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ORIENTATION OF CHROMOPHORES IN REACTION CENTERS OF *RHODOPSEUDOMONAS SPHAEROIDES*: A PHOTOSELECTION STUDY

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Summary

The relative orientation of the pigments of reaction centers from *Rhodopseudomonas sphaeroides* has been studied by the photoselection technique.

A high value (+0.45) of $p = (\Delta A_V - \Delta A_H)/(\Delta A_V + \Delta A_H)$ is obtained when exciting and observing within the 870 nm band which is contradictory to the results of Mar and Gingras (Mar, T. and Gingras, G. (1976) Biochim. Biophys. Acta 440, 609—621) and Shuvalov et al. (Shuvalov, V.A., Asadov, A.A. and Krakhmaleva, I.N. (1977) FEBS Lett. 76, 240—245). It is shown that the low values of p obtained by both groups were erroneous due to excitation conditions.

Analysis of the polarization of light-induced changes when exciting with polarized light in single transitions (spheroiden band and bacteriopheophytin Q_x bands) enable us to propose a possible arrangement of the pigments within the reaction center. It is concluded that the 870 nm band corresponds to a single transition and is one of the two bands of the primary electron donor (*P*-870). The second band of the bacteriochlorophyll dimer is centred at 805 nm. The Q_y transitions of the molecules constituting the bacteriochlorophyll dimer are nearly parallel (angle less than 25°).

The two bacteriopheophytin molecules present slightly different absorption spectra in the near infra-red. Both bacteriopheophytin absorption bands are subject to a small shift under illumination. The angle between the Q_y bacteriopheophytin transitions is 55° or 125°. Both Q_y transitions are nearly perpendicular to the 870 nm absorption band. Finally, the carotenoid molecules makes an angle greater than 70° with the 870 nm band and the other bacteriochlorophyll molecules.

Introduction

During the past few years considerable progress has been made in understanding the mechanism of the primary reaction in photosynthetic bacteria. These advances are mainly due, to the availability of photoactive particles, called reaction centers, which are devoid of antenna pigments and were first isolated by Reed and Clayton [1] and to the advent of picosecond absorption techniques allowing detection of very short-lived intermediate states [2,3].

On the basis of chemical studies, it is now recognized that each reaction center contains four bacteriochlorophyll and two bacteriopheophytin molecules [4—6]. This seems to be the case for different species of bacteria. These pigments give rise to absorption bands around 760 nm (bacteriopheophytin Q_y transitions) and around 870 and 800 nm (bacteriochlorophyll Q_y transitions). After a charge separation between a primary electron donor *P*-870 (demonstrated to be a bacteriochlorophyll dimer by EPR and ENDOR spectroscopy [7—10]) the electron is stabilised on a quinone molecule within 200 ps [2,3]. However, little is known about the spatial arrangement of these six molecules. The main difficulty comes from the fact that the reaction center protein is hydrophobic and therefore cannot be crystallised. Some information concerning the disposition of the pigments can be gained from the use of spectroscopic techniques involving linearly polarized light, such as linear dichroism of oriented preparations, and photoselection experiments (photodichroism in polarized light and polarization of fluorescence). So far, rather contradictory conclusions have been drawn from these different approaches. From photodichroism studies of the bleaching of the 870 nm band, Mar and Gingras [11] found a polarization value of $p = (\Delta A_V - \Delta A_H)/(\Delta A_V + \Delta A_H)$ of +0.25 when exciting within the band. They concluded that the 870 nm band is composed of two non-parallel monomeric transitions and that the 800 nm band is due to either a strongly interacting dimer or two monomers. Vermeglio and Clayton [12] deduced from linear dichroism experiments on oriented chromatophores that the 870 nm band is composed of a single transition and the primary electron donor (*P*-870) absorbs also at 810 nm. More recently, and during completion of this work, Shuvalov et al. [13] reported a *p* value (+0.22) for the 870 nm band similar to the one observed by Mar and Gingras [11]. They proposed that the two Q_y transitions of the bacteriochlorophyll monomers constituting the *P*-870 dimer are parallel and that excitonic coupling of the transitions led to a single allowed transition at 870 nm. Their interpretation is in contradiction with their observation of a *p* value of +0.22, as for a single transition a value of +0.5 is expected. The low polarization value obtained by both groups cannot be easily reconciled with both the constant dichroic ratio observed by Vermeglio and Clayton [12] in the 870 nm band and the polarization value ($p = +0.5$) of the fluorescence observed by Ebrey and Clayton [14].

We shall demonstrate in this paper that these low values of *p* obtained by photodichroism were erroneous due to experimental factors. The polarization value *p* is equal to +0.45 (±0.02) when the excitation and observation are confined to the 870 nm band, as expected for a single band and in agreement with fluorescence polarization data [14]. Analysis of the polarization of the light-induced absorption changes when exciting this single band led us to refine the previous interpretation of Vermeglio and Clayton [12]. It seems more likely that the light-induced absorption changes observed around 800 nm are due to both the bleaching at 810 nm of the second band of the dimeric primary electron donor and an absorption shift due to pigments exhibiting absorption maxima around 800 nm. Finally, excitation of carotenoid transitions and bacteriopheophytin Q_x transitions enable us to propose a possible arrangement of the different pigments within the reaction center.

Materials and Methods

Reaction centers from *Rhodopseudomonas sphaeroides* strain R_{26} and strain 241 were prepared as described previously [15,16].

The classical right-angle geometric arrangement was used to excite and observe light-induced absorption changes. The directions of propagation of both the analysing and the excitation light beams are contained in a horizontal plane (H). The measuring beam was polarized with a Rochon crystal. Excitation light was provided by a quartz iodine lamp (650 W) filtered through interference filters (Pomfret) with 3 nm half-bandwidth. The excitation light was polarized by a polarizing sheet (Polaroid HR for wavelengths greater than 800 nm HN7 for wavelength between 750 and 850 nm, and HN38 for wavelength smaller than 750 nm). The duration of the exciting light in most of the experiments was 33 or 66 ms and could be increased up to a few seconds. We also used short pulses (approx. 500 ns) from a dye laser (Electrophotonics, model 23). Rhodamine 6G and Coumarin 6 were used for excitation around 600 and 535 nm, respectively. The excitation wavelength was adjusted with the tuning prisms set in the laser cavity. The half bandwidth was approx. 0.1 Å.

Measurements were performed at low temperatures using a gas (nitrogen or helium) flow cryostat (Meric). Any temperature value in the range 30—300 K was obtainable. We used either a square cell (10 × 10 mm) or a thin cuvette (1 mm optical pathlength).

Reaction centers were dispersed in a medium containing 66% glycerol and 33% 0.01 M Tris buffer, pH 7.8, 0.3% LDAO to insure a transparent sample. The absorbance of the sample was approximatively 0.3 at 870 nm for all of the experiments.

Results

A. Preliminary remarks on photoselection studies

Besides the fact that the exciting and measuring lights must be properly polarized, two conditions must be fulfilled in photoselection experiments: (1) the rotational motion of the molecules or particles must be much slower than the life-time of the photoinduced species; (2) one must work at a low excitation level (10%) in order to be far from saturation.

Fig. 1 shows light absorption changes induced by 900 nm exciting light polarized either vertically (Fig. 1A) or horizontally (Fig. 1B) and observed at 870 nm. The time duration of the excitation light was 33 ms. The intensity was low enough to oxidize only 10% of the total reaction centers. The temperature

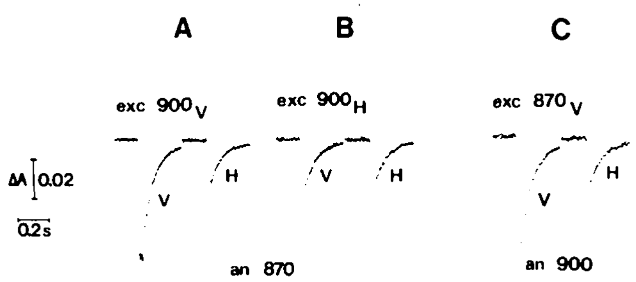


Fig. 1. Kinetics of light-induced changes of R_{26} reaction centers for both horizontal (H) and vertical (V) polarization of the analyzing beam. The reaction centers were dispersed in 66% glycerol and 33% 0.01 M Tris buffer, pH 7.8/0.3% LDAO. The temperature was 160 K. The excitation time was 33 ms. A. Excitation with 900 nm light polarized vertically; analyzing wavelength 870 nm for both V and H. B. Excitation with 900 nm light polarized horizontally; analyzing wavelength 870 nm for both V and H. C. Excitation with 870 nm light polarized vertically; analyzing wavelength 900 nm for both V and H.

was 160 K, the polarization value defined as $p = (\Delta A_V - \Delta A_H)/(\Delta A_V + \Delta A_H)$ (ΔA_V and ΔA_H being respectively the absorbance changes for vertical and horizontal polarization of the analysing beam) is equal to +0.45 when exciting with vertically polarized light (Fig. 1A). The reverse experiment, i.e. excitation by 870 nm light and observation at 900 nm, gives the same value of p (Fig. 1C). In a control experiment we excited with horizontally polarized light (Fig. 1B). As expected, the amplitudes of the absorption changes are equal, whatever the polarization of the analysing beam, and are equal to the changes observed for vertical polarization of the excitation, but horizontal polarization of the analysing beam.

Fig. 2 shows polarization values obtained at different temperatures for experiments similar to the one shown in Fig. 1A. For high temperature values, depolarization by rotational motion of the reaction center protein occurs, and we therefore obtained low value of p. For temperatures lower than 240 K, the particles are immobilized, at least in the time scale of the relaxation of the absorption changes, and high polarization values are observed.

At first sight, the polarization we obtained in these experiments ($p = +0.45 \pm 0.02$) seems contradictory to that reported independently by Mar and Gingras [11] and by Shuvalov and al. [13] ($p = +0.25$ and $p = +0.22$, respectively). However both groups utilized much longer excitation intervals than was

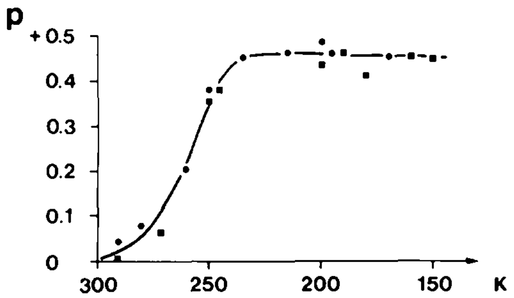


Fig. 2. Plot of polarization values p, calculated from experiments similar to the ones shown in Fig. 1A, as a function of the temperature of the sample.

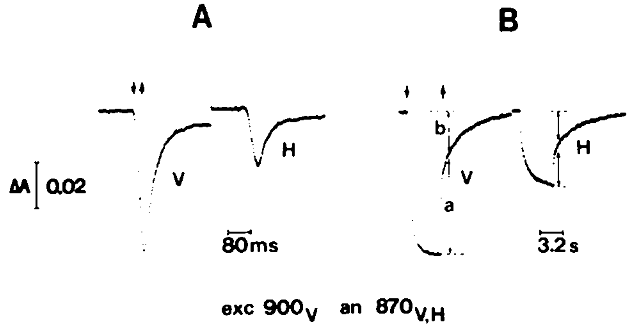


Fig. 3. Kinetics of absorbance changes induced in R_{26} reaction centers by 900 nm vertically polarized light. The analyzing beam was 870 nm and polarized either vertically (V) or horizontally (H). The temperature was 150 K. No orthophenanthroline was added. A. Pulse duration of the excitation light 33 ms. B. Pulse duration of the excitation light 4 s.

the case for the data reported in Fig. 1. We therefore compared absorbance changes induced by a short (33 ms) (Fig. 3A) or a long (4 s) (Fig. 3B) excitation pulse using the same sample. The light intensity was adjusted in order to obtain the same amount of oxidized reaction centers (approx. 10%). We obtained a smaller polarization value (+0.30) for absorbance changes induced by a long excitation time than for a short one (p = +0.45). This difference is explained by the fact that the reaction centers are inhomogeneous as far as the half time of the back reaction is concerned (see Fig. 3). Most of the oxidized reaction centers have a relaxation half-time of 30 ms, but some of them exhibit half-times of the order of seconds. These two half-times can be ascribed respectively to the back reaction between P^+ and the primary electron acceptor for the "fast" one, and to the back reaction between P^+ and secondary electron acceptor for the "slow" one. The relative amount of "fast" and "slow" reaction centers depends on the temperature. The lower the temperature, the smaller the amount of "slow" reaction centers. The rise time for reaching the steady state is equal to $1/(\sigma I + k)$ where k is the time constant of the back reaction and σI, proportional to the light intensity I, is the time constant of the oxidation of reaction centers. As a consequence, for a given intensity, the "fast" reaction centers will reach the steady-state level more rapidly than the "slow" ones. Moreover, lengthening the excitation time and reducing the light intensity, will favor oxidation of "slow" reaction centers as compared to the "fast" ones. These "slow" reaction centers, although present in small amounts, can be completely photooxidized even when 10% of the total amount of the reaction centers are saturated. In other words, these "slow" reaction centers have all been photooxidized. Therefore, they contribute to the absorbance changes to the same extent for both polarizations of the analysing beam and lead to a lower value of p. This is clearly shown on Fig. 3B where, after the light is turned off, the amplitude of the rapid component (indexed as "a") is nearly three times grater for the vertically polarized analysing beam than for horizontally polarized light ($p \approx +0.5$). On the other hand, only a small difference for the two polarizations of the analysing beam is observed for the amplitudes of the slow component (indexed as "b"). Similar effects can be observed on the kinetics of the absorbance changes reported by Shuvalov et al.

[13]. To minimize the type of artefact, we always utilized short excitation times. Furthermore, orthophenanthroline was added to the samples. This compound inhibits electron transfer from the primary to the secondary electron acceptor. Thus a homogeneous sample with respect to relaxation times is obtained, since the reaction centers relax by way of a fast back reaction.

B. Polarization of the excitation spectrum of the bleaching at 870 nm

The absorption spectrum of reaction centers of *Rhodopseudomonas sphaeroides* strain 241 measured at 150 K is shown in Fig. 4. We have omitted the absorption spectrum of reaction centers prepared from the carotenoidless mutant R_{26} which is almost identical, except for the carotenoid absorption band region (420—525 nm). Polarization values for the bleaching at 870 nm for different excitation wavelengths are plotted in Fig. 4 (lower traces) for both the wild-type and the carotenoidless reaction centers. The polarization is positive and equal to 0.45 at 870 nm. It is negative in the pheophytin absorption band (—0.17) and around 605 nm (—0.18).

C. Polarization of light induced changes upon excitation at 900 nm measured at different temperatures

Additional information may be obtained upon excitation within a single absorption band and by observing the polarization of the light-induced changes,

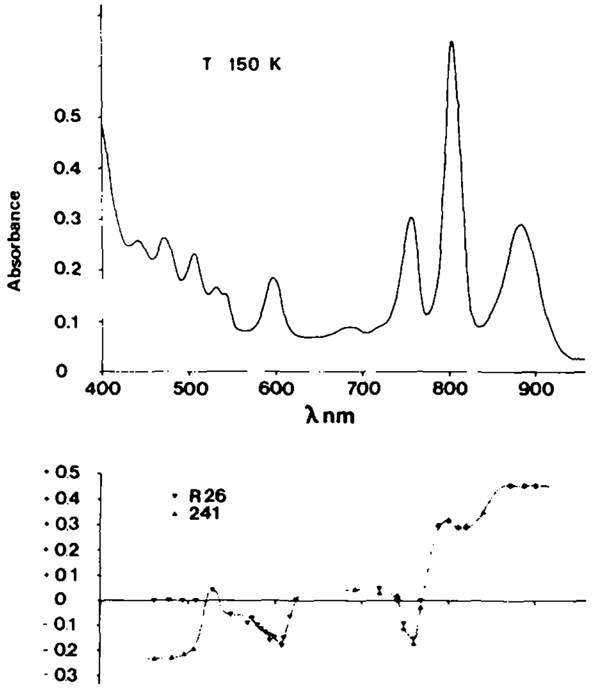


Fig. 4. Absorption spectrum of 2.4.1 reaction centers recorded with a Cary 17 spectrophotometer at 150 K and polarization spectrum of the bleaching observed at 870 nm for both 2.4.1. (▲) and R_{26} (▼) reaction centers. The polarization values *p* were calculated from similar data to the ones shown in Fig. 1A but for different excitation wavelengths.

since not all of the pigments are involved in the light minus dark spectrum, as is the case for the excitation spectrum. We therefore excited at 900 nm and looked for the polarization of the light-induced changes.

The results are shown in Fig. 5 for R_{26} reaction centers. They were obtained at 160 K. At 870 nm the polarization is large and equal to 0.45.

In order to appreciate the contributions of bleaching within the band and absorption band shifts on the light-induced changes, in particular around 800 nm, we performed the same experiments at different temperatures. Fig. 6 shows the light minus dark difference spectrum for the two polarizations of the analyzing beam at 160, 230, 260 K and room temperature. For the experiments done at 260 K and at room temperature the reaction centers were dried onto a slide in order to prevent rotational motion which occurs at these temperatures in glycerol medium (cf. paragraph A). It appears clearly that the polarization around the crossing point (near 800 nm) varies with the temperature.

D. Excitation within the carotenoid absorption band

Light-induced absorption changes are plotted in Fig. 7 for the two polarizations of the analyzing beam when exciting the carotenoid molecules at 496 nm. As a control, we did the same experiment with R_{26} reaction centers and found that for the same concentration of reaction centers, excitation of R_{26} reaction centers at 496 nm induced 27% of the light changes observed in the case of

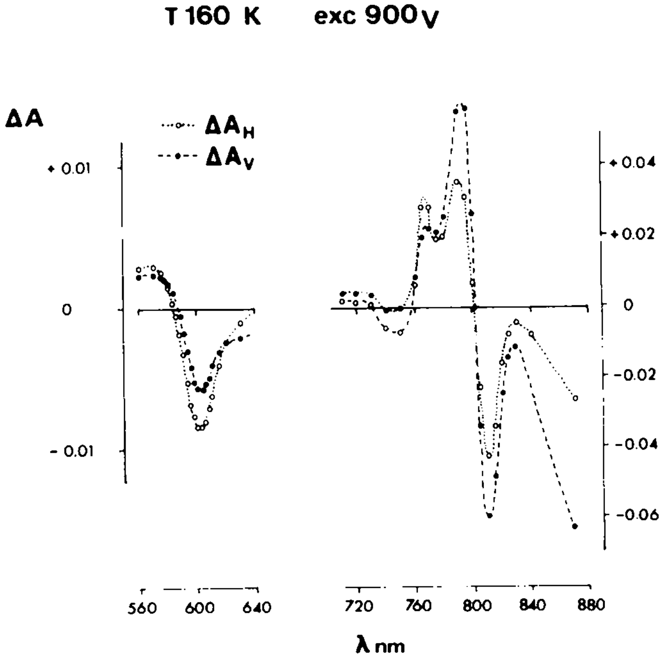


Fig. 5. Difference spectra induced by 900 nm vertically polarized light for both polarizations of the analyzing beam (V and H). The temperature was 160 K for R_{26} reaction centers. ○······○, ΔA_H; ●- - - - - -●, ΔA_V.

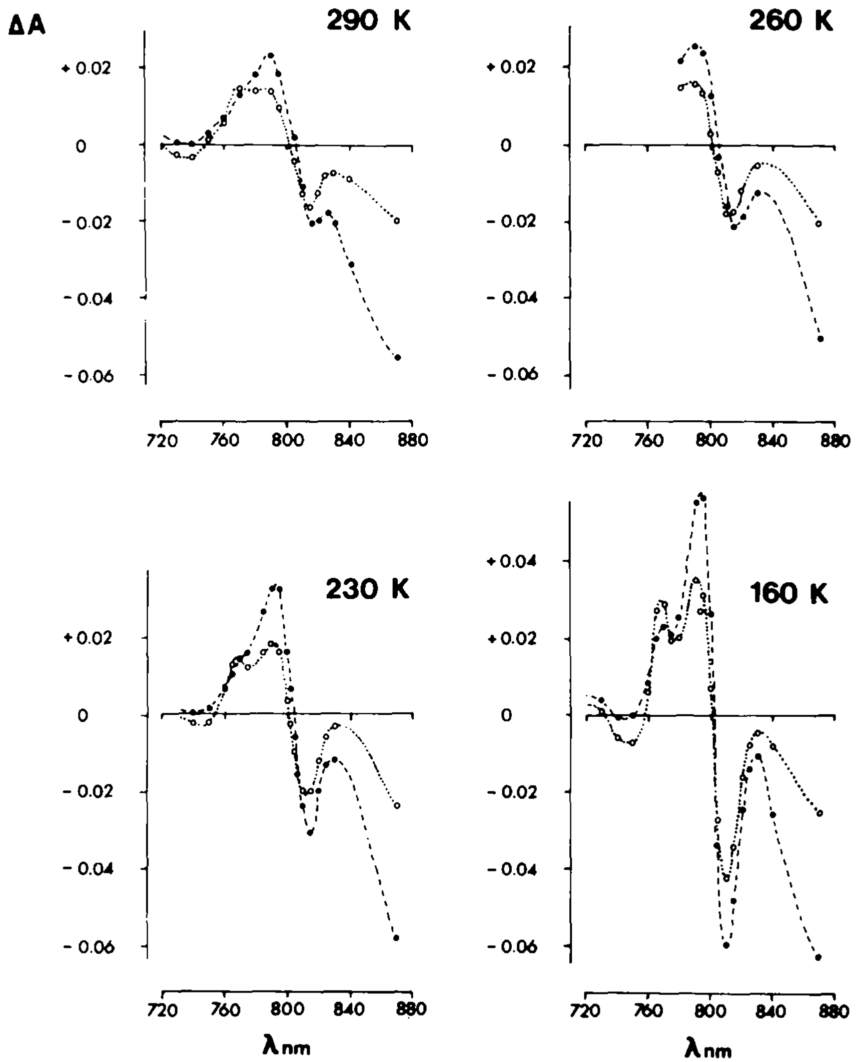


Fig. 6. Difference spectrum induced by 900 nm vertically polarized light for both polarizations of the analyzing beam (V and H) observed at different temperatures. For room temperature and 260 K the R_{26} reaction centers were dried onto a plate (same symbols as in Fig. 5).

wild-type reaction centers under the same excitation. However, this 27% does not depend on the polarization of the analyzing beam. The light induced absorbance changes reported in Fig. 7 are corrected for this 27% effect to reflect only the changes induced by the carotenoid absorption.

E. Excitation within the bacteriopheophytin Q_x absorption bands

Each reaction center contains two molecules of bacteriopheophytin. At low temperature these two molecules present two resolved Q_x transitions absorbing respectively at 530 and 546 nm [17]. On the other hand, charge separation within the reaction center induces absorption band shifts of the Q_y transition of the bacteriopheophytin molecule(s). In order to decide if both of the

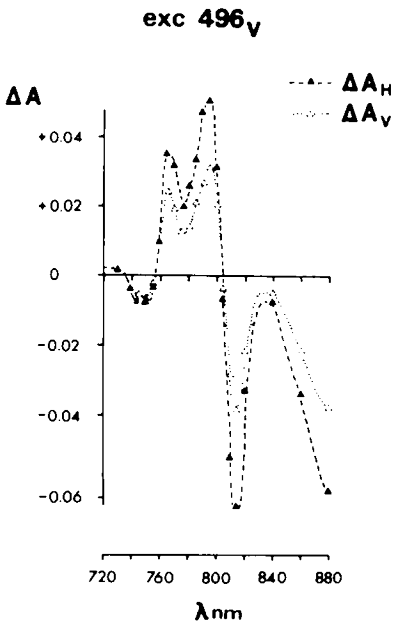


Fig. 7. Difference spectra induced by 496 nm vertically polarized light for both polarization (V and H) of the analyzing beam. The temperature was 150 K for 2.4.1. reaction center.

bacteriopheophytin molecules, or only one of them, are subject to the local electrical field induced by the separated charges, we excited separately, with the dye laser, the two Q_x transitions and observed the polarization of the absorption changes attributed to the bacteriopheophytin Q_y transition shift. In these experiments the temperature was 50 K to insure a good spectral resolution of the two Q_x absorption bands. At this temperature some cracks appeared within the sample. We verified that these cracks did not induce a depolarization of the analyzing or exciting beams by remeasuring the polarization value

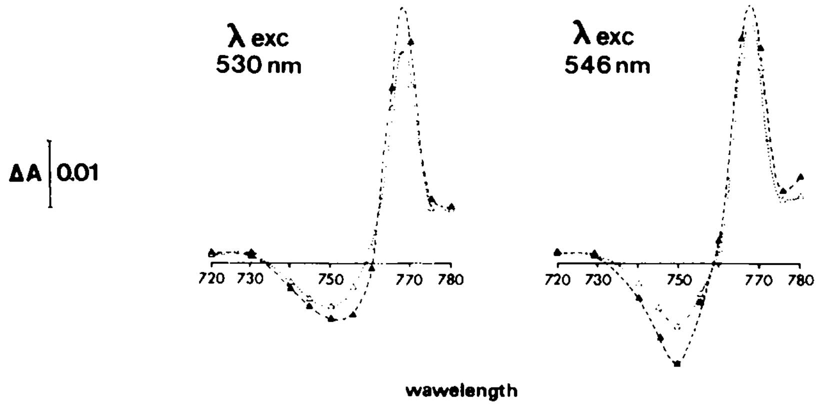


Fig. 8. Difference spectra induced by 530 nm and 546 nm vertically polarized light for both polarizations of the analyzing beam. The temperature was 50 K for R_{26} reaction centers. ▲------▲, ΔA_H; △······△, ΔA_V.

of the 870 nm band, which was found to be +0.45. Absorption changes induced by 530 and 546 nm exciting light observed between 720 and 780 nm are shown in Fig. 8. Polarization values when observing the bleaching at 870 nm were $p = -0.06 \pm 0.02$ and $p = +0.05 \pm 0.02$ when exciting at 546 and 530 nm, respectively.

Discussion

In principle, the mutual orientation of the six pigments within the reaction center complex can be deduced from photoselection experiments. The polarization value p is related to the angle α between the axis of the excitation transition moment and the axis of the transition moment detected by the analyzing light by the formula $p = (3\cos^2\alpha - 1)/(\cos^2\alpha + 3)$. p varies from +0.5 ($\alpha = 0°$) to −0.33 ($\alpha = 90°$). However, if overlap between different pigments occurs either in the absorption or in the light-induced changes, the situation is more complex. Instead of working with the values ΔA_V or ΔA_H, it is more advantageous to calculate the variations of absorption parallel ($\Delta A_\parallel$) or perpendicular ($\Delta A_\perp$) to the transition involved in the excitation (cf. Appendix). In any case the interpretation of the $\Delta A_\parallel$ and $\Delta A_\perp$ values is simplified when the excitation is confined to a single transition. Within the reaction center, we expect that there is only one pure transition in the following four cases exciting at: (1) the 870 nm band, (2) the carotenoid transition band (between 450 and 510 nm) and (3) and (4) the two bacteriopheophytin Q_x bands (530 and 546 nm) which are spectrally well resolved at low temperatures. Excitation of each band will define a particular direction in the reaction center.

(1) Excitation within the 870 nm band: problem of the structure of the dimeric primary electron donor

We have found upon excitation and observation within the 870 nm band a high value of p (+0.45) at all temperatures investigated. This result is in very good agreement with the value reported by Ebrey and Clayton [14] from fluorescence polarization data, but in contradiction to the one reported by Mar and Gingras [11] and by Shuvalov and coworkers [13] using absorption photoselection techniques. We have shown that the low value of p reported by these two groups is due to their experimental conditions. It is clear (Fig. 3) that under condition of illumination similar to those used by these authors (illumination time greater than a few seconds), partial depolarization is observed.

The high value of p observed implies that the 870 nm absorption band is due to a single transition, or to two parallel transitions. Then a particular axis is defined in the reaction center: the direction of the transition moment of the 870 nm band. The analysis of the polarization of the light-induced changes when exciting within this band is therefore simplified. It was generally assumed that the changes around 800 nm and 760 nm are due respectively to bacteriochlorophyll [13] and bacteriopheophytin absorption band shifts. Recently Vermeglio and Clayton [12] proposed from linear dichroism results on oriented chromatophores that the bleaching at 810 nm and the absorption increase at 790 nm are due respectively to the disappearance of a dimer band

and to the appearance of a monomer-like band, respectively. This hypothesis implies that the 810 nm band and the 870 nm band are perpendicular to each other [18]. Fig. 9 shows the light-induced changes observed perpendicular ($\Delta A_\perp$) and parallel ($\Delta A_\parallel$) to the 870 nm band for different temperatures. These light minus dark spectra were calculated from the results depicted on Fig. 6 using the equations derived in the appendix. At all temperatures, it is clear that the absorption changes $\Delta A_\perp$ and $\Delta A_\parallel$ occurring near 800 nm are not proportional (this is also the case for ΔA_V and ΔA_H). For example the crossing points ($\Delta A = 0$) are different. The assymetry of the absorption changes may be characterized by the ratio R of the maximal absorption changes $\Delta A_\parallel$ and $\Delta A_\perp$ occurring around 790 and 810 nm. Around 790 nm this ratio R is equal to 1.6 and does not depend on the temperature. This is not the case for the R value measured around 810 nm. It varies with the temperature: 0.8 at room temperature, 0.7 at 260 K and 1.2 at 160 K. This is not compatible with a simple absorption band shift occurring in the light minus dark difference spectrum. It implies that around 800 nm there are at least two transitions which contribute

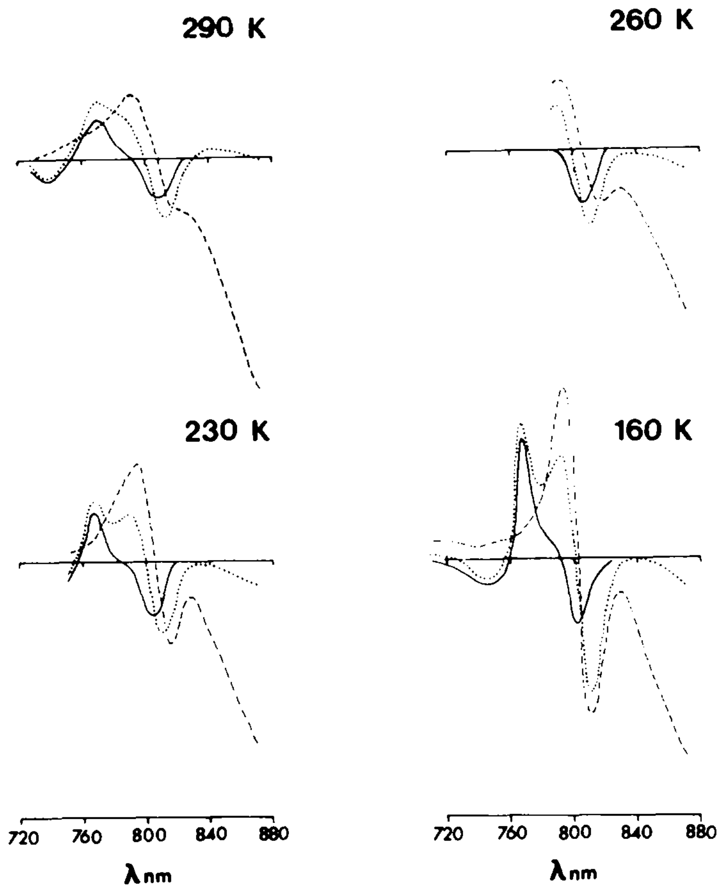


Fig. 9. Difference spectra ($\Delta A_\parallel$, $\Delta A_\perp$ and $\Delta A_\perp - \Delta A_\parallel/1.6$) induced by 900 nm vertically polarized light calculated from the data reported on Fig. 6 by using the equations derived in the Appendix. ······, $\Delta A_\perp$; - - - - - -, $\Delta A_\parallel$; ———, $\Delta A_\perp - \Delta A_\parallel/1.6$.

to the absorption changes. We have assumed that the contribution of one of these bands is an absorption band shift giving $\Delta A_{\parallel} = 1.6\ \Delta A_{\perp}$. Then the other contribution may be deduced by plotting the difference $\Delta A_{\perp} - \Delta A_{\parallel}/1.6$. The results are shown in Fig. 9.

For each temperature, besides the absorption changes occurring around 760 nm which will be discussed in the next paragraph, a negative band with a maximum around 805 nm is observed in the quantity ($\Delta A_{\perp} - \Delta A_{\parallel}/1.6$). This negative band centred at 805 nm is therefore perpendicular to the 870 nm absorption band. We interprete the absorption changes between 780 and 820 nm as being due to two contributions: (1) an absorption band shift [13] with a polarisation value of +0.23 (implying that, if it is a single band, the transition makes an angle of 37° with the 870 nm band); and (2) the bleaching of a transition absorbing at 805 nm which is perpendicular to the 870 nm absorption band. This suggests that these two perpendicular transitions (805 and 870 nm) are the two transitions of the primary electron donor as predicted by the excitonic theory of Kasha [18] and is consistent with the hypothesis of Vermeglio and Clayton [12]. Nevertheless it must be noticed (Fig. 9) that the band width of these two transitions are different (44 nm for the 870 nm band and 14 nm for the 805 nm band). This can result from at least two physical processes which may take place in the case of the special pair: a non-negligible dependance of the resonance interaction on the nuclei motion [19] and the configuration interaction between charge-transfer states and the neutral singlet states corresponding to the 870 nm absorption band [20].

Examining now the polarization of the absorption changes observed around 605 nm when exciting at 900 nm (Fig. 5) should give us the relative orientation of the Q_x and the 870 nm transitions of the dimer. The polarization value is equal to −0.17 and does not vary significantly within the light induced changes (Fig. 5). This result implies that both Q_x transitions of the dimer are nearly perpendicular to the 870 nm band. As a consequence and according to the theory of Kasha, the two Q_y transitions of the bacteriochlorophyll constituting the dimer are nearly parallel. The angle between the two Q_y transitions of the bacteriochlorophyll molecules constituting the dimer can be calculated more accurately from the surfaces of the two absorption bands. We estimate the ratio of these surfaces (870/805) to be greater than 20, implying that the angle between the two Q_y transition directions is less than 25°.

(2) Orientation of the bacteriopheophytin molecules

From the excitation spectrum shown in Fig. 4, it is clear that both Q_y bacteriopheophytin transitions are nearly perpendicular (angle >70°) to the 870 nm absorption band. This conclusion has also been drawn earlier [12,13,21]. This result is confirmed when looking at the absorbance change ($\Delta A_{\perp}$ and $\Delta A_{\parallel}$) occurring near 760 nm upon excitation at 900 nm (Fig. 9): all of the changes related to the bacteriopheophytin absorption band shift are seen in the $\Delta A_{\perp}$, but not in the $\Delta A_{\parallel}$ difference spectrum. This is true at all temperatures (Fig. 9). Excitation within the two spectrally resolved Q_x transitions (530 and 546 nm) provides further information. Firstly let us suppose that the two Q_x transitions are not due to excitonic interactions. Let us call Q_{y1}, Q_{x1} the transitions of the bacteriopheophytin molecule which absorbs at 546 nm and

Q_{y2}, Q_{x2} the transition of the bacteriopheophytin absorbing at 530 nm. As indicated in the appendix, we calculated the two parallel difference spectra $\Delta A_{\parallel,1}$ and $\Delta A_{\parallel,2}$, which correspond to the absorption changes parallel to Q_{x1} and Q_{x2}, respectively. Fig. 10 shows these two spectra and the ΔA difference spectrum obtained with unpolarized light. In the case of non interacting molecules Q_{y1} will be perpendicular to Q_{x1} and idem for Q_{y2} and Q_{x2}. This implies that $\Delta A_{\parallel,1}$ corresponds to the only shift of the Q_{y2} transition and vice versa. The two parallel absorption changes being different, this means that the two bacteriopheophytin molecules have slightly different absorption bands in the near infrared, and both absorption band shifts. One may write the following equations (cf. Appendix):

$$\Delta A_{\parallel,1} = \Delta A_2 \cos^2\phi_{12}$$

$$\Delta A_{\parallel,2} = \Delta A_1 \cos^2\phi_{21}$$

where ΔA_1 and ΔA_2 are the absorption changes related to the Q_{y1} and Q_{y2} transitions. ϕ_{12} is the angle between Q_{x1} and Q_{y2}, ϕ_{21} the angle between Q_{x2} and Q_{y1}. As $\Delta A = \Delta A_2 + \Delta A_1$ we may write:

$$\Delta A = \frac{\Delta A_{\parallel,1}}{\cos^2\phi_{12}} + \frac{\Delta A_{\parallel,2}}{\cos^2\phi_{21}}$$

We obtained from the experimental data by applying the least-squares

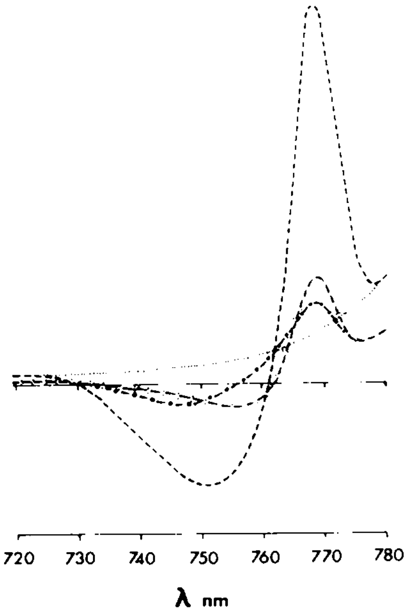


Fig. 10. Difference spectra calculated from the results depicted in Fig. 8. ΔA corresponds to the difference spectrum obtained with unpolarized excitation and analyzing light. $\Delta A_{\parallel,1}$ (-·-·-·) corresponds to the difference spectrum parallel to the $Q_{x,1}$ (546 nm) transition. $\Delta A_{\parallel,2}$ (-:-:-:) corresponds to the difference spectrum parallel to the $Q_{x,2}$ (530 nm) transition. - - - - - -, ΔA.

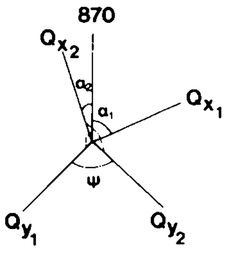


Fig. 11. Schematic representation of the transition moments (870 nm, bacteriopheophytin Q_{x1}, Q_{y1} and Q_{x2}, Q_{y2}) arrangement within the reaction center.

method, the following:

$$\Delta A = 2.1\ \Delta A_{\parallel,1} + 2.5\ \Delta A_{\parallel,2} \tag{1}$$

and therefore $\cos^2 \phi_{12} = 0.48$ ($\phi_{12} = 46°$) and $\cos^2 \phi_{21} = 0.40$ ($\phi_{21} = 51°$). Now if α_1 and α_2 are the angles between Q_{x1} and the 870 nm band and between Q_{x2} and this same transition one may write (see Fig. 11):

$$\cos^2\phi_{21} = \sin^2\alpha_2 \sin^2\phi$$

$$\cos^2\phi_{12} = \sin^2\alpha_1 \sin^2\phi$$

where ϕ is the angle between the two bacteriopheophytin Q_y transitions. $\mathrm{Sin}^2\, \alpha_1$ and $\sin^2 \alpha_2$ may be calculated from the polarization values of the bleaching at 870 nm when exciting at 546 and 530 nm, respectively (see Results). One finds that $\sin^2 \alpha_1 = 0.73$ ($\alpha_1 \approx 60°$) and $\sin^2 \alpha_2 = 0.60$ ($\alpha_2 \approx 50°$). One may also verify that $\cos^2 \phi_{12}/\cos^2 \phi_{21} = 1.20$ is nearly equal to $\sin^2 \phi_1/\sin^2 \phi_2 = 1.22$. $\sin^2 \phi$ is therefore equal to 0.66 and ϕ to 55° or 125°.

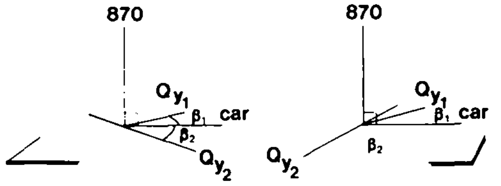
Now if the two Q_x transitions are in excitonic interaction the two $\Delta A_\parallel$ observed must be equal:

$$\Delta A_{\parallel,1} = \Delta A_{\parallel,2} = \tfrac{1}{2}(\Delta A_1 \cos^2\phi_{21} + \Delta A_2 \cos^2\phi_{12})$$

This is not observed (Fig. 10). However the small differences between $\Delta A_{\parallel,1}$ and $\Delta A_{\parallel,2}$ may be due to a small electronic interaction between the Q_y transitions. In a similar manner as in the previous case (monomeric) we may calculate the angle ϕ between the two Q_y transitions and we find again $\phi = 55°$ (or 125°). In this hypothesis the angles between Q_x transitions and the 870 nm band are equal to 90° and 36°.

(3) Orientation of the carotenoid molecule

From the results depicted in Fig. 7, high negative values of p are derived. For example p is equal to -0.24 for the bleaching at 870 nm. The carotenoid molecule is therefore nearly perpendicular to the 870 nm band (angle >72°) and to the other bacteriochlorophyll molecule. The p values for the bacteriopheo-



$\beta_1 + \beta_2 = 55°$ $\beta_1 + \beta_2 = 125°$

Fig. 12. Schematic representation of the transition moments (870 nm, carotenoid and bacteriopheophytin Q_{y1} and Q_{y2} transition moments) within the reaction center. The 870 nm transition moment is perpendicular to the plane containing the transition moment of the carotenoid molecule (car) and the transition moments of the two bacteriopheophytin molecules (Q_{y1} and Q_{y2}). Four possible arrangements are represented if the carotenoid transition moment is taken as the reference axis:

(1) $\beta_1 + \beta_2 = 55°$ $\beta_1 = \beta_2$
(2) $\beta_1 + \beta_2 = 55°$ $\beta_1 = 180° + \beta_2$
(3) $\beta_1 + \beta_2 = 125°$ $\beta_1 = \beta_2$
(4) $\beta_1 + \beta_2 = 125°$ $\beta_1 = 180° + \beta_2$

phytin are less high. If β_1 and β_2 are the angles between the transitions Q_{y1} and Q_{y2} of the bacteriopheophytin and the carotenoid (Fig. 12) and if $\Delta A_{\parallel}$ is the absorbance change parallel to this carotenoid transition, one may write the following equation:

$$\frac{\Delta A_{\parallel}}{\Delta A} \approx \tfrac{1}{2}(\cos^2\beta_1 + \cos^2\beta_2) = \tfrac{1}{2}(1 + \cos(\beta_1 + \beta_2)\cos(\beta_1 - \beta_2))$$

since $\Delta A_{\parallel}/\Delta A = 0.2$ and $\cos(\beta_1 + \beta_2) = \cos\phi = \pm 0.58$, we find that $\beta_1 - \beta_2 = 0^\circ$ or 180°.

Thus, in the plane perpendicular to the 870 nm transition moment, the Q_{y1} and Q_{y2} transitions of the two bacteriopheophytin molecules and the one of the carotenoid molecules can occupy the four possible dispositions depicted in Fig. 12.

Conclusions

The 870 nm band corresponds to a single transition. The absorption changes around 800 nm are attributed to both an absorption band shift and the bleaching of the second band of the dimer at 805 nm.

The two Q_y transitions of the molecules constituting the bacteriochlorophyll dimer are nearly parallel (angle smaller than 25°).

Both bacteriopheophytin absorption bands in the red are subject to a small shift when a charge separation occurs within the reaction center. These two bacteriopheophytin molecules present slightly different absorption spectra in the near infrared. The angle between the Q_y bacteriopheophytin transitions is 55 or 125°. Both Q_y transitions are nearly perpendicular to the 870 nm absorption band.

The carotenoid molecule is nearly perpendicular to the 870 nm band and the other bacteriochlorophyll molecules.

Appendix

In the case where the transitions implicated in the excitation and the detection are linearly polarized and make an angle α, one may write:

$$\Delta A_{\mathrm{V}} = \frac{\dfrac{3\Delta A}{2}\displaystyle\int_0^{\pi/2} \cos^2\theta(\cos^2\theta(3\cos^3\alpha - 1) + \sin^2\alpha)\sin\theta\,\mathrm{d}\theta}{\displaystyle\int_0^{\pi/2}\cos^2\theta\,\mathrm{d}\theta} \tag{1}$$

$$\Delta A_{\mathrm{H}} = \frac{\dfrac{3\Delta A}{2}\displaystyle\int_0^{\pi/2} \cos^2\theta(\cos^2\theta(1 - 3\cos^2\alpha) + 1 + \cos^2\alpha)\sin\theta\,\mathrm{d}\theta}{\displaystyle\int_0^{\pi/2}\cos^2\theta\,\mathrm{d}\theta} \tag{2}$$

where ΔA_{V} and ΔA_{H} are the absorbance changes analysed with vertically or horizontally polarized light and θ is the angle between the analyzing beam and the transition moments implied in the absorption, it follows that

$$\Delta A_V = \frac{\Delta A(2\cos^2\alpha + 1)}{5} \tag{3}$$

$$\Delta A_H = \frac{\Delta A(2 - \cos^2\alpha)}{5} \tag{4}$$

the polarization $p = (\Delta A_V - \Delta A_H)/(\Delta A_V + \Delta A_H)$ is then equal to $(3\cos^2\alpha - 1)/(3 + \cos^2\alpha)$.

If more than one transition is implicated either in the absorption or in the light induced changes, the value of p is not significant, in particular around a crossing point ($\Delta A = 0$). It is more interesting to introduce different quantities. From the Eqns. 3 and 4 one may derive:

$$\Delta A_V + 2\Delta A_H = \Delta A \tag{5}$$

$$2\Delta A_V - \Delta A_H = \Delta A\cos^2\alpha = \Delta A_{\parallel} \tag{6}$$

$$3\Delta A_H - \Delta A_V = \Delta A\sin^2\alpha = \Delta A_{\perp} \tag{7}$$

$\Delta A_{\parallel}$ and $\Delta A_{\perp}$ are the absorption changes observed parallel or perpendicular to the direction of the excitation, respectively. This result can be generalized for the case when several transitions are involved by a simple summation. Compared to ΔA, the values of $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ give information which is more easily interpretable than p or ΔA_V and ΔA_H;

Eqns. 5, 6 and 7 correspond to the case where one excites at one particular wavelength and measures the polarization of absorption changes at different

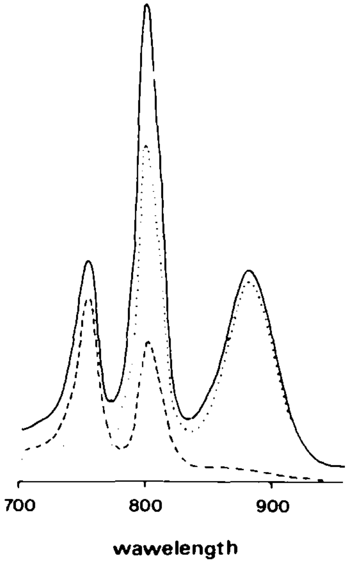


Fig. 13. Absorption spectrum (A, ———) of 241 reaction centers measured at 150 K. Absorption spectrum parallel to the 870 nm transition moment ($\Delta A_{\parallel}$, ·······) and absorption spectrum perpendicular to the 870 nm transition moment ($\Delta A_{\perp}$, ------) calculated from the data reported on Fig. 4 using the equations derived in the Appendix.

wavelengths. These equations are also valid when exciting at different wavelengths and observing the polarisations of absorbance changes at a particular wavelength. In that case one must substitute ΔA, $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ by A, $A_{\parallel}$ and $A_{\perp}$, respectively. An example is given on Fig. 13 showing the plot of the absorbance spectrum of 2.4.1 reaction centers at 150 K and the $A_{\parallel}$ and $A_{\perp}$ quantities calculated from this spectrum and the value of p reported in Fig. 4.

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