the 800 nm band.

Accepting that T'_{ij} has rank 3, we found that there are large numbers of sets of parameters g_i^{λ}'s, $h_j^{\lambda'}$'s and α_{ij}'s which could fit the experimental data. The matrices $\mathbf{G}'_{\lambda i}$ and $\mathbf{H}'_{j\lambda'}$ can have ranks of either 2 or 3. For $\mathbf{G}'_{\lambda i}$ (or $\mathbf{H}'_{j\lambda'}$) to have rank 3 in a three-dimensional matrix implies that, within the 800 nm band, there are three non-parallel transition dipoles with different absorption spectra (or change of absorption spectra). For $\mathbf{G}'_{\lambda i}$ (or $\mathbf{H}'_{j\lambda'}$) to have rank 2 in a three-dimensional matrix implies that the matrix has only two linearly independent columns (or rows). The latter case would obtain with two non-parallel transition dipoles with proportional absorption spectra (or change of absorption spectra). Our polarization data cannot distinguish between the two cases.

Although Eqn. 2 cannot be solved uniquely, we tested whether any one of the three transitions in the 800 nm band could be orthogonal to the transition of the 870 nm band. Assuming one of the angles to be 90°, we could not find any set of parameters g_i^{λ}'s, $h_j^{\lambda'}$'s and α_{ij} that would fit the experimental data of the preparation from strain G9. The largest angle between any one of the 800 nm band transitions and the 870 nm transition was found to be approx. 70°. In the case where the matrix $\mathbf{H}'_{j\lambda'}$ is nonsingular (i.e., does not contain two proportional rows or columns), we can show analytically that no absorption dipole in the 800 nm band is orthogonal to the 870 nm transition dipole (appendix C).

It is possible to fit the polarization data ob-

TABLE I

POLARIZATION DATA IN MATRIX FORM

Presented in matrix form are the ratios of $\Delta\mathbf{A}_{\parallel}$ to the total absorbance change $\Delta\mathbf{A}_{\mathrm{T}}$ as a function of λ, the wavelengths of the exciting light and λ', the wavelengths of the measuring light for two species of *R. rubrum*

S1

$\lambda \backslash \lambda'$	806	808	810	812
790	0.568	0.539	0.494	0.482
795	0.586	0.552	0.518	0.488
800	0.583	0.551	0.519	0.503
805	0.570	0.553	0.534	0.526
810	0.534	0.552	0.560	0.563
815	0.503	0.554	0.572	0.578
820	0.473	0.507	0.548	0.596

G9

$\lambda \backslash \lambda'$	806	808	810	812	814
790	0.568	0.484	0.443	0.444	0.333
795	0.597	0.524	0.492	0.465	0.431
800	0.615	0.552	0.517	0.486	0.453
805	0.615	0.567	0.525	0.499	0.478
810	0.503	0.531	0.539	0.530	0.526
815	0.333	0.420	0.463	0.536	0.583
820	0.152	0.236	0.298	0.444	0.496

tained with the photoreaction centers from strains S1 and G9 by using the same set of angles smaller than 70°. However, the polarization data of the wild type preparation also can be fitted by larger angles, including an angle of 90°, between the 870 nm transition dipole and one of the dipoles of the 800 nm band. A possible reason for this difference may be that the three absorption components of the 800 nm band are better resolved in the preparation from strain G9 than in the preparation from strain S1. This better resolution is indicated by a clearly discernable shoulder at 810 nm in the preparation from strain G9 but that is not seen in the preparation from strain S1 [27]. This shoulder probably indicates the presence of a predominant component in the red wing of the 800 nm band. The contribution of this component to the polarization in the 810 nm range would be more important in the preparation from G9, since it is better resolved there. This would explain why the polarization varies more as a function of wavelength in the G9 than in the S1 photoreaction center where the polarization throughout the band are close to the average value (Fig. 3). Hence, the greater separation of the components may allow the polarization data to be fitted with a smaller set of angles in the preparation from strain G9. Since it appears unlikely that the orientation of BChl would be different in these preparation from the same bacterial species, we suggest that 70° is the upper limit for the angle formed by the 870 nm dipole with any of the 800 nm band transition dipoles.

This conclusion is in apparent contradiction with our recent CD studies of oriented photoreaction center which indicate that the 810 and 870 nm bands are exciton bands [22]. Indeed, symmetric exciton theory predicts that exciton bands should have mutually orthogonal transitions [23]. Another prediction, that the excitonic transitions should have equal bandwidths [28] seems to be violated by the observation that the 810 nm CD band is much narrower than the 870 nm band. It can be shown, however, that those predictions only apply if the exciton is equally shared between two identical molecules. An exciton formalism which takes into account the effect of the environment on the electronic transition energies indicates that the properties of the special pair may be determined not only by the interaction between chemically identical BChl molecules but also by their interaction with other surrounding molecules in the photoreaction center environment [29].

Appendix A

Depolarization can have several different causes: light scattering, improper polarizers, the intensity of the exciting and measuring light are not infinitely weak (at saturating light intensity, all BChl will be excited and the polarization will vanish), the sample is not at infinite dilution and improper optics. Improper optics comes mainly from the use of lenses to focus both the exciting and measuring light beams onto the sample. This means that the light rays which pass outside the center of the lenses are not orthogonal to each other. Since it is difficult to correct the measured polarization for each depolarization effect individually, we correct the overall measured polarization by assuming that the changes of absorbance consist of two parts – a polarized component and an unpolarized component due to instrumental depolarization. To check whether our polarization measurements were vitiated with instrumental artifacts, we calibrated our instrument with fluorescein, an organic dye known to have a long-lived metastable excited state [30].

Exciting fluorescein dissolved in ethanol at 120 K with 440 nm linearly polarized light and observing the polarization of the change of absorbance at 436 nm, we found a p value of 0.447 instead of the theoretical 0.5. This indicates that depolarization effects can account for the deviation of the p value from the theoretical limit in photoreaction center. The measured polarization p is defined as:

$$p = \frac{\Delta A_V - \Delta A_H}{\Delta A_V + \Delta A_H} \qquad \text{(A-1)}$$

If there is an unpolarized component due to scattering or imperfect optics then the corrected polarization p_c would be:

$$p_c = \frac{\Delta A_V - \Delta A_H}{\Delta A_V + \Delta A_H - 2\Delta A_0} \qquad \text{(A-2)}$$

where ΔA_0 is the unpolarized component. Defining k as the ratio of the total depolarized absorbance change to that of the total measured absorbance change at any wavelength:

$$k = \frac{3\Delta A_0}{\Delta A_\mathrm{V} + 2\Delta A_\mathrm{H}} \tag{A-3}$$

we can easily see from the three definitions that:

$$p_\mathrm{c} = \frac{p}{1 - \frac{k}{3}(3 - p)} \tag{A-4}$$

We can safely assume that k is independent of wavelength, at least in the 700–920 nm range, since p (and hence k) is constant from 860 to 920 nm with a value of 0.429 and since with fluorescein a p value of 0.447 was obtained at 436 nm; this means that p decreased only by 0.018 from 436 to 920 nm. From these considerations, $k = 0.166$.

Appendix B

$\Delta A_\parallel$ and $\Delta A_\perp$ for exciting one transition i and observing the absorbance change at another transition j are defined after Vermeglio et al. [6] as:

$$\begin{aligned} (\Delta A_\parallel)_{i,j} &= \Delta A_{\mathrm{i,j}} \cos^2 \alpha_{i,j} \\ (\Delta A_\perp)_{i,j} &= \Delta A_{\mathrm{i,j}} \sin^2 \alpha_{i,j} \end{aligned} \tag{B-1}$$

If there are several j transitions involved in the change in absorbance:

$$(\Delta A_\parallel)_i = \sum_j (\Delta A_\parallel)_{i,j} = \sum_j h_j (\Delta A_\mathrm{T})_i \cos^2 \alpha_{i,j} \tag{B-2}$$

where $h_j = \Delta a_{i,j}/(\Delta A_\mathrm{T})_i$ and $(\Delta A_\mathrm{T})_i = \sum_j \Delta A_{i,j}$. If the exciting light is absorbed by several i transitions:

$$\Delta A_\parallel = \sum_i g_i (\Delta A_\parallel)_i = \sum_i g_i \sum_j h_j \Delta A_\mathrm{T} \cos^2 \alpha_{i,j} \tag{B-3}$$

$$\Delta A_\perp = \sum_i g_i (\Delta A_\perp) i = \sum_i g_i \sum_j h_j \Delta A_\mathrm{T} \sin^2 \alpha_{i,j} \tag{B-4}$$

Appendix C

From Fig. 3b, we see that the polarization is nearly constant at excitation wavelength of 809 nm regardless of the wavelength of observation. Denoting N as the constant value of $(\Delta A_\parallel/\Delta A_\mathrm{T})^{809,\lambda'}$, we can rewrite Eqn. 2 as:

$$\sum_{j=1}^{3} h_j^{\lambda'} \left(\sum_i^3 g_i^{809} \cos^2 \alpha_{ij} - N \right) = 0 \tag{C-1}$$

The above equation will be satisfied if either:

$$\sum_i^3 g_i^{809} \cos^2 \alpha_{ij} = N \tag{C-2}$$

or the matrix $\mathbf{H}'_j$ is singular. If the 890 nm band consist of a single transition dipole, then:

$$\left(\frac{\Delta A_\parallel}{\Delta A_T} \right)^{809,890} = M = \sum_i^3 g_i^{809} \cos^2 \alpha_{i,4} \tag{C-3}$$

where α_{i4} is related to the angle α_{ij} by the law of cosines in spherical trigonometry:

$$\cos \alpha_{i4} = \cos \alpha_{ij} \cos \alpha_{j4} + \sin \alpha_{ij} \sin \alpha_{j4} \cos s_{ij} \tag{C-4}$$

where s_{ij} is the angle opposite the side α_{i4} of a spherical oblique triangle whose sides are α_{i4}, α_{ij} and α_{j4}. If there exist a transition j in the 800 nm band which is orthogonal to the transition of the 870 nm band, $\cos \alpha_{j4}$ will be equal to 0 and $\sin \alpha_{j4}$ will be equal to 1. Then Eqn. C-4 reduces to:

$$\cos \alpha_{i4} = \sin \alpha_{ij} \cos s_{ij} \tag{C-5}$$

In the case where the matrix $\mathbf{H}'_{j\lambda'}$ is not singular, on combining Eqns. C-2 and C-5:

$$N + M = \sum_i^3 g_i^{809} \left(\cos^2 \alpha_{ij} + \sin^2 \alpha_{ij} \cos^2 s_{ij} \right) \tag{C-6}$$

since $\cos^2 s_{ij} \leqslant 1$, $\mathrm{N} + \mathrm{M} \leqslant 1$ if there exist a transition in the 800 nm band which is orthogonal to the 870 nm transition. Experimentally $\mathrm{N} + \mathrm{M}$ are equal to 1.20, which is greater than 1. This implies that there are no orthogonal angles in the case where all three transition dipoles have different absorption and change of absorbance spectra. If $\mathbf{H}'_{j\lambda'}$ is singular, in the special case where two transition dipoles have similar changes of absorbance spectrum, we can show by a similar argument that the third transition dipole which has different change of absorbance spectrum from the other two transitions is not orthogonal to the 870 nm transition.

Acknowledgement

This research was supported by the National Research Council of CAnada and the Ministère de l'Education du Québec.

References

1 Straley, S.C., Parson, W.W., Mauzerall, D. and Clayton, R.K. (1973) Biochem. Biophys. Res. Comm. 73, 501–506

2 Van Der Rest, M. and Gingras, G. (1974) J. Biol. Chem. 249, 6446–6453

3 Ebrey, T.G. and Clayton, R.K. (1969) Photochem. Photobiol. 10, 109–117

4 Mar, T. and Gingras, G. (1976) Biochim. Biophys. Acta 440, 609–621

5 Shuvalov, C.A., Asadov, A.A. and Krakhmaleva, I.V. (1977) FEBS Lett. 76, 240–245

6 Vermeglio, A., Breton, J., Paillotin, G. and Cogdell, R. (1978) Biochim. Biophys. Acta 501, 514–530

7 Penna, F.J., Reed, D.W. and Ke, B. (1974) in Proceedings of the 3rd International Congress on Photosynthesis Research, Rehovot (Avron, M., ed), Vol. 1, pp. 421–425

8 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500–515

9 Rafferty, C.N. and Clayton, R.K. (1978) Biochim. Biophys. Acta 502, 52–60

10 Rafferty, C.N. and Clayton, R.K. (1979) Biochim. Biophys. Acta 545, 105–122.

11 Clayton, R.K., Rafferty, C.N. and Vermeglio, A. (1979) Biochim. Biophys. Acta 545, 58–69

12 Thurnauer, M.C. and Norris, J.R. (1976) Biochem. Biophys. Res. Comm. 73, 501–506

13 Boxer, S.G. and Roelofs, M.G. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5636–5640

14 Paillotin, G., Vermeglio, A. and Breton, J. (1979) Biochim. Biophys. Acta 545, 249–264

15 Shuvalov, C.A. and Asadov, A.A. (1979) Biochim. Biophys. Acta 545, 296–308

16 Frank, H.A., Friesner, R., Nairn, J.A., Dismukes, G.C. and Sauer, K. (1979) Biochim. Biophys. Acta 547, 484–501

17 Abdurakhmanovm, I.A., Prokhorenko, I.R., Serdiuk, O.P. and Erokhin, I.E. (1980) Dokl. Akad. Nauk SSSR 251, 219–222

18 Junge, W. and Eckhof, A. (1974) Biochim. Biophys. Acta 357, 103–117

19 Junge, W., Schaffernicht, H. and Nelson, N. (1977) Biochim. Biophys. Acta 462, 73–85

20 Norris, J.R., Scheer, H., Druyan, M.E. and Katz, J.J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 4897–4900

21 Feher, G., Hoff, A.J., Isaacson, R.A. and Ackerson, L.C. (1975) Ann. N.Y. Acad. Sci. 224, 239–259

22 Mar, T. and Gingras, G. (1984) Biochim. Biophys. Acta 764, 86–92

23 Kasha, M., Rawls, H.R. and Ashraf El-Bayoumi, M. (1965) Pure Appl. Chem. 11, 371–392

24 Noël, H., Van Der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta 275, 219–230

25 Vadeboncoeur, C., Noël, H., Poirier, L., Cloutier, C. and Gingras, G. (1979) Biochemistry 18, 4301–4307

26 Weber, G. (1952) Biochem. J. 51, 145–149

27 Vadeboncoeur, C. (1980) Ph.D. Thesis, Université de Montréal

28 Hemenger, R.P. (1978) J. Chem. Phys. 68, 1722–1728

29 Mar, T. and Gingras, G. (1984) Biochim. Biophys. Acta 764, 283–294

30 Lewis, G.N., Lipkin, D. and Magel, T.T. (1941) J. Am. Chem. Soc. 63, 3003–3018