Biochimica et Biophysica Acta, 449 (1976) 500–515

BBA 47212

ORIENTATION OF CHROMOPHORES IN REACTION CENTERS OF *RHODOPSEUDOMONAS SPHAEROIDES*

EVIDENCE FOR TWO ABSORPTION BANDS OF THE DIMERIC PRIMARY ELECTRON DONOR

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(Received May 14th, 1976)

SUMMARY

Chromatophores from *Rhodopseudomonas sphaeroides* were oriented by allowing aqueous suspensions to dry on glass plates. Orientation of reaction center pigments was investigated by studying the linear dichroism of chromatophores in which the absorption by antenna bacteriochlorophyll had been attenuated through selective oxidation. Alternatively the light-induced absorbance changes, in the ranges 550–650 and 700–950 nm, were studied in untreated chromatophores. The long wave transition moment of reaction center bacteriochlorophyll (*P*-870) was found to be nearly parallel to the plane of the membrane, whereas the long wave transition moments of bacteriopheophytin are polarized out of this plane. For light-induced changes the linear dichroic ratios, defined as $\Delta a_v/\Delta a_h$, are nearly the same for untreated and for oxidized chromatophores. Typical values are 1.60 at 870 nm, 0.80 at 810 nm, 1.20 at 790 nm, 0.70 at 765 nm, 0.30 at 745 nm, and 0.50 at 600 nm. The different values for the absorbance decrease at 810 nm (0.80) and the increase at 790 nm (1.20) are incompatible with the hypothesis that these changes are due to the blue-shift of a single band. We propose that the decreases at 870 and 810 nm reflect bleaching of the two components of a bacteriochlorophyll dimer, the "special pair" that shares in the photochemical donation of a single electron. The increase at 790 nm then represents the appearance of a monomer band in place of the dimer spectrum, as a result of electron donation. This hypothesis is consistent with available data on circular dichroism. It is confirmed by the presence of a shoulder at 810 nm in the absorption spectrum of reaction centers at low temperature; this band disappears upon photooxidation of the reaction centers. For the changes near 760 nm, associated with bacteriopheophytin, the polarization and the shape of the "light-dark" difference spectrum (identical to the first derivative of the absorption spectrum) show that the 760 nm band undergoes a light-induced shift to greater wavelengths.

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INTRODUCTION

The primary photochemical electron transfer in bacterial photosynthesis is effected in reaction centers: complexes that contain four molecules of bacteriochlorophyll and two of bacteriopheophytin bound to characteristic polypeptides [1, 2]. These entities are components of the photosynthetic (chromatophore) membrane. Strong interactions between the bacteriochlorophyll molecules in a reaction center are suggested by circular dichroism studies [3, 4]. Also, when the bacteriochlorophyll has given up an electron through photooxidation, the unpaired electron is shared by two of the four bacteriochlorophyll molecules, as shown by electron spin resonance [5] and electron-nuclear double resonance [6] data. This dimeric structure of the primary electron donor is seen also in reaction centers of higher plants [7, 8] and algae; it may be a necessary characteristic of the photochemical systems of photosynthesis. The dimeric bacteriochlorophyll complex, coupled with a bacteriopheophytin molecule, is apparently involved [9] in the transient light-induced state P^F, discovered by Parson et al. [10] and implicated as an intermediate in the photochemistry [11, 12].

New information concerning the orientations of pigments in photosynthetic membranes has come from measurements with polarized light: in measurements of linear dichroism [13–15], of the polarization of fluorescence [16] and in photoselection experiments [17]. By applying such techniques to reaction centers one can hopefully reveal the relative orientations of the six chromophores. In linear dichroism studies it is necessary to achieve an anisotropic sample by orienting a large number of organelles. Several different techniques have been used to orient photosynthetic membranes: flow gradient, [14, 18] mechanical spreading, [16, 19] air-drying, [13, 14, 19] sedimentation, [20] and magnetic field [15, 19, 21]. Most of these techniques are based on the relatively large size of the organelle, and have therefore succeeded with chromatophores of several species of photosynthetic bacteria [14, 22]. On the other hand, Penna et al. [23] attempting to orient purified reaction center particles by the spreading method of Breton [19], detected only slight linear dichroism. Their results indicated either that the chromophores are randomly oriented in the reaction centers, or, more likely, that poor orientation was achieved because of the small sizes of the reaction center particles.

We report here an investigation of linear dichroism in chromatophores that have been oriented by drying onto glass plates. We have studied the orientations of reaction center components in two ways. First, we examined the polarization of light-induced absorbance changes, associated with the reaction center pigments, in purified chromatophores. Second, we treated chromatophores with the oxidant potassium chloroiridate so as to bleach the antenna pigments selectively and irreversibly, giving chromatophores with the absorption spectrum of reaction centers [24, 25]. Dichroic absorption by the reaction center pigments could be studied directly in such material. Our measurements showed a high degree of orientation for the reaction center components with respect to the membrane.

Our study of the linear dichroism of light-induced changes also gave new insights into the interpretation of these changes. We shall propose that the changes in the near infrared are not due simply to a bleaching of the 865 nm absorption band and a blue-shift of the 803 nm band. Rather, they are due to bleaching of both components of the electron-donating dimer, around 865 and 810 nm, and the appearance of a

monomeric bacteriochlorophyll band near 790 nm, a property of the singly oxidized dimer. The changes near 760 nm associated with bacteriopheophytin arise simply from a bathychromic shift of this band. These interpretations are confirmed by analysis of the first derivative of the absorption spectrum and of the light-induced absorbance changes in purified reaction centers at low temperatures.

MATERIALS AND METHODS

Biological materials. Rhodopseudomonas sphaeroides, wild type strain 2.4.1 and carotenoidless mutant strain R-26, was grown as described earlier [26]. The methods of preparing purified chromatophores [27] and reaction centers [28] have also been described. Purified chromatophores, suspended in 0.01 M Tris · HCl, pH 7.5, were treated with K_2IrCl_6 (listed as iridic potassium chloride by K and K Chemicals, Plainview, N.Y.) as described earlier [24], so as to bleach the absorption bands of antenna bacteriochlorophyll completely while causing relatively little damage to the reaction center chromophores. We found that these oxidized chromatophores have a tendency to aggregate, yielding scattering suspensions. Finer particles could be separated from coarser ones by differential centrifugation and/or fractionation on a sucrose gradient after sonication.

Absorption measurements. Absorption spectra were measured with a Cary 14R Spectrophotometer. For polarization measurements we placed a Glan-Thompson polarizing crystal in both the reference and the sample beam; the beam passed through the crystal before the sample. Side illumination of the sample was provided with a tungsten-iodine lamp (Sylvania Sun Gun, 650 W); the beam from this lamp was passed through 1 inch of water and concentrated onto the sample cuvette. Complementary color filters prevented scattered exciting light from reaching the detector.

Signals from the Cary 14R, taken from the outputs of relays C and I, were sent through a home-made differential amplifier for smoothing and linear-log conversion. The voltage output of this amplifier was proportional to the absorbance of the sample within the range 0–0.3 absorbance; the system was non-linear at higher absorbances. We took care to stay within the linear range. The output of this amplifier was received by a Tracor-Northern TN-1500 Signal Averager. Spectra could thus be stored and manipulated: multiplied by any factor and then added or subtracted, differentiated, etc. Data acquisition by the averager was triggered by a photodiode coupled to the wavelength dial of the Cary 14R. In processing the spectra, baselines were subtracted separately for horizontal and vertical polarizations, since the two baselines were slightly different. We usually recorded and averaged several such spectra to improve the signal/noise ratio. In doing so we alternated light and dark, horizontal and vertical polarization, etc., to check any progressive changes in the sample. For low temperature spectra we used a cryogenic helium refrigerator from Cryogenic Technology Inc., Waltham, Mass., U.S.A.

Kinetic experiments were made with a split-beam absorption spectrometer described earlier [29], with a polarizing crystal placed in the measuring path before the sample. The intensity of the measuring beam received by the chromotophores was kept low enough, and equal for both polarizations to avoid any artifacts due to excitation by this beam.

Orientation of chromatophores and control experiments. Chromatophores

suspended in distilled water were oriented by air drying onto microscope slides [13, 14]. In this method the chromatophores are imagined to lie down flat on the slide, so that the photosynthetic membranes are mainly parallel to the slide, but with random angular orientation in this plane. In our experiments the measuring beam was horizontal and the slide was in a vertical plane. To detect any orientation of chromophores with respect to the membrane, one must tilt the slide in the measuring beam. With the slide normal to the measuring beam, both horizontal and vertical polarizations lie in the plane of the membrane. With the slide tilted about a vertical axis, the smaller the angle between the plane of the slide and the axis of the measuring beam, the larger the difference to be expected between absorption measurement with vertically and horizontally polarized measuring light. We tested the relationship $\Delta a = (a_v - a_h) =$ const. $(\sin^2\theta/\cos\theta)$ where Δa is the linear dichroism value and θ is the angle between the axis of the measuring beam and the normal to the slide [14, 19]. We found that this relationship held for $\theta \leqslant 45$ °C, but not at higher values of θ. Nevertheless we used $\theta = 60$ ° and took into account the departure from linearity when estimating the orientation of chromophores.

To check for trivial artifacts arising from the optical arrangement we made measurements in two situations where linear dichroism should be absent: first with oriented chromatophores on a slide perpendicular to the measuring beam; second, with purified reaction centers dried, apparently with random orientations, onto an slide and placed with 30° between the slide and the measuring beam. In both cases, no differences could be detected between horizontal and vertical polarization, for absolute absorption measurements and for light-induced changes.

RESULTS

Linear dichroism of reaction center chromophores in chloroiridate-treated chromatophores.

Fig. 1 shows absorption spectra of a dry film of K_2IrCl_6-treated chromatophores from *Rps. sphaeroides* strain R-26, for both horizontal and vertical polarizations of the measuring beam. The slide was tilted about a vertical axis to make an angle of 30° between the plane of the slide and the axis of the measuring beam; thus for vertical polarization the electric vector was in the plane of the slide, and for horizontal polarization this vector was predominantly perpendicular to the slide, making a 60° angle. Baselines for blank slides had been subtracted in obtaining these spectra; the dashed lines show an additional estimated baseline correction, necessitated by the strongly scattering nature of the K_2IrCl_6-treated chromatophores. All the absorption bands are representative of those in reaction centers, except the band at 690 nm, which is due to oxidized bacteriochlorophyll. The difference between the two spectra of Fig. 1 is plotted in Fig. 2; this shows the strong polarization in all the long wave absorption bands. We emphasize that if the slide was perpendicular to the measuring beam, the two polarizations gave identical spectra. The linear dichroism spectrum of Fig. 2 is very similar to the one reported by Penna et al. [23], although the latter was made with purified reaction centers and with a more sophisticated detection system.

Dichroic ratios, a_v/a_h, are listed in Table I for several wavelengths. These represent averages obtained with fifteen different slides of K_2IrCl_6-treated chromatophores, made from four different chromatophores preparations.

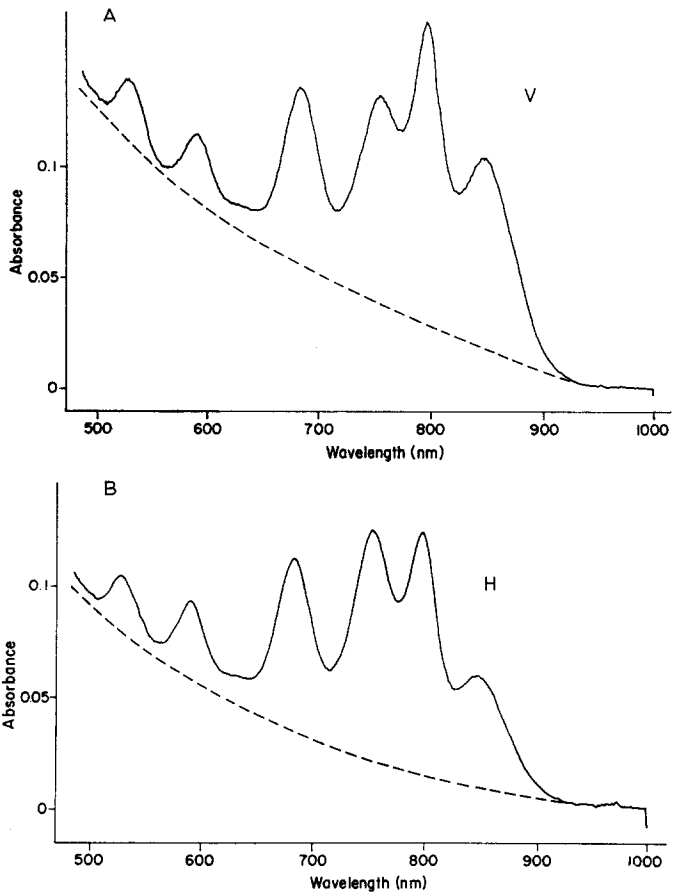

Fig. 1. Absorption spectra of a dried film of chloroiridate-treated chromatophores from *Rps. sphaeroides*, for vertical (A) and horizontal (B) polarization of the measuring beam. The dashed lines are base lines to correct for scattering, estimated from absorption spectra of purified reaction center.

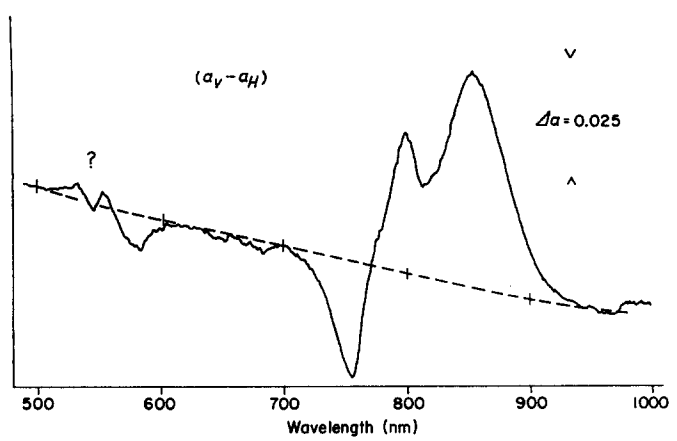

Fig. 2. Linear dichroism spectrum, $a_v - a_h$, derived from the spectra of Fig. 1. The question mark indicates details that were reproducible but of doubtful significance.

TABLE I

LINEAR DICHROIC RATIOS, a_v/a_h, FOR DRIED FILMS OF CHLOROIRIDATE-TREATED CHROMATOPHORES OF *RPS. SPHAEROIDES*

Values of a_v and a_h were taken from spectra like those of Fig. 1 after subtraction of the estimated "scattering" baseline. Each value is an average of 15 measurements with different films. Confidence limits are based on rough estimates of the precision of measurements.

Wavelength (nm)	a_v/a_h
530	1.05±0.05
600	0.95±0.05
760	0.80±0.05
800	1.15±0.05
870	1.50±0.05

Linear dichroism of light-induced changes in chloroiridate-treated chromatophores

Further information could be obtained from the linear dichroism of light-induced absorbance changes. Using side-illumination with blue light (Corning 4-96 filter) in the Cary 14R Spectrophotometer, we recorded "light minus dark" spectra for both horizontal and vertical polarizations of the measuring beam. The material and the optical arrangement were the same as in the preceding section. Fig. 3 shows the resulting difference spectra, light minus dark, for the two polarizations. Both difference spectra show the changes typical of reaction centers [30] or chromatophores [24]: absorbance decreases centered near 860, 812 and 745 nm, and increases centered near 785 and 770 nm. The relative amplitudes of these changes depend on the polarization

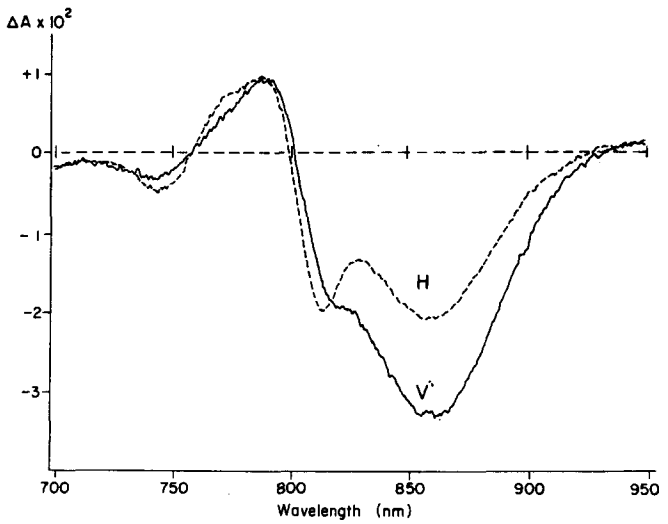

Fig. 3. Light minus dark absorption difference spectra of a dried film of chloroiridate-treated chromatophores. Solid curve, vertical polarization of the measuring beam; dashed curve, horizontal polarization. The intensity of the exciting light was such that the photochemistry was half-saturated in the steady state.

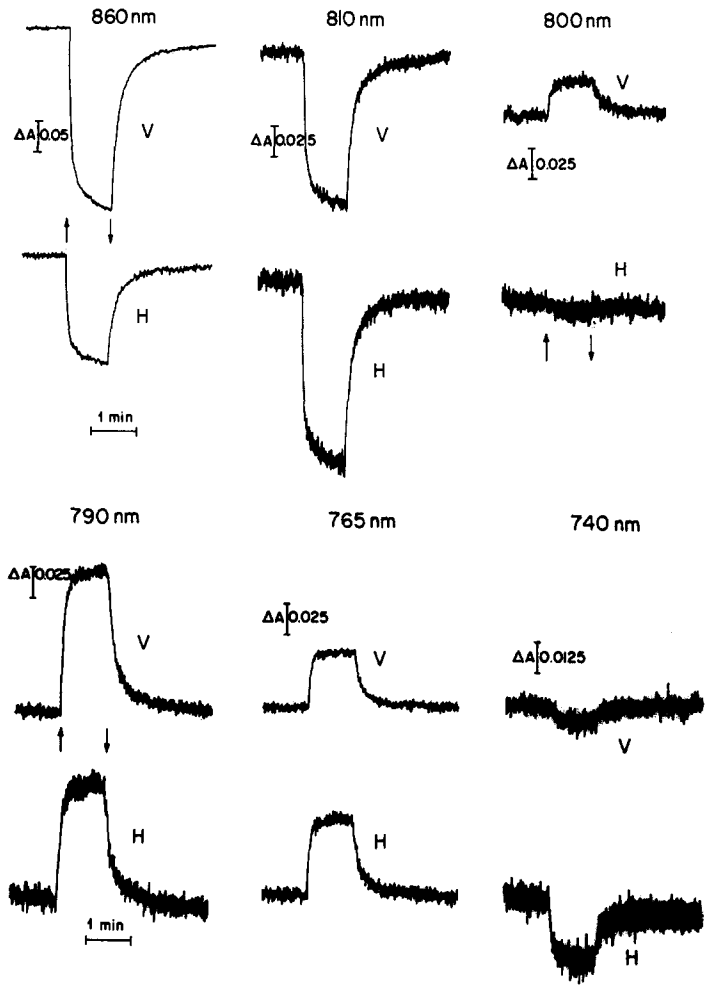

Fig. 4. Kinetics of light-induced absorbance changes in a dried film of chloroiridate-treated chromatophores, recorded with a split beam spectrophotometer at selected wavelengths for both horizontal (H) and vertical (V) polarizations of the measuring beam. The changes correspond to 50% saturation. The arrows (up and down) indicate exciting light on and off, respectively.

of the measuring beam; the amplitudes at 860 and (marginally) at 785 nm are larger for vertical than for horizontal polarization, and the inverse for 812, 770 and 745 nm. Also the null near 800 nm is at slightly different wavelengths for the two polarizations. Similar results were obtained when measuring the light-induced changes at selected wavelengths with the split beam spectrometer, as shown in Fig. 4. These measurements also showed that the changes have the same kinetics regardless of the polarization. Measurements near 600 and 1250 nm also showed strong dichroic effects, shown in Figs. 5 and 6.

These data for light-induced absorbance changes are summarized by the dichroic ratios listed in Table II, along with values obtained with untreated chromatophores as described in the next section.

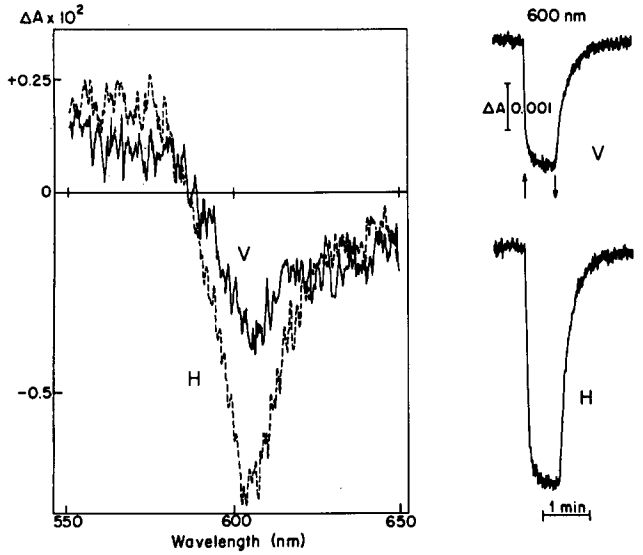

Fig. 5. Same material and conditions as in Figs. 3 (difference spectra) and 4 (kinetic traces).

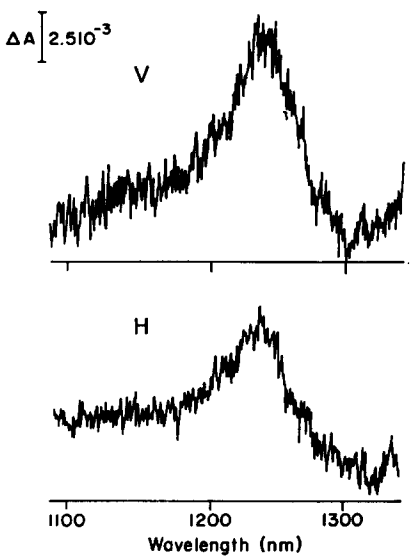

Fig. 6. Same material and conditions as in Fig. 3.

TABLE II

LINEAR DICHROIC RATIOS, $\Delta a_v/\Delta a_h$, FOR LIGHT-INDUCED CHANGES MEASURED IN CHLOROIRIDATE-TREATED AND UNTREATED CHROMATOPHORES

As described in the text and depicted in Figs. 3–6 and 9.

Wavelength (nm)	Dichroic ratio, $\Delta a_v/\Delta a_h$	
	Chloroiridate-treated chromatophores	Untreated chromatophores
600	0.50±0.02	0.42±0.05
745	0.31±0.04	
765	0.74±0.02	0.70±0.05
790	1.17±0.02	1.21±0.05
810	0.82±0.02	0.71±0.05
860	1.60±0.02	1.50±0.05
1250	1.50±0.10	

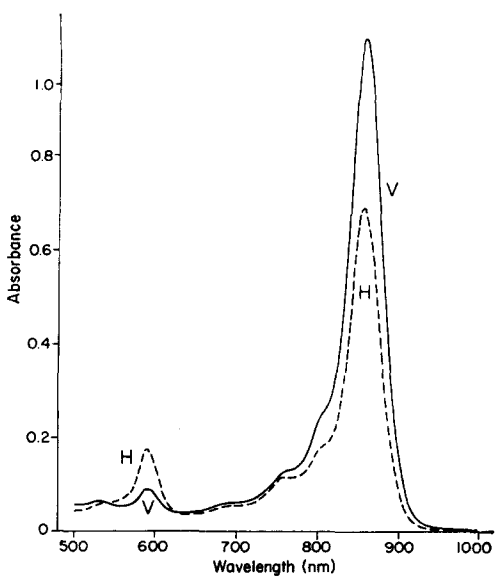

Fig. 7. Absorption spectra of a dried film of purified chromatophores from *Rps. sphaeroides* strain R-26, for vertical (V) and horizontal (H) polarizations of the measuring beam. The plane of the slide made an angle of 30° with the measuring beam. A base-line correction has not been applied in this figure. After correction the dashed curve (H) lies above the solid curve (V) at 760 nm as implied in Fig. 8.

Linear dichroism of light-induced changes in untreated chromatophores

We repeated the foregoing experiments with untreated purified chromatophores from *Rps. sphaeroides* R-26, to see whether the K_2IrCl_6 treatment had altered appreciably the orientation of the reaction center pigments with respect to the chromatophore membrane.

Absolute absorption spectra for both polarizations, and the linear dichroism spectrum, are plotted in Figs. 7 and 8; these results agree very well with those obtained by Breton [22] using a different optical system. To Breton's discussion we need only

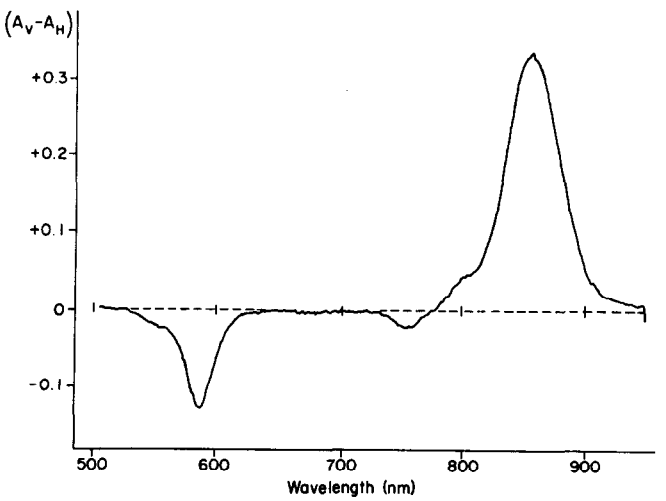

Fig. 8. Linear dichroism spectrum, $a_v - a_h$, derived from the spectra of Fig. 7.

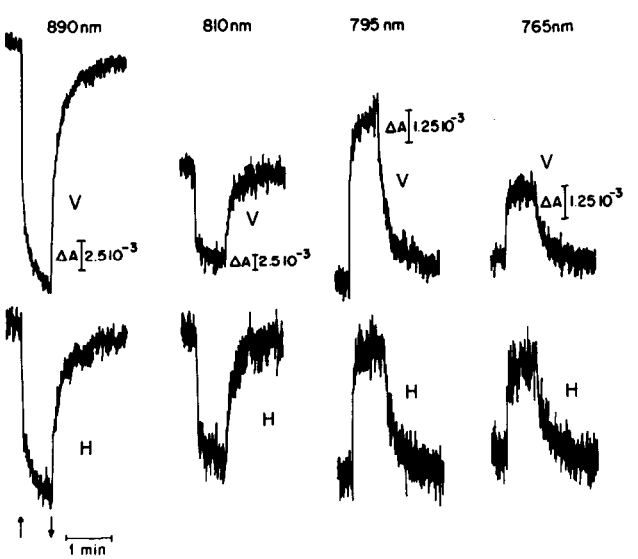

Fig. 9. Same as Fig. 4, but for a dried film of untreated purified chromatophores from *Rps. sphaeroides*. The changes correspond to 40 % saturation.

add that the negative value of $a_v - a_h$ around 760 nm is attributable to the bacteriopheophytin of the reaction center.

Light-induced changes measured with dry films of untreated chromatophores are shown in Fig. 9; in these measurements the exciting light was strong enough to cause 40 % of the maximum possible change in the steady state. These changes showed dichroic ratios similar to those observed in K_2IrCl_6-treated chromatophores, as listed in Table II.

We could also compare the orientations of the antenna pigment and reaction center pigment, on the same sample. Typical dichroic ratios for the antenna pigment are 1.40 ± 0.03 at the long wave absorption maximum and 0.40 ± 0.02 at 590 nm. For the light-induced bleaching at 870 and 600 nm, the values are 1.44 ± 0.05 and 0.42 ± 0.05, respectively. The difference between these values for antenna and reaction center pigments are probably not significant within the precision of our measurements.

With wild type *Rps. sphaeroides* chromatophores we confirmed Breton's observations [22] that the antenna components *B*-850 and *B*-800 are oriented in the membrane plane and that the carotenoids are tilted out of the plane. In a few confirmatory measurements we found, for light-induced change in dried chromatophore films the same wavelength dependence of dichroic ratios with wild type *Rps. sphaeroides* as with K_2IrCl_6-treated or untreated strain R-26.

DISCUSSION

In general we observed that absorption and linear dichroism bands have the same shape and the same maxima, with both positive and negative bands appearing in the linear dichroism spectra. We assume therefore that artifacts such as those arising from textural dichroism were negligible, and that the dichroic effects we observed are due to orientation of the chromophores in the chromatophore membrane.

The oxidation of antenna pigments by K_2IrCl_6 did not appear to change the orientation of the reaction center pigments with respect to the membrane. We conclude this because the dichroic ratios for light-induced absorbance changes were the same, at each wavelength tested, in K_2IrCl_6-treated as in untreated chromatophores. We also found the same negative values of linear dichroism for absorption by the bacteriopheophytin of the reaction center (around 760 nm) in both treated and untreated chromatophores. Consequently we shall analyze only the results obtained with treated chromatophores, which provided better signal/noise ratios.

The linear dichroism spectrum shown in Fig. 2 for reaction center pigments is in excellent agreement with that reported by Penna et al. [23]. The reaction center particles that they used probably gave very little scattered light. We suppose therefore that our measurements were not affected by the high degree of scattering by the K_2IrCl_6-treated chromatophores. We observed dichroic effects ($a_v - a_h$) about 100 times greater than did Penna et al. [23], presumably because the orientation with chromatophores was much better than that with the smaller reaction center particles. Also in our measurements the orientation of the reaction center pigments could be related sensibly to the plane of the chromatophore membrane. Of course, we lack direct information about the orientation of the chromatophores. We assume that they tend to lie flat on the slide when dried, but we do not know the exact degree of this

orientation. Nevertheless we can estimate [14, 19] that the long wave (865 nm) transition moment of bacteriochlorophyll in the reaction centers makes an angle of less than 20° with the plane of the membrane, and that the transition moment(s) of bacteriopheophytin at 760 nm is tilted out of the membrane plane at more than 45°. The orientations of the transitions near the 800 nm absorption band of bacteriochlorophyll are harder to determine. Penna et al. [23] have already noticed a small negative band centered at 811 nm in their linear dichroism spectrum of reaction center particles. At least two transition moments having opposite contributions in the linear dichroism spectrum make up the absorption around this wavelength, leaving the analysis difficult.

The most interesting and unexpected finding was that the polarization was of opposite sign for the absorption increase at 790 nm ($\Delta a_v - \Delta a_h > 0$) and for the bleaching at 810 nm ($\Delta a_v - \Delta a_h < 0$); see Figs. 3 and 4. This result is not consistent with the hypothesis, generally assumed, of a blue shift of the 803 nm band. For the shift of a single band we expect the same dichroic value for both limbs of the resultant difference spectrum. We therefore propose an alternative hypothesis: the absorption decreases at 870 and 810 nm are due to the bleaching of the two bands in the dimer spectrum of the "special pair" bacteriochlorophyll that acts as primary electron donor. The increase at 790 nm corresponds to the appearance of a monomeric bacteriochlorophyll band, a property of the singly oxidized dimer $(Bchl)_2^+$. This hypothesis accounts fully for our observations of dichroism in the light-induced absorbance changes; we need only assume that the two transition moments of the dimer are not parallel.

One can find further support for this hypothesis from other experiments and theory. The view that two molecules of bacteriochlorophyll in the reaction center share in the photochemical donation of a single electron arose from electron spin resonance [5] and electron-nuclear double resonance [6] data indicating that the primary oxidized entity is a dimeric cation radical, $(Bchl)_2^+$. For the neutral dimer $(BChl)_2$, the molecular excition model of Kasha [31] predicts that the splitting of excited state energy levels will produce two absorption bands in place of one for the monomer. The difference in energy between the two new levels depends on the geometrical relationships between the two molecules of the dimer [31]. The theory of circular dichroism spectra also predicts [32] that in the case of strong interactions and nondegenerate transitions, the number of resolved components of both positive and negative sign will be less than or equal to the number of interacting molecules, in this case two. Light minus dark or oxidized minus reduced difference spectra of circular dichroism reported by Sauer et al. [3] and by Reed and Ke [4] show just two bands of opposite sign, centered respectively at 865 and 811 nm, in reaction centers from *Rps. sphaeroides*. This is exactly what we expect if the two absorption bands at 865 and 811 nm represent the two transition moment of a dimeric complex, and if the exciton interaction disappears along with the long wave transitions as a result of oxidation [33].

Our hypothesis is also supported by Feher's observation [34] of a peak at 810 nm in the second derivative absorption spectrum of reduced reaction centers at 77 K. This peak is absent in oxidized reaction centers [34]. We confirm this finding, but we find that the clear presence of a resolved band at 810 nm depends on the way in which the reaction centers have been prepared and brought to low temperature.

Perhaps the band is sometimes at a slightly shorter wavelength and is not resolved from the larger absorption band centered at 803 nm. In Fig. 10 (upper trace) we show the absorption spectrum of reaction centers prepared by column chromatography on diethyl aminoethyl ceullulose, dried onto a slide, and recorded in the dark at 35 K. A shoulder can be discerned near 810 nm. This 810 nm component can be seen more clearly in the first derivative spectrum; it disappears upon illumination of the reaction centers (Fig. 10, lower traces).

We observed that at 35 K as well as at room temperature, the linear dichroism spectrum in the region of the long wave band had exactly the same shape as the absolute absorption spectrum and as the spectrum of light-induced absorbance changes. These results are in contradiction to those of Penna et al. [23] who found two bands at 870 and 880 nm in the linear dichroism spectrum of reaction center particles at 77 K. Reed and Ke [4] also observed fine structure in the 880 nm region of the circular dichroism spectrum of *Rps. sphaeroides* reactions centers at 77 K. We propose that the fine structure was an artifact due to sharp structure in the emission spectrum of the xenon arc lamp used by both groups. We therefore believe that the 865 nm band represents a single component.

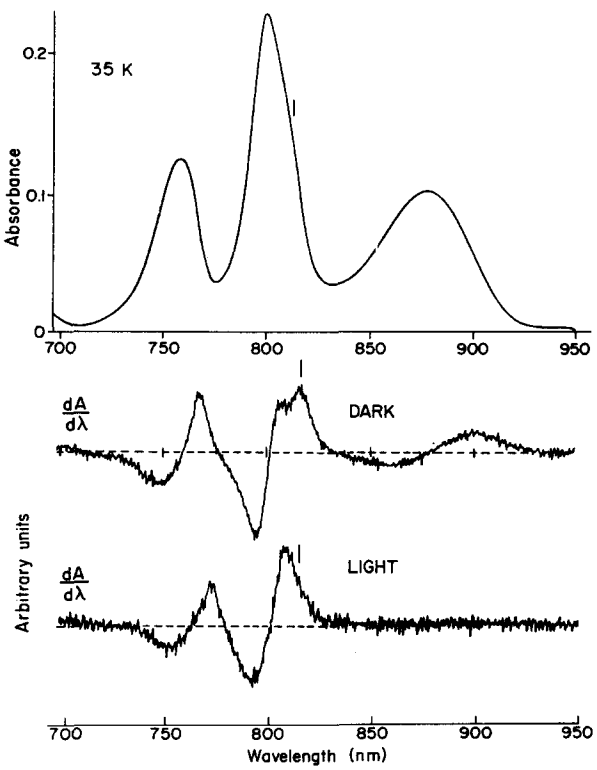

Fig. 10. Upper trace: absorption spectrum of a dried film of reaction centers from *Rps. sphaeroides* purified by chromatography on diethylaminoethyl cellulose. Middle trace: first derivative of the absorption spectrum of the upper trace, recorded with weak measuring light. Lower trace: first derivative absorption spectrum recorded under strong illumination, sufficient for more than 80% saturation of the photochemistry. The vertical bars indicate the 810 nm component. Temperature 35 K.

All the foregoing data and arguments are consistent with our hypothesis that the absorbance changes around 800 nm are due not to the blue-shift of a band, but to the bleaching of a dimer component at 810 nm and the appearance of a new band, assigned to $(BChl)_2^+$, at 790 nm. In contrast, the changes centered around 760 nm do seem to be due to a bathychromic shift of the 760 nm band of bacteriopheophytin. First, we find the same polarization for these light-induced changes as for the absolute absorption near 760 nm ($a_v < a_h$). Second, in the light-induced changes the dichroism is of the same sign and magnitude for the increase at 765 nm as for the decrease at 745 nm (Figs. 3 and 4), if we take into account the fact that part of the change at 765 nm is associated not with the 760 nm band but with changes due to bacteriochlorophyll, centered at greater wavelengths. Finally at low temperature, with the 760 nm absorption band well resolved, it is clear that the light minus dark difference spectrum has the same shape as the first derivative absorption spectrum around this band (Fig. 11).

We found nearly the same values for the linear dichroic ratios at 1250 nm and 870 nm (Figs. 3 and 6; Table II). This agrees with a molecular orbital calculation [35] which shows, at least for a monomer, that the long wave transition moment in the cation radical (1250 nm) will be polarized in the same direction as that of the parent molecule. The same results have been obtained in spinach chloroplasts [36, 37] for the long wave absorption band of *P*-700 at 703 nm and for the absorption band of its cation radical around 820 nm.

The 600 nm band and the long wave band of monomeric bacteriochlorophyll correspond to Q_x and Q_y transition moments, respectively; these are mutually perpendicular and in the tetraphyrole plane. From the high degree of polarization of the

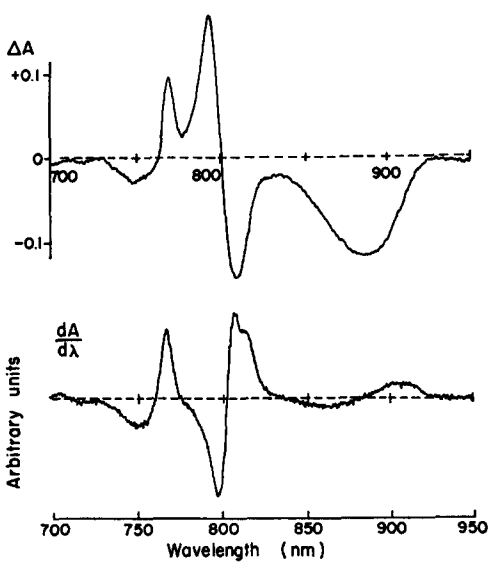

Fig. 11. Upper trace: light minus dark absorption difference spectrum of reaction centers from *Rps. sphaeroides* in 80 % glycerol: 20 % 0.01 M Tris · Cl buffer, pH 7.6, with 0.3 % lauryl dimethylamine oxide. Lower trace: first derivative of the absolute absorption spectrum measured in weak light. Temperature 35 K.

light-induced bleaching at 600 nm ($\Delta a_v/\Delta a_h = 0.5$), we can speculate on the orientation of the electron donating bacteriochlorophyll dimer with respect to the membrane. If we suppose that the two molecules forming the dimer are nearly coplanar* with the resultant Q_x and Q_y moments in the plane of the dimer, then the dimer is nearly perpendicular to the plane of the membrane. This follows because the Q_y moment for the 865 membrane is approximately in the plane of the membrane, while the Q_x moment (600 nm) is approximately perpendicular to this plane. The same conclusion was drawn by Breton [37] for the orientation of the electron donating dimer (*P*-700) of green plant Photosystem I.

CONCLUSIONS

Our studies of linear dichroism in reaction center pigments have led us to reinterpret the light-induced absorbance changes in the near infrared. We proposed that the decreases centered at 865 and 810 nm are due to bleaching of the two bands of a bacteriochlorophyll dimer upon photooxidation. This dimer acts as the primary electron donor. The absorbance increase at 790 nm reflects the appearance of the cation radical $(BChl)_2^+$, with some spectral properties of monomeric bacteriochlorophyll.

We confirm that the 760 nm band of bacteriopheophytin undergoes a bathychromic shift upon illumination of reaction centers.

Linear dichroic ratios for the light-induced changes at 870 nm ($\Delta a_v/\Delta a_h = 1.60$) and at 600 nm ($\Delta a_v/\Delta a_h = 0.50$) suggest that the bacteriochlorophyll dimer is nearly perpendicular to the plane of the membrane. The Q_y transition moments of bacteriopheophytin are also tilted out of the plane. The other two bacteriochlorophylls, not directly involved in the electron donation, are less clearly oriented.

ACKNOWLEDGEMENTS

Andre Vermeglio wishes to thank Dr. J. Breton for many helpful suggestions, discussions and for recording some linear dichroism spectra. This project was supported by Contract No. AT (11-1)-3162 with the U.S. Energy Research and Development Administration and by Grant No. BMS 74-07701 from the National Science Foundation.

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* This is the case in the model Katz and Norris have proposed for the primary electron donor (*P*-870) [38].

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