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THE ORIENTATIONS OF REACTION CENTER TRANSITION MOMENTS IN THE CHROMATOPHORE MEMBRANE OF *RHODOPSEUDOMONAS SPHAEROIDES*, BASED ON NEW LINEAR DICHROISM AND PHOTOSELECTION MEASUREMENTS

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Summary

Chromatophore membranes from *Rhodopseudomonas sphaeroides* were oriented by drying suspensions on the surfaces of glass slides. Polarized spectra of light-induced absorption changes were obtained between 500 and 1000 nm. As observed earlier, these spectra showed negative bands, reflecting photooxidation of the bacteriochlorophyll 'special pair' in the reaction centers, centered near 870, 810, 630 and 600 nm. These bands have been designated BY_1, BY_2, BX_1 and BX_2, respectively, corresponding to two Q_y transitions and two Q_x transitions of the dimeric special pair. We found the BY_1 and BX_1 transition moments to be parallel (within 20°) to the plane of the membrane, whereas the BX_2 moment makes an angle of 55—63° with the plane.

Using the photoselection technique we found that the angle between the BY_1 and BX_1 transition moments is 30°, while that between BY_1 and BX_2 is 75°. The BX_1 and BX_2 moments were found to be orthogonal, consistent with the prediction of molecular exciton theory for a dimer.

By combining these data, we have calculated the orientations of the transition moments of the bacteriochlorophyll dimer in spherical polar coordinates, with the pole of the coordinate system normal to the plane of the membrane. The orientations of the Q_y and Q_x transition moments of the two bacteriopheophytin molecules in the reaction center were also computed in this coordinate system by transforming the data reported by Clayton, C.N., Rafferty, R.K. and Vermeglio, A. ((1979) Biochim. Biophys. Acta 545, 58—68). We have derived the transformation equations for two polar coordinate systems: in one, the pole is an axis of symmetry as defined by the orientations of purified reaction centers in stretched gelatin films (Rafferty, C.N. and Clayton, R.K. (1979)

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Introduction

The chromatophore membrane from *Rhodopseudomonas sphaeroides* is the isolated membrane component which is associated with both reaction centers and the accessory antenna pigments. The reaction center is an intrinsic membrane protein to which six chromophores, four molecules of bacteriochlorophyll and two of bacteriopheophytin, are non-covalently bound. Two of the bacteriochlorophyll molecules, the 'special pair', are thought to share a positive charge following the primary photooxidation-reduction reaction. The initial electron acceptor is thought to be one of the two bacteriopheophytin molecules [1,2].

Evidence for strong electronic interaction between the two bacteriochlorophyll molecules while in the reduced state has been provided by circular dichroism [3,4], absorption [5], linear dichroism [5—7], and photoselection [8] studies on chromatophores and purified reaction centers from *Rp. sphaeroides.* Absorption bands near 870 * and 810 nm have been tentatively assigned to Q_y transitions of the bacteriochlorophyll special pair [5—9]. Recently, an additional negative band was detected in the light minus dark difference spectrum near 630 nm for purified reaction centers in solution and in oriented specimens (stretched gelatin films) [7]. This new band and the well documented negative band at 600 nm have been tentatively assigned to Q_x transitions of the bacteriochlorophyll special pair [7,9]. These assignments are considered as a simple working hypothesis. Other interpretations are possible [7,9]. Arguments have been presented by Clayton et al. [9] that each of the two bacteriopheophytin molecules can be treated as monomers for an adequate description of their electronic absorption spectra.

Considerable information has been obtained about the orientations of these transition dipole moments. Linear dichroism measurements on oriented chromatophore membranes gave approximate angles that these transition moments make with the chromatophore membrane [5]. Linear dichroism measurements on purified reaction centers in stretched gelatin films gave angles that these transition moments make with an axis of symmetry in the reaction center [6,7]. Photoselection measurements gave angles that these transition moments make with each other [8]. Combining the data obtained from the stretched film measurements, from the photoselection measurements of Vermeglio et al. [8], and from additional photoselection measurements presented formally in this paper, Clayton et al. [9] computed the orientations of the Q_y and Q_x transitions of the bacteriochlorophyll special pair and of the two bacteriopheophytin molecules. The orientations were given in spherical polar coordinates with the reaction center symmetry axis as the pole.

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* The band near 870 nm varies between 850 and 870 nm depending on the environment of the reaction center; we shall refer to it generically as the 870 nm band.

The object of this study was to determine transition moment orientations in relation to the chromatophore membrane; i.e. in a polar coordinate system with the pole normal to the plane of the membrane. To achieve this we have determined angles between the 870 nm transition moment and each of the Q_x (630 and 600 nm) transition moments of the special pair; also the angle between the 630- and 600-nm transition moments, using the photoselection technique. From measurements of linear dichroism we determined the angle that each of these transition moments makes with the plane of the membrane. By comparing these data with earlier data obtained with purified reaction centers [7—9], we have developed equations for transforming polar coordinates between two frames of reference: In one (present experiments), the pole is normal to the plane of the membrane; in the other [6,7], the pole is an axis of symmetry as defined by the orientations of reaction centers in stretched gelatin films. As a result we have deduced the orientations of the 'special pair' transition moments, of the transition moments of the bacteriopheophytin of the reaction centers, and of the reaction center symmetry axis, all in the frame of reference defined by the plane of the membrane.

Materials and Methods

Preparation of chromatophore membranes and reaction centers. Carotenoidless mutant strain R-26 of *Rp. sphaeroides* was grown phototrophically by the method of Clayton [10]. Purified chromatophore membranes were then isolated as described by Clayton and Clayton [11]. Chromatophores were suspended in distilled water and stored at 4°C until use. Reaction centers were isolated as described by Clayton and Wang [12]. Reaction centers were stored in a solution of 0.3% (v/v) lauryl dimethyl amine oxide (Onyx Chemical Company, Jersey City, NJ) and 0.01 M Tris-HCl, pH 7.5, and were kept at —20°C until use.

Method of orienting chromatophore membranes. The method has been described in detail elsewhere [5,13,14]. Aqueous suspensions were pipetted onto the surfaces of glass microscope slides and dried under a forced stream of room air.

Optical measurements on oriented chromatophores. Dark absorption spectra (no actinic illumination) were recorded with a Cary 14R Spectrophotometer. Light-induced absorption changes were measured with a split-beam absorption spectrophotometer [15] in conjunction with a Tracor-Northern TN-1500 Signal Averager. Both spectrophotometers were fitted with Glan-Thompson polarizers in their measuring beams. Actinic light was provided by a Xenon flash lamp with a decay half lifetime of 200 μs. The axis of the actinic flash was set normal to the measuring beam of the split-beam spectrophotometer. A slide bearing dried chromatophores was mounted in the measuring beam of one of the two spectrophotometers and rotated around a vertical axis so that a line drawn normal to the slide made an angle of 60° to the measuring beam axis. With this geometry, the electric vector of vertically polarized light was parallel to the plane of the slide, whereas the electric vector of horizontally polarized light made an angle of 60° with the plane of the slide. By measuring a series of light-induced changes at different wavelengths, light minus dark difference spectra were constructed. To avoid artifacts due to photoselection, the intensity of the actinic light was set so that the photochemistry was saturated. Experiments on oriented chromatophores were carried out at 20—25°C.

Photoselection measurements on purified reaction centers. The techniques employed were essentially those developed by Vermeglio et al. [8]. The flash spectrophotometer used for these experiments is described above. An additional polarizer was inserted between the flash lamp and sample. A Glan-Thompson polarizer was used for excitation in the near infrared; a sheet polarizer for excitation in the visible. Actinic light was always polarized so that the electric vector was vertical. Interference filters with half bandwidths of approximately 10 nm determined the wavelength of the actinic light. Thermal rotation of reaction centers was minimized by maintaining samples at 150 K using a helium cryostat (Cryogenic Technology Inc., Waltham, MA). The composition of reaction center samples was 0.3% lauryl dimethyl amine oxide, 0.01 M Tris-HCl, pH 7.5 and 67% (w/v) glycerol. Light-induced changes were measured for both vertically and horizontally polarized light. The intensity of the actinic light was adjusted so that the photochemical response was approximately 10% of that at saturation.

Mathematical treatment

Polarized absorption of oriented chromatophores

The equations relating transition moment orientation and polarized absorption have been derived previously [5,13,14]. In this treatment, we modify these equations to correct for imperfect orientation of the membranes on the glass slides. The initial forms of the equations are based on four assumptions.

(1) Chromatophore membranes dry so that the membrane planes are precisely parallel to the surface of the slide. (2) There is no long range lateral order in the plane of the chromatophore film. (3) The angle that a particular transition moment makes to the membrane plane is the same for each reaction center or antenna pigment in the membrane. (4) The measured absorbance is associated with a single linear transition dipole moment. The absorbances of vertically and horizontally polarized light (A_V and A_H, respectively) are then given by

$$A_V = \tfrac{1}{2} A_\mu \cos^2\alpha_p \tag{1}$$

$$A_H = \tfrac{1}{2} A_\mu \cos^2\beta \cos^2\alpha_p + A_\mu \sin^2\beta \sin^2\alpha_p \tag{2}$$

where A_μ is the absorbance of light with the electric vector parallel to a linear transition moment, for an arbitrary sample concentration and pathlength, α_p is the angle made by the transition moment to the membrane plane, and β is the angle between the measuring beam axis and the normal to the plane. β is the angle of refraction of the measuring beam inside the chromatophore film, not the angle of incidence outside the film. For an angle of incidence of 60°, β was found * to be 41.9 ± 1.8° (95% confidence limits).

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[*] β (called ϕ in Ref. 6) was determined by a method similar to that used by Rafferty and Clayton [6] for unstretched gelatin films. At any one wavelength, $\cos\beta = A_V(0)/A_V(60)$ where $A_V(0)$ and $A_V(60)$ are the absorbances of vertically polarized light for a chromatophore film at angles of incidence of 0 and

Eqns. 1 and 2 can be modified in a simple way to correct for imperfect alignment of the membrane planes and the slide surface. The way we choose to express imperfect alignment is to partition the population of a particular transition moment into a perfectly oriented fraction f and a randomly oriented fraction $(1 - f)$ *. The fraction f is correctly described by the foregoing equations. The absorbance of the fraction $(1 - f)$ is $A_\mu/3$ for both vertically and horizontally polarized light. Eqns. 1 and 2 then become

$$A_V = \tfrac{1}{2} f A_\mu \cos^2\alpha_p + \tfrac{1}{3} A_\mu(1 - f) \tag{3}$$

$$A_H = \tfrac{1}{2} f A_\mu \cos^2\beta \cos^2\alpha_p + f A_\mu \sin^2\beta \sin^2\alpha_p + \tfrac{1}{3} A_\mu(1 - f) \tag{4}$$

The dichroic ratio is given by

$$A_V/A_H = [\tfrac{1}{2} f \cos^2\alpha_p + \tfrac{1}{3}(1 - f)]/[\tfrac{1}{2} f \cos^2\beta \cos^2\alpha_p + f \sin^2\beta \sin^2\alpha_p + \tfrac{1}{3}(1 - f)] \tag{5}$$

When a light-induced absorbance change is measured, the symbols A_V, A_H, and A_μ are simply replaced by ΔA_V, ΔA_H, and ΔA_μ, respectively, in Eqns. 1—5.

Photoselection data for purified reaction centers

Vermeglio et al. [8] have derived equations which give the angle that two transition dipoles make with each other when orientation is induced by photoselection. In this treatment, we modify these equations to correct for imperfect photoselection. The initial forms of the equations are based on two pertinent assumptions: (1) Reaction center transition moments have fixed orientations relative to each other. (2) Both excitation and measurement of the induced absorption changes in reaction centers are carried out at wavelengths where absorption and absorption changes, respectively, are associated with single linear transition moments. The absorbance changes measured with vertically and horizontally polarized light (ΔA_V and ΔA_H, respectively) induced by excitation with vertically polarized light are given by

$$\Delta A_V = \tfrac{1}{5} \Delta A_\mu (2 \cos^2\gamma(i, j) + 1) \tag{6}$$

$$\Delta A_H = \tfrac{1}{5} \Delta A_\mu (2 - \cos^2\gamma(i, j)) \tag{7}$$

where ΔA_μ is the absorbance change measured with light with the electric vector parallel to the associated linear transition dipole, for an arbitrary sample concentration and pathlength, and $\gamma(i, j)$ is the angle between two transition moments, one of which is associated with the wavelength of measurement and the second of which is associated with the excitation wavelength.

Imperfect photoselection may occur for several reasons. Two possibilities are partial depolarization of the excitation beam and excitation within an

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60°, respectively. $A_V(0)$ and $A_V(60)$ were measured at 855 and 590 nm for four different films with maximal film absorbances ($A_V(0)$) ranging from 0.22 to 2.2 Within experimental error, the ratio $A_V(0)/A_V(60)$ was found to be independent of film thickness and wavelength. Using Snell's Law, the index of refraction of dried chromatophore films was calculated to be 1.32 ± 0.05 (95% confidence limits).

* The method, originally proposed by Fraser [22], was used to allow for imperfect orientation of purified reaction centers of *Rp. sphaeroides* in stretched gelatin films [7].

absorption band associated with more than one transition moment. A useful correction is one analogous to that applied to the equations for imperfect chromatophore orientation *. Applying this correction to Eqns. 6 and 7, the dichroic ratio is given by

$$\Delta A_{\mathrm{V}}/\Delta A_{\mathrm{H}} = [\tfrac{1}{5}g(2\cos^2\gamma(i,j) + \tfrac{1}{3}(1-g)]/[\tfrac{1}{5}g(2-\cos^2\gamma(i,j) + \tfrac{1}{3}(1-g)] \qquad (8)$$

where g may be interpreted as that fraction of reaction centers which is perfectly photoselected according to Eqns. 6 and 7 and $(1-g)$ is that fraction which is randomly excited **. Note that an equally acceptable angle between two transition moments is $(180° - \gamma(i, j))$.

The spherical polar coordinates of reaction center transition moment orientation in relation to the chromatophore membrane

In the report of Clayton et al. [9] the orientations of transition moments were specified in a polar coordinate system with the pole aligned with an axis of symmetry of the reaction centers as defined by the axis of a stretched gelatin film. In the present report we use a coordinate system with the pole normal to the plane of the membrane. The angles α_p and $\gamma(i, j)$, computed from linear dichroism and photoselection measurements, yield coordinates in this frame of reference through the equations of spherical trigonometry [9]. Coordinates of transition moments of the two bacteriopheophytin molecules are also obtained by transforming the data provided by Clayton et al. [9]. In a similar way the orientation of the axis of symmetry of the reaction center can be computed in the new coordinate system. We will show that the axis of symmetry is nearly parallel to the plane of the membrane.

Results

The polarized absorption spectra of oriented chromatophore membranes

Absorption spectra of oriented chromatophores were recorded using vertically and horizontally polarized light. Samples were mounted so that the measuring beam axis made an angle of 60° to a line drawn normal to the plane of the slide. Two major bands were apparent in the near infrared and visible at 855 and 590 nm. These bands are attributed primarily to the Q_y and Q_x transitions of the antenna bacteriochlorophyll [5,13,14]. The dichroic ratios (A_V/A_H) of these bands were determined for a series of samples; we observed no significant dependence of the dichroic ratios on the amount of chromatophores deposited on the slides, although the measured absorbance at 855 nm (slide plane normal to the measuring beam axis) varied over a ten-fold range, 0.22–2.2. This result implies that the chromatophore membranes stack with remarkable regularity onto the surfaces of the glass slides. The average values of the dichroic ratios at 855 and 590 nm are listed in Table I. These values agree well with those reported by Vermeglio and Clayton [5].

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* We introduce an analogous orientation factor g which is used in the context of photoselection (Table II). The orientation factor f applies only to the linear dichroism of chromatophores (Tables I and IV).

** Similar corrections have been used in other photoselection studies and are discussed in some detail by Albrecht [23]. The fraction $(1-g)$ has been referred to as the 'randomization factor'.

TABLE I

THE DICHROIC RATIO AND THE ANGLE α_p FOR VARIOUS TRANSITION MOMENTS IN CHROMATOPHORE MEMBRANES OF *RP. SPHAEROIDES*

The dichroic ratios were obtained from measurements of polarized absorption (antenna) or light-induced absorbance changes (reaction center) using oriented membrane fragments. α_p is the angle of inclination of a transition moment to the membrane plane. It was calculated from the listed dichroic ratios using Eqn. 5 for two values of f, the equivalent fraction of ideally oriented membranes. The experimental error is given as approximate limits for a 95% confidence interval.

Wave-length (nm)	Chromophore	Transi-tion	Desig-nation	Dichroic ratio		α_p (deg.)	
				A_V/A_H	$\Delta A_V/\Delta A_H$	$f = 1.00$	$f = 0.72$
855	antenna bacteriochlorophyll	Q_y		1.40 ± 0.04		23 ± 1	17 ± 2
590		Q_x		0.43 ± 0.02		55 ± 1	63 ± 1
855	reaction center bacteriochlorophyll special pair	Q_y	BY_1		1.55 ± 0.05	18 ± 2	0 ± 9
810		Q_y	BY_2		≈1	≈35	≈35
630		Q_x	BX_1		1.54 ± 0.07	18 ± 3	0 ± 12
600		Q_x	BX_2		0.425 ± 0.032	55 ± 1	63 ± 2

The spectra of light-induced absorbance changes, measured with vertically and horizontally polarized light, are shown in Fig. 1. Negative bands are centered at 855, 810, 630, and 600 nm. Excepting the 810 nm band, the dichroic ratios ($\Delta A_V/\Delta A_H$) are constant across these bands: for the 855 nm band from 830 to 880 nm, for the 630 nm band from 630 to 650 nm, and for the 600 nm band from 595 to 610 nm. Average values of the dichroic ratios are listed in Table I. The dichroic ratios did not show a dependence on the amount of chromatophore membranes deposited on the slides. The dichroic ratios for the 855 and 600 nm bands agree with those reported by Vermeglio and Clayton [5]. The negative band at 630 nm was first detected for purified reaction centers that were oriented in stretched gelatin films [7]. We confirm its presence in the difference spectra of oriented chromatophore membranes. Consistent with previous interpretations [5—9], we attribute the negative 855 and 810 nm bands to disruption of exciton coupling between the Q_y transitions of the bacteriochlorophyll 'special pair' dimer in reaction centers of *Rp. sphaeroides*, upon photooxidation of the dimer. Similarly, we attribute the negative 630 and 600 nm bands to disruption of exciton coupling between the Q_x transitions of the dimer.

The angle α_p that each of the transition moments makes to the chromatophore membrane was calculated from the dichroic ratio for the corresponding band using Eqn. 5. These values of α_p are listed in Table I. α_p was calculated for the case that chromatophore membranes were ideally oriented ($f = 1$), and for $f = 0.72$, which represents the greatest degree of disorder compatible with the data *.

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* The smallest angle that a transition moment can make to the membrane plane is 0°. When $f = 0.72$ and $\alpha_p = 0$, Eqn. 5 predicts a dichroic ratio of 1.55, the experimentally observed value for the 855 and 630 nm bands. For this dichroic ratio, a value of $f < 0.72$ gives the meaningless condition $|\cos^2\alpha_p| > 1$.

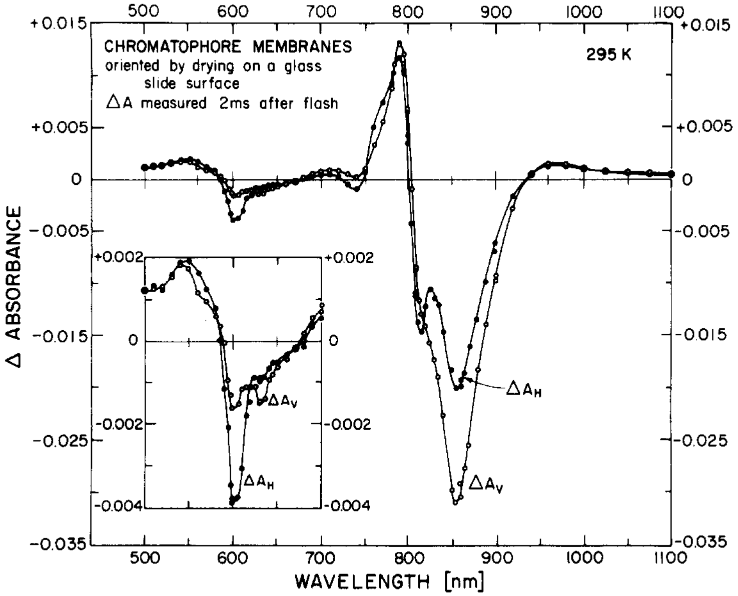

Fig. 1. The polarized light minus dark difference absorption spectra of chromatophore membranes from *Rp. sphaeroides*, oriented by drying on a glass slide surface. The inset shows the difference spectra between 500 and 700 nm in a four fold expanded absorbance scale. We measured the light-induced absorbance changes in response to a brief unpolarized actinic flash. The plane of the slide was vertical and at 30° and 60°, respectively, to the mutually perpendicular axes of the measuring and actinic beams. Thus the electric vector of the vertically polarized measuring beam was parallel to the slide plane whereas the electric vector of the horizontally polarized measuring beam made an angle of 60° with the slide plane. We averaged four measurements at each wavelength. The samples did not deteriorate appreciably in the course of several hundred flashes. For measurements above 650 nm the actinic light passed through a blue (Corning 4-96) filter and the photomultiplier was shielded by a red (Corning 2-58) filter. Below 700 nm we used an infrared-insensitive photomultiplier and placed a near infrared (Corning 7-69) filter in the actinic path. The actinic light was saturating with both the blue filter and the near infrared filter.

Photoselection experiments on purified reaction centers

The results are summarized in Table II. Excitation within the 870 nm band generates a dichroic ratio ($\Delta A_V/\Delta A_H$) of the induced absorbance changes, measured within the same band, which closely approaches three, the value predicted by Eqn. 8 ($g = 1$) for a band which arises from a single transition moment (rows a and b, Table II). From the extent of deviation of the experimental and ideal dichroic ratios, the equivalent fraction g of reaction centers which is ideally photoselected can be calculated using Eqn. 8. For excitation within the 870 nm band, $g = 0.894 \pm 0.022$.

With excitation within the 870 nm band, absorbance changes were measured at several wavelengths between 650 and 580 nm using vertically and horizontally polarized light. The spectra of these absorbance changes, 2 ms following the excitation flash, are shown in Fig. 2. Negative bands are centered near 630 and 600 nm. The dichroic ratios are constant across both bands: for the 630 nm band between 650 and 625 nm and for the 600 nm band between 610 and 590 nm. The average values of the dichroic ratios for these two bands together with the calculated angles between the 870 nm transition moment and the 630 and 600 nm moments are listed in Table II, rows c and d. These angles were calculated using Eqn. 8 for $g = 1$ and $g = 0.894$. We prefer the values cal-

TABLE II

SUMMARY OF PHOTOSELECTION EXPERIMENTS ON PURIFIED REACTION CENTERS FROM *Rp. sphaeroides*

$\gamma(i,j)$ is the angle between two transition moments in the reaction center. It was calculated from the listed dichroic ratios and values of g using Eqn. 8. g is the equivalent fraction of ideally photoselected reaction centers and was determined as described in the text. The experimental error of the dichroic ratio is given as limits for a 95% confidence interval. The polarization value P is defined as $(\Delta A_V - \Delta A_H)/(\Delta A_V - \Delta A_H)$ and is listed parenthetically to facilitate comparison with other studies. The error of g is given as limits for a 95% confidence interval. The error of $\gamma(i,j)$ is given as limits for a 95% confidence interval and was based on either the uncertainty in the experimental dichroic ratio or on the uncertainty in f, whichever was larger.

Row	Excitation wavelength (nm) (transition i)	Measuring wavelength (nm) (transition i)	Dichroic ratio $\Delta A_V/\Delta A_H$ (polarization) value P	g	$\gamma(i,j)$ (deg.)
a	870 (PY_1)	902 (PY_1)	2.7 ± 0.3 (0.46 ± 0.04)	1.000	14^{+7}_{-14}
				0.894 ± 0.022	0 ± 16
b	902 (PY_1)	870 (PY_1)	2.67 ± 0.06 (0.455 ± 0.01)	1.000	15.5 ± 2
				0.894 ± 0.022	0 ± 7
c	902 (PY_1)	650—625 (PX_1)	1.85 ± 0.14 (0.30 ± 0.04)	1.000	33 ± 3
				0.894 ± 0.022	30.5 ± 3
d	902 (PY_1)	610—590 (PX_2)	0.62 ± 0.02 (—0.235 ± 0.015)	1.000	72.5 ± 1.5
				0.894 ± 0.022	75.5 ± 2
e	600 (PX_2)	870 (PY_1)	0.74 ± 0.02 (—0.15 ± 0.015)	1.000	65.5 ± 1
				0.590 ± 0.050	75.5 ± 5.5
f	600 (PX_2)	630 (PX_1)	0.69 ± 0.035 (—0.185 ± 0.025)	1.000	68 ± 2
				0.590 ± 0.050	90 ± 15

culated for $g = 0.894$ because this value of g, with excitation in the 870 nm band, gives the result predicted by theory for measurement within the same band. We also list values for $g = 1$ to facilitate comparison with other studies.

We expect that excitation near 600 nm should produce a significantly smaller fraction of ideally photoselected reaction centers than excitation in the 870 nm band. This follows from the mixed origin of the band near 600 nm in the dark spectrum of reaction centers from *Rp. sphaeroides* [16]. The major contribution is from the two bacteriochlorophylls/reaction center which do not participate as electron donors. It this case, Eqn. 8 predicts that excitation at 600 nm and measurement of the absorbance changes in the 870 nm band should produce a higher dichroic ratio (closer to unity) than the converse experiment. This expectation was realized; compare row e to row d in Table II. An estimate of the equivalent fraction of ideally photoselected reaction centers was calculated as follows. The preferred value of the angle between the 870- and 600-nm transition moments is 75.5 ± 2° and was obtained by excitation within the 870 nm band and measurement at 600 nm, using $g = 0.894$ for

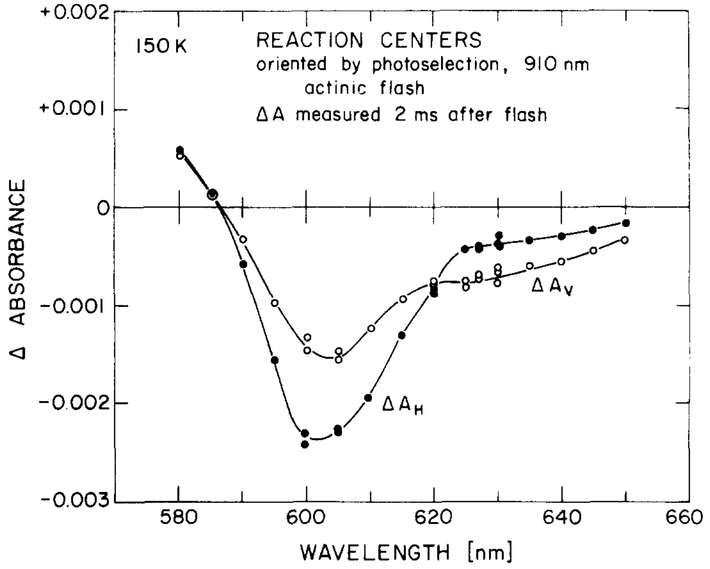

Fig. 2. The polarized light minus dark difference absorption spectra of purified reaction centers from *Rp. sphaeroides*, oriented by photoselection. We measured the light-induced absorbance changes using vertically and horizontally polarized light in response to a brief actinic flash at 910 nm. The actinic flash was vertically polarized and gave changes which were approximately 10% of those at saturation. The axes of the measuring and actinic beams were mutually perpendicular. We averaged eight measurements at each wavelength. The samples did not deteriorate appreciably in the course of several hundred flashes. We used an infrared-insensitive photomultiplier to minimize flash artifacts.

870 nm excitation (see earlier). The experimental dichroic ratio for excitation at 600 nm and measurement within the 870 nm band is 0.74 ± 0.02. Substituting $\gamma = 75.5^\circ$ and $\Delta A_V/\Delta A_H = 0.74$ in Eqn. 8 gives $g = 0.590 \pm 0.05$ for excitation at 600 nm.

The experimental dichroic ratio for excitation at 600 nm and measurement in the 630 nm band was found to be 0.69 ± 0.035 (row f, Table II). The angle between the 600 and 630 nm transition moments was then calculated using Eqn. 8 for this ratio and $g = 0.590$. The calculated angle is $90 \pm 15^\circ$.

The results of this photoselection study are fully compatible with those reported by Vermeglio et al. [8] but are in disagreement with those reported by Mar and Gingras [17] and by Shuvalov et al. [18]. Vermeglio et al. [8] have shown that reports of lower polarization values for excitation and measurement within the 870 nm band [17,18] were in error due to the presence of a mixed population of reaction centers with different reaction kinetics.

The spherical polar coordinates of reaction center transition moment orientation in relation to the chromatophore membrane

Fig. 3a shows a polar view of a spherical polar coordinate system. The chromatophore membrane plane is coincident with the equatorial XY′ plane. The polar axis (Z′-axis) is thus normal to the membrane plane. The orientations of the 870 nm (BY_1), 630 nm (BX_1), and 600 nm (BX_2) transition moments of the bacteriochlorophyll special pair in reaction centers from *Rp. sphaeroides* are represented by unit vectors drawn from the origin into the northern hemisphere (positive Z'axis). (Only the BY_1 and BX_1 transition moments are shown

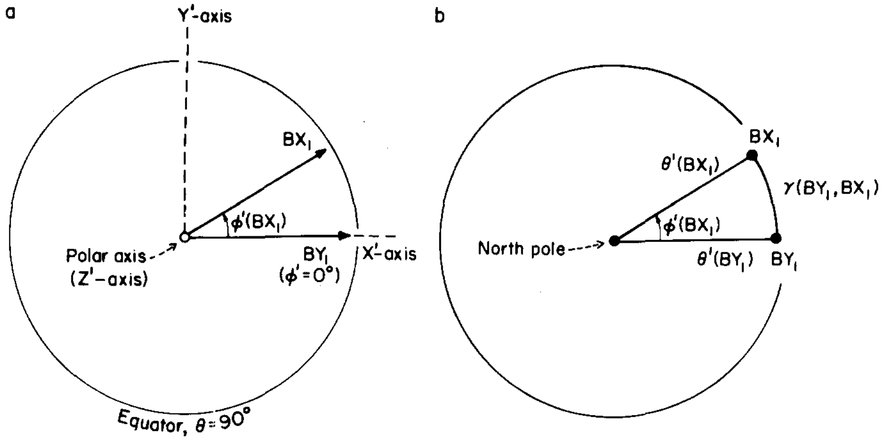

Fig. 3. (a) Polar view of a spherical coordinate system with the pole set normal to the plane of the chromatophore membrane of *Rp. sphaeroides*. Optical transition moments of a reaction center are represented by unit vectors drawn from the origin into the north hemisphere. The BY$_1$ (870 nm) transition moment is set arbitrarily at $\phi' = 0$. The BX$_1$ (630 nm) transition moment is also shown. (b) Polar view of great circle lines which lie on the surface of a unit sphere and connect the termini of the transition moments to each other and to the north pole. The angle between the transition moments is $\gamma(i, j)$.

in Fig. 3a.) The terminus of each of the transition moments has coordinates θ' (declination from the north polar axis) and ϕ' (longitude). ϕ' is measured counter clockwise from the X'-axis. We arbitrarily set $\phi' = 0°$ for the BY$_1$ transition moment. Note that there is redundancy in that a transition moment with coordinates θ', ϕ' also has coordinates $(180° - \theta')$, $(180° + \phi')$ as it intersects the opposite side of the sphere.

For each transition moment θ' is given by $(90° - \alpha_p)$. Values of α_p are taken from Table I. Values of ϕ' for the BX$_1$ and BX$_2$ transition moments are calculated by an application of the law of cosines in spherical trigonometry. The terminus of each transition moment occupies the surface of a unit sphere which is centered at the origin of the coordinate system. Great circle lines connecting the termini of the transition moments to each other and to the northern pole lie on the surface of the unit sphere. A polar view of the great circle lines for the BY$_1$ and BX$_1$ transition moments is shown in Fig. 3b. The lengths of the great circle lines connecting the transition moments are the angles between the transition moments $\gamma(i, j)$ determined from photoselection measurements. The best values of $\gamma(i, j)$ (see the previous section; choices of g) are listed in Table III. The lengths of the great circle lines connecting the transition moments and the north pole are the angles θ' for each transition moment. For the BX$_1$ transition, ϕ' is given by

$$\cos \phi'(\mathrm{BX}_1) = [\cos \gamma(\mathrm{BY}_1, \mathrm{BX}_1) - \cos \theta'(\mathrm{BY}_1) \cos \theta'(\mathrm{BX}_1)]/[\sin \theta'(\mathrm{BY}_1) \sin \theta'(\mathrm{BX}_1)] \tag{9}$$

An analogous equation can be written for the BX$_2$ transition.

We shall first calculate allowed values of ϕ' using values of $\theta' = 90° - \alpha_p$ obtained for the case of ideally oriented chromatophore membranes (Table I,

TABLE III

Best values (basis for preference discussed in text) of the angles between various transition moments in reaction centers of *Rp. sphaeroides*, taken from Table II.

Wavelengths (nm)	Transition i	Transition j	Angle $\gamma(i,j)$ (deg.) between the transition moments *
870, 630	BY_1	BX_1	30.5 ± 3 or 149.5 ± 3
870, 600	BY_1	BX_2	75.5 ± 2 or 104.5 ± 2
630, 600	BX_1	BX_2	90 ± 15

* An alternative value of the angle between two transition moments is $(180^\circ - \gamma(i,j))$.

$f = 1$). For the angle between the BY_1 and BX_1 transitions, the two equivalent values are $\gamma(BY_1, BX_1) = 30.5$ or 149.5°. If $\gamma(BY_1, BX_1) = 149.5^\circ$, we compute $|\cos\phi'(BX_1)| > 1$ which is an impossible condition. We reject this value and keep $\gamma(BY_1, BX_1) = 30.5^\circ$. We then find $\phi'(BX_1) = \pm 32 \pm 4^\circ$. We have no basis for preferring the positive or the negative value. We shall take the positive value here and in the subsequent calculations, but will point out at the end that an equally acceptable configuration is one in which every value of ϕ' is reversed in sign.

Turning to BX_2, we have $\gamma(BY_1, BX_2) = 75.5$ or 104.5°. Application of Eqn. 9, with BX_2 in place of BX_1, does not allow us to reject either of these values; Eqn. 9 gives $\phi'(BX_2) = \pm 90 \pm 7^\circ$ for the former and $\pm 160 \pm 20^\circ$ for the latter.

The multiplicy of alternative positive and negative signs in these ϕ' coordinates can be reduced through calculations based on the experimental finding that the BX_1 and BX_2 transitions are approximately orthogonal. The law of cosines gives

$$\cos\gamma(BX_1, BX_2) = \cos\theta'(BX_1)\cos\theta'(BX_2) + \sin\theta'(BX_1)\sin\theta'(BX_2)\cos[\phi'(BX_2) - \phi'(BX_1)] \quad (10)$$

We require that the computed value of $\gamma(BX_1, BX_2)$ must fall within the range 90 ± 15° obtained experimentally.

Applying Eqn. 10, with $\phi'(BX_1) = 32 \pm 4^\circ$, we find for the four possible values of $\phi'(BX_2)$:

$\phi'(BX_2)$	$\gamma(BX_1, BX_2)$
90 ± 7°	57 ± 7° (reject)
−90 ± 7°	92 ± 7° (accept)
160 ± 20°	93 ± 13° (accept)
−160 ± 20°	107 ± 5° (reject marginally)

leaving acceptable values of $\phi'(BX_2) = -90 \pm 7^\circ$ or $160 \pm 20^\circ$.

If we repeat these calculations with values of $f < 1$ (imperfect membrane orientation on the slide), we encounter a larger number of acceptable solutions. The least value of f compatible with the experimental data is 0.72; for smaller

TABLE IV

POLAR COORDINATES OF TRANSITION MOMENTS IN REACTION CENTERS OF *RP. SPHAEROIDES*

Data are calculated from the data of Tables I and III. In this coordinate system, the polar axis is fixed normal to the chromatophore membrane plane. Angles are in degrees. Wavelengths of transitions in nm are shown in parenthesis. BY_1 was set at $\phi' = 0°$. For $f = 0.72$, two alternative values of $\phi'(BX_1)$ arise; these are distinguished in the last two columns. Beyond that, an alternative set of values with the sign of ϕ' reversed throughout (mirror image) is equally acceptable. The experimental values of θ' were carried over from Table I ($\theta' = 90° - \alpha_p$).

Transition	Calculated for $f = 1.00$		Calculated for $f = 0.72$		
	θ'	ϕ'	θ'	ϕ' (1st alternative)	ϕ' (2nd alternative)
BY_1 (870)	72 ± 2	0	90 ± 9	0	0
BX_1 (630)	72 ± 3	32 ± 4	90 ± 12	31 ± 3	150 ± 7 *
BX_2 (600)	35 ± 1	−90 ± 7 or 160 ± 20	27 ± 2	−67 ± 13 or 150 ± 30	67 ± 13 or −150 ± 30

* This alternative solution for ϕ' (BX_1) is allowed only when $f \leqslant 0.9$. Its absolute maximum value is $|\phi'(BX_1)| = 180°$ which is obtained when $f = 0.9$.

values application of Eqn. 5 to some dichroic ratios leads * to the impossible condition $|\cos \alpha_p| > 1$. Taking values of α_p for $f = 0.72$ from Table I, with $\theta' = 90° - \alpha_p$, we find that Eqn. 9 is compatible with $\gamma(BY_1, BX_1) = 30.5°$ (as before), and also with $\gamma(BY_1, BX_1) = 149.5°$. The corresponding solutions of Eqn. 9 are: for $\gamma(BY_1, BX_1) = 30.5°$, $\phi'(BX_1) = \pm 31 \pm 3°$, and for $\gamma(BY_1, BX_1) = 149.5°$, $\phi'(BX_1) = \pm 150 \pm 7°$. Again we keep only the positive sign for $\phi'(BX_1)$, and anticipate an alternative configuration with the signs of all ϕ' values reversed. For BX_2, with $\gamma(BY_1, BX_2) = 75.5$ or $104.5°$, and values from Table I with $f = 0.72$, Eqn. 9 gives: for $\gamma(BY_1, BX_2) = 75.5°$, $\phi'(BX_2) = \pm 67 \pm 13°$, and for $\gamma(BY_1, BX_2) = 104.5°$, $\phi'(BX_2) = \pm 150 \pm 30°$. We now apply Eqn. 10, with values arising from $f = 0.72$, and find the following acceptable solutions: for $\phi'(BX_1) = 31 \pm 3°$, $\phi'(BX_2) = -67 \pm 13°$ or $150 \pm 30°$, and for $\phi'(BX_1) = 150 \pm 7°$, $\phi'(BX_2) = 67 \pm 13°$ or $-150 \pm 30°$. We have rejected solutions incompatible with the experimental value $\gamma(BX_1, BX_2) = 90 \pm 15°$.

Values of θ' and ϕ' for BY_1, BX_1 and BX_2 computed thus far are summarized in Table IV.

Turning to the orientations of the two molecules of bacteriopheophytin in the reaction center, we start with the orientations given by Clayton et al. [9] in which the pole of the coordinate system is the axis of symmetry of the reaction center as found in gelatin films. We must transform (rotate) these coordinates to the system with the pole normal to the plane of the membrane. Primed variables (θ', ϕ' or x', y', z') refer to the present coordinate system and unprimed ones to the system of Clayton et al. [9]. A set of transforming equations can be expressed by linear combinations of the cartesian coordinates, chosen to give agreement between the θ and ϕ coordinates and the θ' and ϕ' coordinates of BY_1, BX_1 and BX_2. For the θ' and ϕ' coordinates we took the values in Table IV for $f = 1$; we prefer this over $f < 1$ for reasons to be

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* See footnote p. 195

discussed. The corresponding θ and ϕ coordinates are from Clayton et al. [9]. A best fit (compare Tables IV and V) was obtained with the following transforming equations.

If $\phi(\mathrm{BX}_1)$ is taken as negative, the equations are

$$\begin{aligned} x' &= 0.495x + 0.258y + 0.830z \\ y' &= -0.629x - 0.552y + 0.547z \\ z' &= 0.599x - 0.793y - 0.111z \end{aligned} \tag{11}$$

and $\phi'(\mathrm{BX}_1)$ turns out to be positive. If $\phi(\mathrm{BX}_1)$ is positive (mirror image), the transformation is

$$\begin{aligned} x' &= 0.495x - 0.258y + 0.830z \\ y' &= 0.629x - 0.552y - 0.547z \\ z' &= 0.599x + 0.793y - 0.111z \end{aligned} \tag{12}$$

and $\phi'(\mathrm{BX}_1)$ comes out negative. In applying these equations and reverting to polar coordinates values of θ', ϕ' for $\theta' > 90°$ (south hemisphere) are replaced by $180° - \theta'$, $180° + \phi'$, to give the corresponding projection in the north hemisphere.

Table V shows the values of θ' and ϕ' for BY_1, BX_1 and BX_2 computed from the data of Clayton et al. [9] by these transforming equations. Note that these values of θ' and ϕ' are in excellent agreement with those found in the present study (Table IV, $f = 1$).

The orientation of the symmetry axis of the reaction center is also shown in Table V; it lies nearly parallel to the membrane ($\theta' = 84°$).

The $\mathrm{Q_y}$ and $\mathrm{Q_x}$ transition moments of the two bacteriopheophytin molecules are designated PY_1 (760 nm) and PX_1 (545 nm) for one molecule and PY_2

TABLE V

Data from [9] are transformed into the coordinate system of the present study by Eqns. 11 and 12 in the text. Angles are in degrees. Wavelengths of transitions in nm are shown in parentheses.

Transition	Pole (reaction center symmetry axis in gelatin films [9])		Pole (axis normal to the membrane plane (present study))	
	θ	ϕ	θ'	ϕ'
BY_1 (870)	41 ± 1	0	72 ± 1	0
BX_1 (630)	22.5 ± 3	−50 ± 10	74 ± 3	32 ± 4
BX_2 (600)	64 ± 1	98 ± 7 or 163 ± 17	34 ± 6 or 39 ± 15	162 ± 7 or −90 ± 14
Symmetry axis in gelatin films	0	—	84	−146.5
Mirror image alternative for sign of ϕ and ϕ':				
BX_1 (630)		50 ± 10		−32 ± 4
BX_2 (600)		−98 ± 7 or −163 ± 17		−162 ± 7 or 90 ± 14
Symmetry axis in gelatin films		—		146.5

TABLE VI

Same as Table V, for the transition moments of the two bacteriopheophytin molecules of the reaction center. Four alternatives arise because the signs of $\phi(BX_1)$ and $\phi(PY_1)$ can be taken independently as either + or —. The choices of $\phi(BX_1)$, — or +, respectively, give the choices of sign, + or —, respectively, for ϕ' as listed.

Alternative	Transition	Pole (reaction center symmetry axis in gelatin films [9])		Pole (axis normal to the membrane plane (present study))	
		θ	ϕ	θ'	ϕ'
$\phi(PY_1)$ positive, $215 \pm 10^\circ$	PY_1 (760)	62 ± 3	215 ± 10	85 ± 9	$\pm 275 \pm 4$
	PY_2 (760)	58 ± 3	155 ± 10	36 ± 8	$\pm 255 \pm 12$
	PX_1 (545)	46 ± 1	93 ± 6	48 ± 4	$\pm 180 \pm 5$
	PX_2 (530)	46 ± 1	285 ± 5	54 ± 2	$\pm 53 \pm 4$
$\phi(PY_1)$ negative, $-215 \pm 10 = 145 \pm 10$	PY_1 (760)	62 ± 3	145 ± 10	28 ± 9	$\pm 250 \pm 15$
	PY_2 (760)	58 ± 3	205 ± 10	77 ± 9	$\pm 272 \pm 4$
	PX_1 (545)	46 ± 1	267 ± 6	62 ± 4	$\pm 65 \pm 4$
	PX_2 (530)	46 ± 1	75 ± 5	59 ± 4	$\pm 172 \pm 3$

(760 nm) and PX_2 (530 nm) for the other. Values of θ and ϕ for these transition moments, when transformed by Eqns. 11 and 12, given the θ' and ϕ' coordinates listed in Table VI. The multiplicity of solutions arises because of two independent 'mirror image' choices [9]: The sign of $\phi(BX_1)$ can be taken arbitrarily as either + or —, and independently the sign of $\phi(PY_1)$ can be taken as either + or —.

Discussion

The major results of this study are shown pictorially in Fig. 4. The solid and dashed vectors representing the BX_2 transition show the different orientations of BX_2 that arise when $\gamma(BY_1, BX_2)$ is taken as 75.5 or 104.5°. Alternative orientations of the four bacteriopheophytin transitions are also represented by solid and dashed vectors; the choice is between either the set of solid vectors or the set of dashed vectors. An equally acceptable picture for the complete diagram is a mirror image in which ϕ' has been changed to $-\phi'$ for all the transition moments and the reaction center symmetry axis in stretched gelatin films.

The coordinates of the transition moments depicted in Fig. 4 were calculated under the assumption that chromatophore membranes of *Rp. sphaeroides* were ideally oriented ($f = 1$) in the linear dichroism experiments. The only experimental evidence which supports this assumption is the constancy of the experimental dichroic ratios when different amounts of chromatophore membranes are deposited and dried on the slides. However, this result strongly implies that the planes of the membrane fragments are quite uniformly aligned with the surfaces of the slides. For this reason we prefer the set of coordinates calculated for the ideal case, $f = 1$.

Eqn. 5 relates the experimental dichroic ratio to the angle made by a transition moment to the membrane plane. We have discussed the effect of imperfect membrane alignment with the glass slide. An explicit assumption in the derivation of Eqn. 5 is that the angle made by a particular transition moment to the

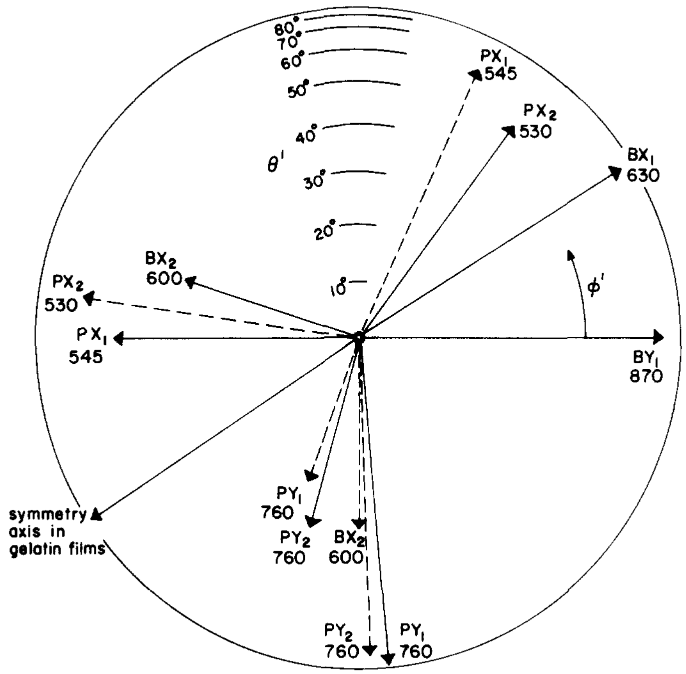

Fig. 4. Polar view of the orientations of optical transition moments of *Rp. sphaeroides*, in a spherical coordinate system with the pole set normal to the chromatophore membrane plane. The transition moments are identified in the text. These orientations were calculated assuming that chromatophore membranes were perfectly oriented ($f = 1.00$) in the measurements of polarized absorption (see text). Alternative orientations of the transition moments are indicated by solid and dashed vectors. For either of the two alternative orientations of the BX_2 transitions, the two alternative sets of orientations for the PY_1, PY_2, PX_1, PX_2 transitions are allowed (see Table VI). Beyond that, an equally acceptable picture would be a mirror image with $-\phi'$ in place of ϕ' for all the transition moments. Numerical values of θ' and ϕ' are given in Tables V and VI.

membrane plane is the same for each reaction center or antenna pigment in the membrane. A statistical distribution of transition moment orientation relative to the membrane plane is equivalent to a decrease in order intrinsic to the membrane structure. It can be shown that such a decrease in order can also be approximated by decreasing the value of the order parameter f. The mean value of the angle that a particular transition makes to the membrane plane is then restricted to the range predicted by Eqn. 5 for $f = 1.00$ and 0.72. Values of α_p for these two cases are listed in Table I. We have no experimental evidence which pertains to possible distributions of the orientations of transition moments in the chromatophore membrane.

The most conservative limits for the coordinates of the BY_1, BX_1, and BX_2 transition moments are given by the values listed in Table IV for the two extreme cases $f = 1$ and $f = 0.72$. The inclination of the reaction center symmetry axis to the membrane plane is then restricted to the range 35—0°. We do not list the coordinates of the bacteriopheophytin transitions for f less than 1 because of the abundance of alternative orientations.

The Q_y transition moments of the antenna bacteriochlorophyll and the BY_1 moment of the bacteriochlorophyll special pair are approximately parallel to the chromatophore membrane whereas the Q_x moments of the antenna bacteriochlorophyll and the BX_2 moment of the special pair are both oriented at 55—63° to the membrane plane. This arrangement could increase the efficiency of energy transfer from the antenna bacteriochlorophyll to the reaction center.

In previous reports [7,9], we have stressed the tentative nature of the assignment of the light-induced negative bands at 870, 810, 630, and 600 nm to disruption of exciton coupling in the bacteriochlorophyll special pair upon photooxidation. Additional interactions between the bacteriochlorophyll special pair and other chromophores in the reaction centers are very likely. The pertinent question is: can the appearance of negative bands in the difference spectrum be adequately explained by a dimer model or must additional interactions be invoked? New evidence is provided by this study that the dimer model is adequate. Exciton theory [19—21] predicts that the maximum number of exciton split bands associated with a single transition moment is equal to the number of monomers in the complex. In the case of a dimer this number is 2. The number of exciton split bands may be reduced depending on the symmetry of the complex. For reaction centers of *Rp. sphaeroides*, only two light-induced negative bands are detected in the Q_y transition region and only two in the Q_x region. Since it is unlikely that identical symmetry restrictions (which might, for example, reduce three bands to only two resolved bands) apply to both the Q_y and Q_x transition, this evidence favors a dimer model. The theory for a dimer also predicts that the components of an exciton split band are polarized orthogonally. The photoselection measurements explicitly verify this prediction for the negative bands at 630 and 600 nm. The relative polarization of the negative bands at 870 and 810 nm is less clear for reasons discussed elsewhere [5—9] although the available evidence is consistent with orthogonal polarization. We recognize that more complicated interpretations, invoking strong excitonic interactions between all four bacteriochlorophylls and perhaps some bacteriochlorophyll-bacteriopheophytin interactions in the Q_y region, should be entertained. We prefer to pursue the 'dimeric special pair' model as long as it remains compatible with the data, for its simplicity and its heuristic value.

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