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ORIENTATION OF REACTION CENTER AND ANTENNA CHROMOPHORES IN THE PHOTOSYNTHETIC MEMBRANE OF *RHODOPSEUDOMONAS VIRIDIS*

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Summary

Whole cells of *Rhodopseudomonas viridis* were oriented in a magnetic field. The degree of orientation of the cells was determined by using a photoselection technique. In order to deduce the orientation of the antennae and chromophores of the reaction centers with respect to the membrane plane, we performed linear dichroism measurements of absolute spectra and light induced difference spectra linked to states P^+I and PI^- on oriented cells. These measurements lead to the following conclusions:

The antennae bacteriochlorophyll molecular plane is nearly perpendicular to the membrane. The Q_y and Q_x transitions moments of these molecules make respectively angles of 20 and 70° with the membrane plane. The antenna carotenoid molecules make an angle of 45° with the membrane.

The primary electron donor possesses two transition moments centered respectively at 970 and 850 nm. The 970 nm transition moment is parallel to the membrane plane, the 850 nm transition is tilted out of the plane. Upon photooxidation of this primary electron donor, a monomer-like absorption band appears at 805 nm. Its transition makes an angle smaller than 25° with the membrane. The photooxidation of the dimer also induces an absorption band shift for the two other bacteriochlorophyll molecules of the reaction center. The absorption band shifts of the two bacteriochlorophyll molecules occur in opposite direction.

One bacteriopheophytin molecule is photoreduced in state PI^-. This photoreduction induces an absorption band shift for only one bacteriochlorophyll molecule. Finally, the geometry of the dimeric primary donor seems to be affected by the presence of a negative charge in the reaction center.

Introduction

The early photochemical steps of purple bacterial photosynthesis occur within a protein complex called the reaction center. Each reaction center contains four molecules of bacteriochlorophyll, two molecules of bacteriopheophytin and a quinone-iron complex [1]. Measurements of fast optical [2,3] changes have shown that absorption of one photon by the reaction center promotes the primary electron donor P to an excited singlet state P^*. In less than 10 ps [4] an electron is then transferred to an intermediate electron carrier I, leading to the state P^+I^- (or P^F [5]). The reduced electron carrier I^- is then reoxidized by the quinone-iron complex (QFe) within 200 ps [2,3]. Comparison of the optical properties of the in vitro bacteriopheophytin radical anion and state P^+I^- led Fajer et al. [6] to propose that the intermediate electron carrier I was a bacteriopheophytin molecule. The state PI^- can be stabilized by continuous illumination at low redox potential for different species of bacteria [7—12]. Very recently Prince et al. [13] have shown that the complex ESR changes accompanying the reduction of I at low temperature are composed of two signals one of which reflects an interaction of the reduced bacteriopheophytin molecules with the reduced quinone iron complex (Q^-Fe). On the other hand, from ESR and ENDOR [14—17] studies, it has been known for several years now that in state P^+I the unpaired electron is delocalized over two of the four bacteriochlorophyll molecules of the reaction center indicating that the primary electron donor is a bacteriochlorophyll dimer.

Even if ESR spectra for both P^+I and PI^- states can be interpreted almost unequivocally, the absorption changes linked to these states are more complex and reflect the bleaching and the appearance of absorption bands together with secondary effects such as band shifts.

Linear dichroism is a very useful tool for the assignment of absorption changes. For example, in the case of *Rhodopseudomonas sphaeroides*, linear dichroism studies on oriented chromatophore membranes [18] or oriented reaction centers in stretched gelatin film [19] as well as photoselection experiments [20] on isolated reaction centers have pointed out that a second band for the bacteriochlorophyll dimer P is underlying the 800 nm band. In addition and certainly more interesting, such measurements can give direct information about the spatial arrangement of the different chromophores in the reaction center and with respect to the plane of the membrane.

In the present study, we analysed the linear dichroism of absorption changes of oriented cells of *Rhodopseudomonas viridis*. The main purposes were to clarify further the origin of the light-induced absorption changes related to the states P^+I and PI^- and to determine the relative orientation of reaction center components versus the membrane plane. The species *Rhodopseudomonas viridis* presents several advantages for our purposes: first of all, due to the cylindrical shape [21] of their membranes, whole cells can be readily oriented in a magnetic field [22] with the long axis of the cylinder perpendicular to the field direction; states P^+I and PI^- can be photoinduced at room temperature [12,13, 23]; finally spectral forms underlying the 830 nm band of reaction centers are more resolved [12] than for bacteriochlorophyll *a*-containing species and are well separated from long wavelength antennae absorption bands in chromatophores.

Materials and Methods

Rhodopseudomonas viridis cells were a generous gift of Professor R.K. Clayton and B.J. Clayton (Cornell University, Ithaca, N.Y.). Whole cells were suspended in 0.01 M Tris buffer, pH 7.8, or in a medium containing 33% glycerol and 0.01 M 66% Tris buffer, pH 7.8. A magnetic field, ranging from 0 to 30 kG, was applied to the suspension of whole cells. Linear dichroism spectra of oriented cells were measured as previously described [24]. The experimental arrangement for measuring the linear dichroism of light-induced absorbance changes is depicted in Fig. 1. The measuring beam was polarized by a Rochon polarizer before falling on the sample. Excitation light was provided either by a dye laser (model 23, Electrophotonics Ltd) operated at 600 nm or a quartz halogen lamp filtered through Corning filters (4-96 or 7-69). Excitation light could be polarized either parallel or perpendicular to the magnetic field. The cell suspension was excited either from the top of the cuvette or along the measuring beam direction (Fig. 1).

Light minus dark difference spectra of unoriented cells were measured with a Cary 17 D spectrophotometer equiped with a side illumination attachment by substracting spectra recorded under continuous illumination from spectra recorded in the dark. Complementary color filters prevented scattered light from reaching the photodetector.

Results

(1) Linear dichroism spectra of oriented Rhodopseudomonas viridis cells

Fig. 2 shows the linear dichroism spectrum ($A_{/\!/} - A_{\perp}$) of cells of *Rhodopseudomonas viridis* oriented in a magnetic field. The strength of the magnetic field was 14.5 kG. Complete saturation of linear dichroism changes occurs for a magnetic field of 20 kG. The magnetoinduced orientation of whole cells of *Rhodopseudomonas viridis* must be related to an anisotropy in the diamagnetic susceptibility of the membranes and to the cooperative effects produced by the regular arrangement of these membranes in the intact cells [21].

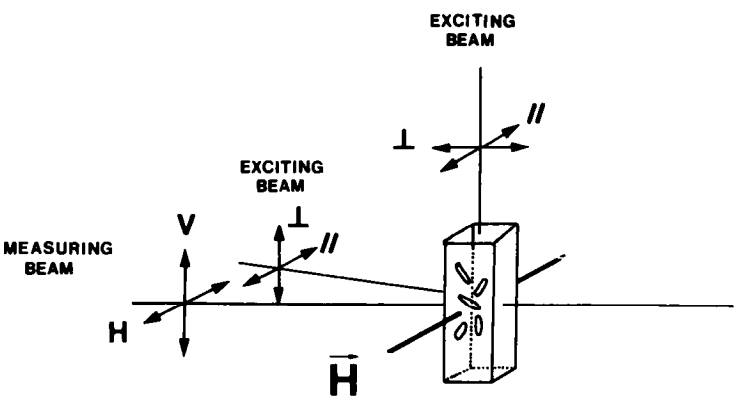

Fig. 1. Diagram of the measuring set up. The cell suspension can be excited by polarized light either from the bottom or from one side of the cuvette.

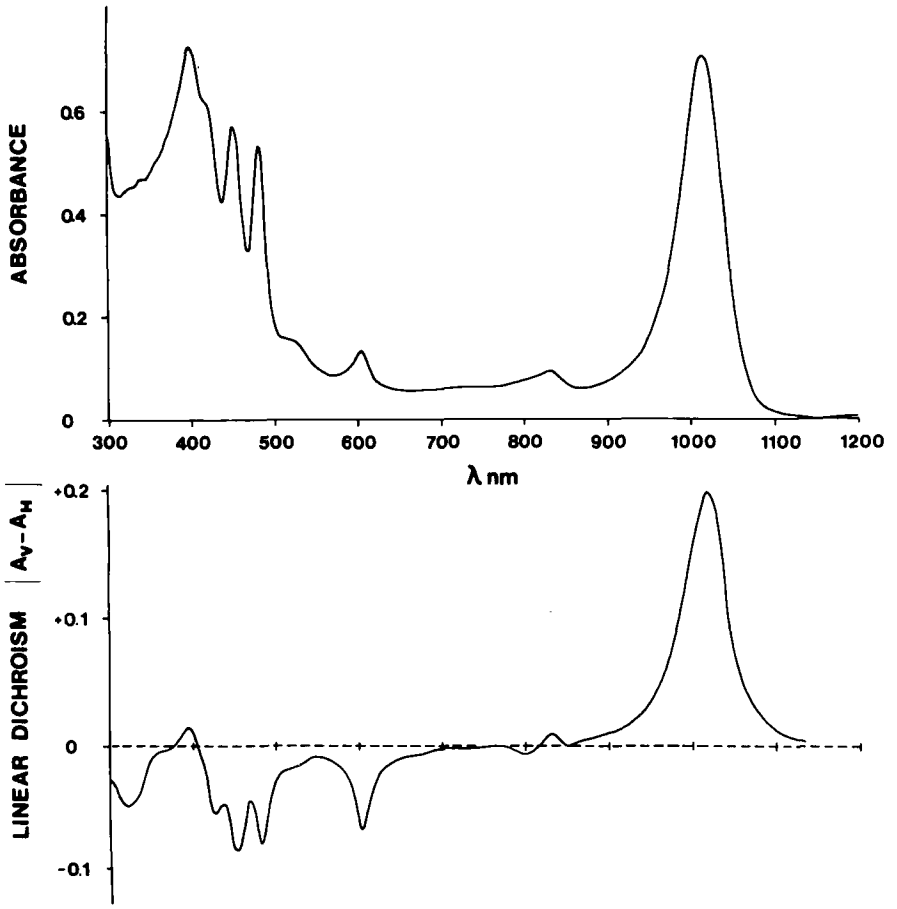

Fig. 2. Top: absorption spectrum of an unoriented suspension of *Rhodopseudomonas viridis* cells suspended in 66% 0.01 M Tris buffer, pH 7.8 and 33% glycerol. Bottom: linear dichroism spectrum of the same cell suspension oriented by a magnetic field (14.5 kG).

The linear dichroism spectrum depicted in Fig. 2 is qualitatively similar to the ones reported [22] for several species of photosynthetic bacteria: the Q_y and Q_x transitions of the bacteriochlorophyll antennae give, respectively, positive and negative values for the linear dichroism and the carotenoid bands give negative linear dichroism values.

Absorption bands around 830 nm are mainly due to reaction center components [25]. Fig. 3 shows linear dichroism of these reaction center bands. Three distincts bands are apparent: two negative ones centered around 850 and 800 nm and a positive one at 830 nm (Fig. 3, part A).

If the cell suspension is subjected to continuous illumination at moderate redox potential (+350, 400 mV) where the state P^+I is expected to accumulate, the 850 nm negative band bleaches and both the 800 nm negative band and 830 nm positive bands slightly decrease in the linear dichroism spectrum (Fig. 3, part B).

At low redox potential (i.e., in presence of few dithionite crystals), the state

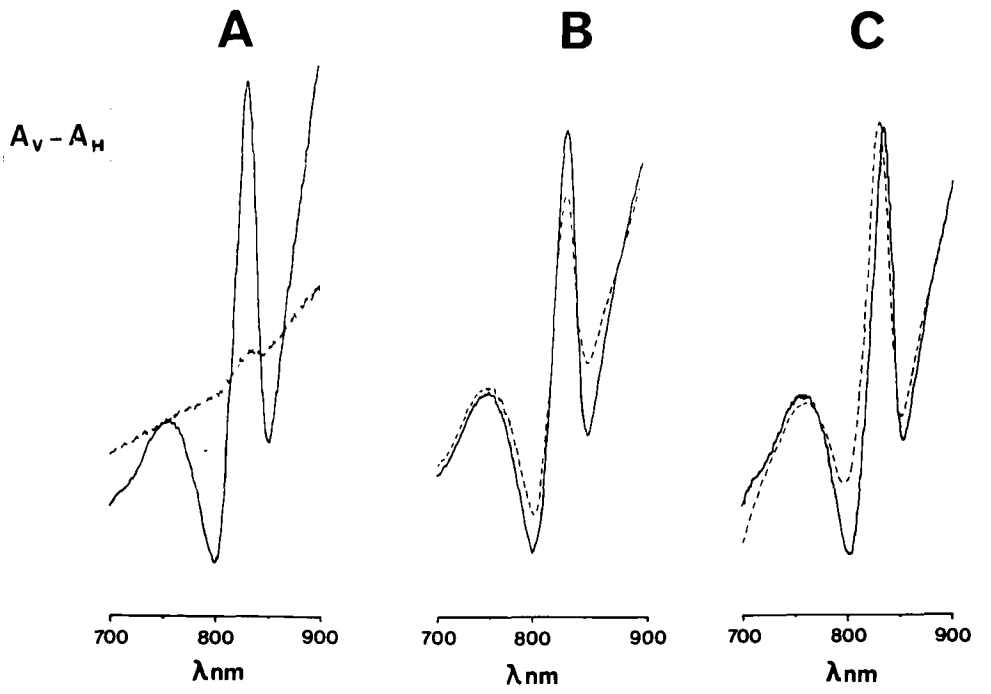

Fig. 3. Linear dichroism spectra of reaction centers bands around 830 nm. A: linear dichroism spectrum recorded in the dark with (———) or without (- - - - -) magnetic field (14.5 kG) B: linear dichroism spectrum recorded in the dark (———) and under blue unpolarized continuous illumination (- - - - -) at moderated redox potential. C: same as B but at low redox potential. The sample had an absorbance at 1010 nm of 0.7 *A* unit.

PI^- is accumulated under continuous illumination [12,13,23]. The changes of linear dichroism spectrum induced by the photoreduction of I are depicted on Fig. 3, part C. Three effects are apparent: (1) bleaching of half of the negative band centered at 800 nm, (2) shift from 830 nm to 820 nm for the positive band, and (3) a slight decrease in the negative band centered at 850 nm.

In the presence of orthophenantroline (2 mM) and ascorbate (10 mM) where continuous illumination can only induce the state cyt^+ PIQ^- no detectable changes in the infrared region of the linear dichroism spectrum are observed (data not shown).

(2) Linear dichroism of absorption changes linked to the oxidation of the primary donor P

Light-induced absorbance changes occuring in unoriented whole cells related to the oxidation of the primary donor P are shown in Fig. 4 (top). These absorption changes are almost identical to those reported previously for oxidation of the primary electron donor in isolated reaction center [12,13,23].

If the *Rhodopseudomonas viridis* cells are oriented in a magnetic field (20 kG) the absorption changes induced by a pulse (66 ms duration) of continuous light depend on the polarization of the analysing beam. Difference spectra recorded point by point are depicted in Fig. 4, bottom, for both

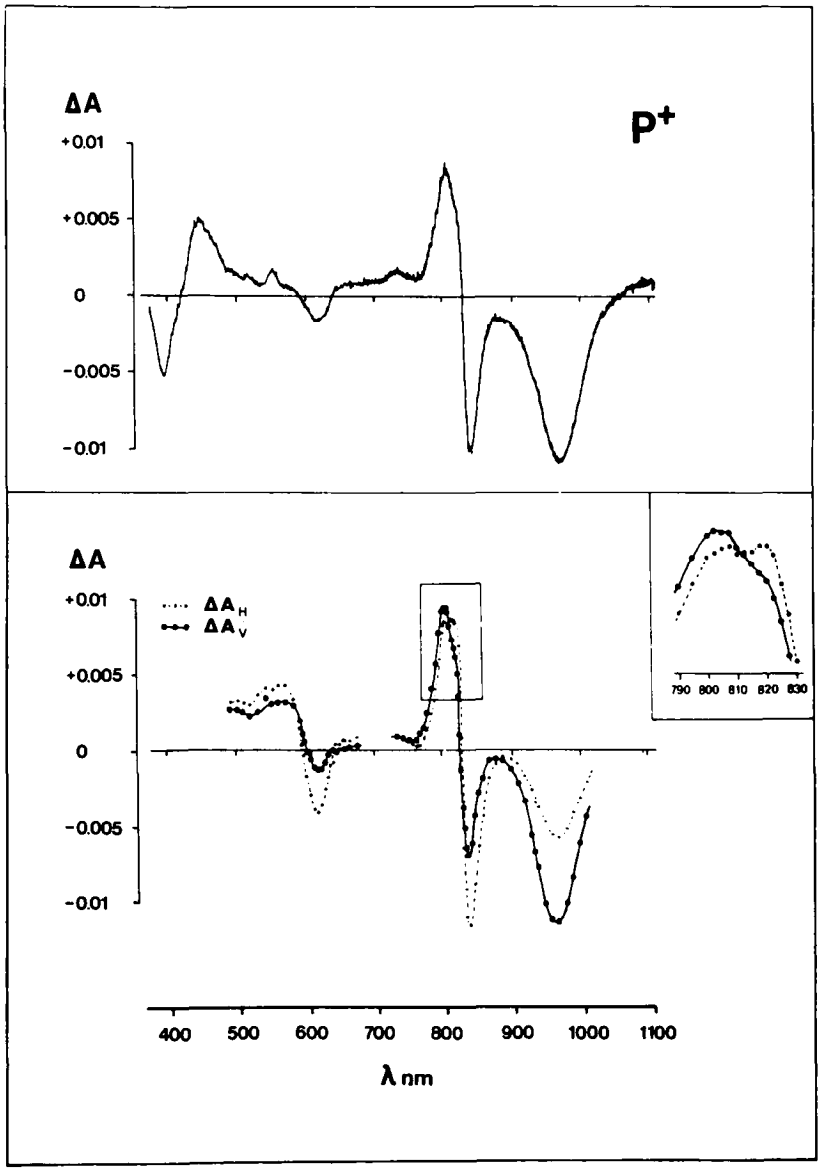

Fig. 4. Top: light minus dark difference spectrum of unoriented cells of *Rhodopseudomonas viridis* recorded at moderate redox potential with a Cary 17 D spectrophotometer. Excitation was provided by unpolarized blue continuous light. Bottom: light minus dark difference spectra of oriented cells of *Rhodopseudomonas viridis* for both polarization of the analysing beam (*------*, ΔA_H; ●———●, ΔA_V). The cells were oriented by a magnetic field (20 kG). Excitation was provided by a pulse (66 ms) on continuous unpolarized blue light. Both samples had an absorbance at 1010 nm of 0.9 *A* unit.

horizontal and vertical polarization of the analysing beam. Qualitatively, the results are similar to those reported previously for chromatophores of *Rhodopseudomonas sphaeroides* oriented by air drying [18]: the long wavelength bleaching (*P*-970) is larger for vertical than for horizontal polarization of the analysing beam while the bleaching centred at 845 nm is greater with the hori-

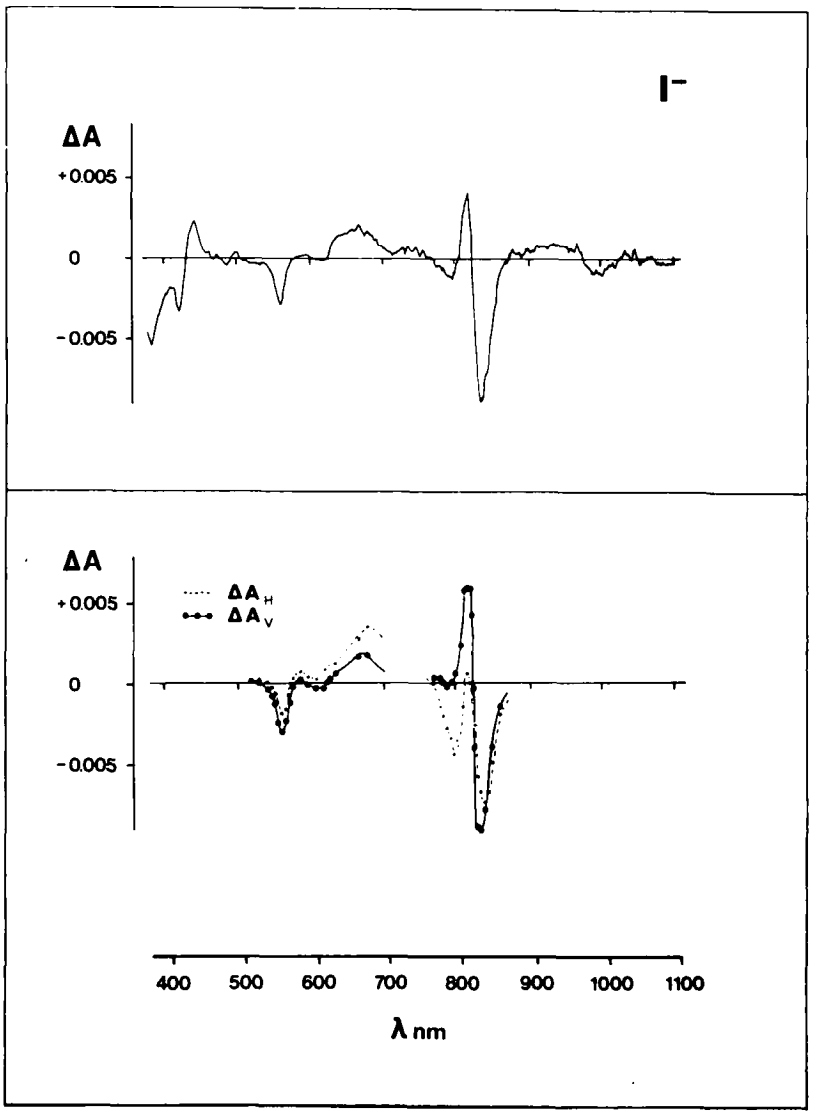

Fig. 5. Same as Fig. 4 but at low redox potential. The time duration of the excitation light was 4 s.

zontal polarization. The situation is more complex for the short wavelength absorption increase (810 nm) as emphasized by the insert, where the vertical changes are larger at 800 nm and smaller at 820 nm than the horizontal ones.

For the bleaching centred at 620 nm, again the situation is analogous to the one reported for chromatophores of *R. sphaeroides* [18] with absorption changes larger for the horizontal polarization of the analysing beam.

(3) Linear dichroism of absorption changes linked to the reduction of the electron carrier I

At redox potentials low enough to chemically reduce the primary electron

acceptor, an intermediate electron carrier I can be accumulated in its reduced form [12,13,23]. Its accumulation is obtained only under strong and prolonged illumination because the back reaction of P^+I^- is about two orders of magnitude faster than the rereduction of P^+ by cytochrome *c*. Fig. 5 (top) shows the light induced changes associated with the reduction of I. No noticeable differences are observed when one compares the difference spectrum obtained in whole cells to those previously reported for isolated reaction centers [12,13, 23].

Fig. 5, bottom, shows the light induced changes related to the reduction of I, for both polarizations of the analysing beam for a magnetically oriented cells suspension. Strong polarization effects are observed specially in the near infrared part where the two difference spectra (H and V) are quite distinct.

Discussion

(1) Calculation of the degree of orientation of whole cells in magnetic field

Measurements of absorption changes, ΔA_V and ΔA_H, polarized along two perpendicular directions on an oriented suspension of membranes are related to both the total absorption changes ΔA and the angle ϕ between the transition implicated in the light-induced variation and the normal to the membrane plane according to the following equations:

$$\Delta A = \Delta A_H + 2\Delta A_V = \sum_i \Delta A_i \tag{1}$$

for a number i of transitions responsible for the absorption changes

$$\Delta D = \Delta A_H - \Delta A_V = S \sum_i S_i \, \Delta A_i \tag{2}$$

where S_i, the order parameter of the ith transition, is equal to:

$$S_i = \frac{3\,\overline{\cos^2}\phi_i - 1}{2} \tag{3}$$

when ϕ_i is defined as the angle between the i^{th} transition and the normal to the membrane plane. S, the order parameter of the suspension is equal to

$$\frac{3\,\overline{\cos^2}\theta - 1}{2} \tag{4}$$

where θ is the angle between the membrane plane and the orientation axis.

Instead of working with the values ΔA_V and ΔA_H, related arbitrarily to the orientation axis, it is more convenient to introduce the quantities $A_{/\!/}$ and $A_\perp$ defined as the absorption changes occurring respectively in the membrane plane and perpendicular to it.

These quantities are determined as follows:

$$\Delta A_\perp = \sum_i \overline{\cos^2}\phi_i \, \Delta A_i \tag{5}$$

and

$$A_{/\!/} = \sum_i \overline{\sin^2}\phi_i \, \Delta A_i = \Delta A - \Delta A_\perp \tag{6}$$

From Eqns. 5, 1 and 2 one may derive:

$$\Delta A_{\perp} = \frac{1}{3}\left(\Delta A + \frac{2\Delta D}{S}\right) \tag{7}$$

and from eqn. 6.

$$\Delta A_{\parallel} = \frac{2}{3}\left(\Delta A - \frac{\Delta D}{S}\right) \tag{8}$$

To calculate $\Delta A_{\perp}$ and $\Delta A_{//}$, we have to know three quantites ΔA, ΔD and S.

ΔA and ΔD can be readily measured experimentally but it is more difficult to estimate the value of the order parameter S. S depends on the degree of orientation but not on the studied transition (see Eqn. 4). It follows that if we can know the quantity $\overline{\cos^2\phi_i}$ (or S_i) for a transition i, the order parameter of the suspension S will be determined. In this respect, the easiest light induced change to study is the bleaching occurring around 970 nm which is related to P^+ because it is due to a single transition well separated from other absorption changes (see Fig. 4). For a single transition Eqn. 2 gives:

$$\frac{\Delta D_{970}}{\Delta A_{970}} = S \cdot S_{970}$$

where S_{970} is the order parameter of the transition absorbing at 970 nm.

Experimentally we measured ΔD_{970} and ΔA_{970} and found that

$$S \cdot S_{970} = -0.14$$

since, for an oriented suspension, S is smaller than unity, $S_{970} < -0.14$.

The smallest possible value of S_{970} is -0.5, obtained for an angle ϕ_{970} of 90°. The value S_{970} is therefore comprised between -0.5 and -0.14.

In order to have a better approximation of the value of S_{970}, one must, if possible, increase the degree of orientation of the particles, in other terms the order parameter S. That cannot be achieved by increasing the magnetic field since we are working at the saturating level. Thus, we have used a method of photoselection of membranes. The rationale of the experiment is the following: the oriented cell suspension absorbs differently light polarized perpendicular or parallel to the magnetic field (Fig. 2). For example at 600 nm one can calculate from Fig. 2 that the absorption parallel to the magnetic field is seven times greater than the absorption perpendicular to the field. If we illuminate the suspension cells with a non saturating 600 nm light propagating perpendicular to the magnetic field direction and polarized parallel to it (see Fig. 1), we are exciting only the cells which have the greater degree of orientation. By this photoselection procedure we are artificially increasing the order parameter S. Using a dye laser operating at 600 nm and for a parallel polarization of the beam, we have observed the light induced bleaching of P-970 for an orientated cells suspension for both horizontal (ΔA_H) and vertical (ΔA_V) polarisations of the analysing beam. Under these conditions the ratio $\Delta D_{970}/\Delta A_{970}$ is equal to -0.23. In fact more information can be gained from this membrane photoselection experiment. Consider a small membrane portion making an angle θ with the magnetic field axis. From Eqns. 1 and 2, the absorption at 600 nm for a

parallel polarisation of the exciting beam is proportional to:

$$1 + (3\cos^2\theta - 1)S_{600}$$

where S_{600} is the order parameter of the pigments absorbing at 600 nm. The absorption changes ΔA_H observed at 970 nm is then proportional to

$$[1 + (3\cos^2\theta - 1)S_{600}][1 + (3\cos^2\theta - 1)S_{970}]$$

After averaging for all the possible values of θ one obtains:

$$\Delta A_H \approx (1 + 2S \cdot S_{600})(1 + 2S \cdot S_{970}) + 9S_{600} \cdot S_{970} \cdot \phi$$

where $\phi = \overline{\cos^4\theta} - (\overline{\cos^2\theta})^2$ is a measure of the fluctuation of the small membrane portion to the orientation axis.

ΔA_H is only related to four parameters S, ϕ which are characteristic of the suspension and S_{600} and S_{970} which are the order parameters associated to the excitation and the analysing wavelengths. It is easy to show that the quantities ΔA_V depend also on the same parameters. If now the absorption changes ΔA_V and ΔA_H are induced by 600 nm excitation light polarized perpendicular to the magnetic field, again ΔA_V and ΔA_H are functions of these four parameters. We therefore possess four different measurements, (ΔA_H and ΔA_V for both vertical and horizontal polarization of the excitation beam) to find four unknown parameters S, ϕ, S_{600} and S_{970}. In fact we have also varied the order parameter S, by decreasing the magnetic field strength and observed light induced changes at other wavelength always for vertical and horizontal polarization of the 600 nm excitation light. Each new experiment introduces a new unknown value but brings four new experimental values, allowing a self consistency check of the equations.

From the average of twenty experiments we have found $S_{970} < -0.45$, indicating that the transition of the dimer absorbing at 970 nm makes an angle smaller than 10° with the membrane plane.

The values of S and ϕ calculated from the preceding photoselection experiments for a saturating magnetic field are $S = 0.32 \pm 0.03$ and $\phi = 0.12 \pm 0.02$.

These values are in good agreement to those calculated for perfect cylindrical membranes [21] oriented perpendicular to the magnetic field axis [22]: $S = 0.25$ and $\phi = 0.125$.

Knowing now the order parameter S of the suspension one can easily calculate, from absorption and linear dichroism spectrum, the absorption occurring perpendicular and parallel to the membrane plane. Fig. 6 gives an example of the result of such a calculation for the reaction center absorption bands peaking at 830 nm. As expected from the linear dichroism spectrum (Fig. 2, part A) a band of low intensity located at 845 nm absorbs mainly perpendicular to the membrane plane. The bacteriopheophytin transitions absorbing around 800 nm are tilted out of the membrane plane as already reported [18] in the case of *Rhodopseudomonas sphaeroides*. The mean value of the angles between the Q_y transitions of the two bacteriochlorophyll molecules absorbing near 830 nm and the plane of the membrane is 25°.

Considering now linear dichroism values of antennae pigments and carotenoids it can be calculated that the carotenoid transitions make an angle of 45° with the membrane plane and that the bacteriochlorophyll molecules of

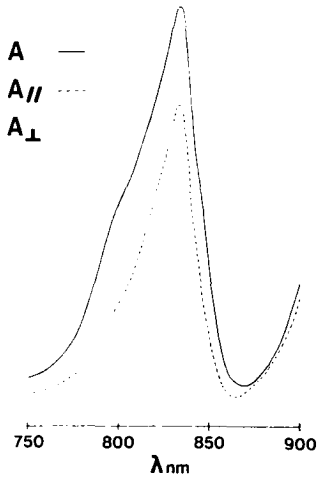

Fig. 6. Absorption of reaction center components in *Rhodopseudomonas viridis* cells (———). Absorptions parallel (- - - - -) and perpendicular (· · · · ·) to the membrane plane were calculated from the equation derived in the discussion and an experimental linear dichroism spectrum as depicted in Fig. 3.

the antennae have their molecular plane nearly perpendicular to the membrane; the Q_x and Q_y transitions making angles of 70° and 20° respectively with the membrane plane. Similar results have been obtained for different species of purple photosynthetic bacteria [22].

(2) Analysis of light induced changes linked to state P^+I

More information concerning the orientation of the reaction center components can be gained from an analysis of the linear dichroism of absorption changes linked to state P^+I and PI^-. Let us first consider solely the absorbance changes related to state P^+I. Fig. 7 shows the decomposition of total absorption changes related to state P^+I in changes occurring perpendicular or parallel to the membrane plane calculated from experiments depicted in Fig. 4. The total absorption changes (Fig. 7, solid line) recalculated from ΔA_V and ΔA_H using Eqn. 1 is in excellent agreement with the difference spectrum recorded with an unpolarized analysing beam on a suspension of unoriented cells (compare Fig. 4, top and Fig. 7, solid line). As already mentionned the 970 nm transition is in the membrane plane: no absorption changes are detectable in the difference spectrum occurring perpendicular to the membrane plane (Fig. 7). For wavelength ranging between 750 and 900 nm the situation is more complex. Both the parallel and perpendicular difference spectra are asymmetrical. Around 850 nm the amplitude of the absorbance changes occurring perpendicular to the membrane plane ($\Delta A_\perp$) is larger than that occurring parallel to the membrane ($\Delta A_{/\!/}$). We interpret this result, as already proposed in the case of *Rhodopseudomonas sphaeroides* reaction centers [18—20], as the disappearance of an electronic transition which is different from the 970 nm band of the dimeric primary electron donor. This interpretation is confirmed by the experiment depicted on Fig. 3, part B, where, in the linear dichroism spectrum, the negative band centered at 845 nm bleaches upon oxidation of *P*-970.

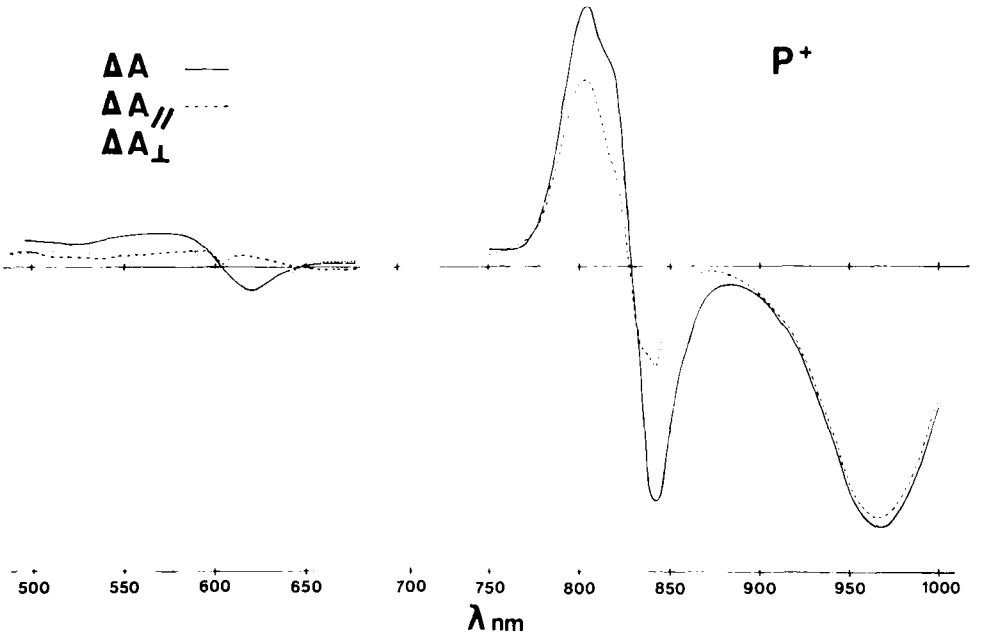

Fig. 7. Light minus dark difference spectra related to state P^+I and calculated from the experimental results depicted in Fig. 5. The total absorption changes (———) are broken down into absorption changes occurring parallel (- - - - - -) and perpendicular (· · · · · ·) to the membrane plane.

Both experiments support the assignment of the 850 nm shoulder in the absorption spectrum of reaction center at 77 K to the primary electron donor by Thornber et al. [25] as well as in the spectra of the absorbance changes associated with the conversion of PI^- to P^*I^- [27]. This second transition (845—850 nm) of the dimer is tilted out of the membrane plane. Due to the low intensity of this band it is difficult to determine precisely the angle between this transition and the membrane. Nevertheless this angle is greater than 54°. Around 805 nm the absorbance changes occur mainly parallel to the membrane plane (see Fig. 7). We attribute these changes to the appearance of the monomer like bacteriochlorophyll transition [18] upon photooxidation of one of the two bacteriochlorophyll molecules of the dimer. The calculated angle between this new transition and the membrane plane is less than 25°. Looking now at the absorption changes occurring in the visible part (Fig. 7) it appears that the main changes occur perpendicular to the membrane plane. However a very small band, centered around 605 nm, can be noticed in the parallel difference spectrum. We tentatively attribute this band which has a small amplitude to the second Q_x band of the primary electron donor.

Putting together the results obtained in both the visible and the near infrared regions, a structure for the bacteriochlorophyll dimer and its position in the membrane can be proposed. We estimate that the angle between the two Q_y transitions is 35°, using the equation $A_{850}/A_{970} = (1 + \cos\beta)/(1 - \cos\beta)$, where A_{850} and A_{970} are the areas of the two near infrared transitions and β the angle between them [28]. A similar value can be estimated for the angle between the two Q_x transitions. Because the resultant Q_y transitions of the dimer absorbing

at 970 nm is in the membrane plane and because the monomer appearing upon its photooxidation makes an angle smaller than 25°, the two Q_y transitions of the bacteriochlorophyll molecules of the dimer must be positionned symmetrically to the membrane plane, making an angle of 17—18° with this plane. This implies that the two bacteriochlorophyll planes are both nearly perpendicular to the membrane. This conclusion is in disagreement with a previous estimation of Clarke et al. obtained from EPR data for the angle between the two bacteriochlorophyll rings in the dimer [29]. However their calculations have been very recently criticized by Hoff and Gorter de Vries [30]. Besides the appearance of a monomer like absorption band near 805 nm and the bleaching of the second band of the dimer centered at 845 nm, there are also absorption band shifts of the bacteriochlorophyll molecules absorbing at 830 nm which give structured features in the $\Delta A_{/\!/}$ and $\Delta A_{\perp}$ difference spectra between 815 and 840 nm (see Fig. 7). These absorption changes due to the bacteriochlorophyll absorption band shifts are more puzzling. First of all, the wavelengths where ΔA is zero, are different for the parallel and perpendicular difference spectra (Fig. 7). This difference will be enhanced if one takes into account the changes due to the appearance of the monomer and the disappearance of the second band of the dimer. This result implies that both bacteriochlorophyll molecules are subjected to an absorption band shift and that the amplitude of the shift is different for each of them and/or that they are absorbing at slightly different wavelengths. On the other hand the amplitude of the shift is greater in the perpendicular light minus dark difference spectrum than in the parallel one. This is at first sight in contradiction with the finding that the average angle for the two bacteriochlorophyll molecules absorbing around 830 nm is smaller than 25°, as we deduced from the linear dichroism spectrum. We expected from the linear dichroism value of the 830 nm band that the $\Delta A_{/\!/}$ absorption changes would have been five times greater than the $\Delta A_{\perp}$ ones. The best way to solve this discrepancy is to suppose that the two bacteriochlorophyll molecules absorbing near 830 nm do not have the same orientation with respect to the membrane plane and that they are shifting in opposite directions. The $\Delta A_{/\!/}$ will be nearly equal to the $\Delta A_{\perp}$ changes if the bacteriochlorophyll molecule which is in the membrane plane is shifting from a short to a long wavelength and if the bacteriochlorophyll molecule which is oriented more out of the membrane plane is shifting from long to short wavelengths. The best fit for both $\Delta A_{/\!/}$ and ΔA difference spectra is observed if one bacteriochlorophyll molecule is flat in the membrane plane and if the other molecule makes an angle of 50° with this plane. The bacteriochlorophyll molecule laying flat in membrane plane must shift from short to long wavelengths. The bacteriochlorophyll molecule tilted out of the plane must shift from long to short and the amplitude of this shift must be three times greater than the previous one. Unpublished results from photoselection studies on isolated reaction centers and low temperature (4.2 K) light minus dark difference spectra confirm this hypothesis.

(3) Analysis of linear dichroism of absorption changes linked to state PI^-

The current interpretation, first suggested by van Grondelle et al. [9], of the light-induced changes of state PI^- is that they are the result of both a bacterio-

chlorophyll band shift and the bleaching of a bacteriopheophytin molecule transition [13,25]. The observed changes in the linear dichroism spectrum (Fig. 3, part C) due to the reduction of I definitively confirm this interpretation. Both effects, the bleaching of one of the bacteriopheophytin molecule and the shift from 830 to 820 nm of the bacteriochlorophyll absorption bands, are clearly seen in the linear dichroism spectrum observed in state PI^-. Only one bacteriopheophytin molecule is bleached in state PI^- as shown by the disappearance of half of the negative band centered at 800 nm in the linear dichroism spectrum (Fig. 3, part C). This result is in agreement with previous reports [4,25]. Decomposition of the absorbance changes observed perpendicular and parallel to the membrane plane for the changes related to the photoreduction of I is shown in Fig. 8. In the parallel difference spectrum only an absorption band shift is observed for both Q_x and Q_y bacteriochlorophyll transitions. An equivalent shift is not observed in the perpendicular difference spectrum which implies that only one molecule has its transition moment subjected to a shift and that the Q_y and Q_x transition of this molecule are respectively parallel and perpendicular to the membrane plane. This is consistent with the interpretation of the absorption band shift linked to state P^+I (preceding paragraph) where we proposed that one of the bacteriochlorophyll molecules has its Q_y transition parallel to the membrane plane. In the perpendicular difference spectrum, besides the bleaching of both Q_y (800 nm) and Q_x

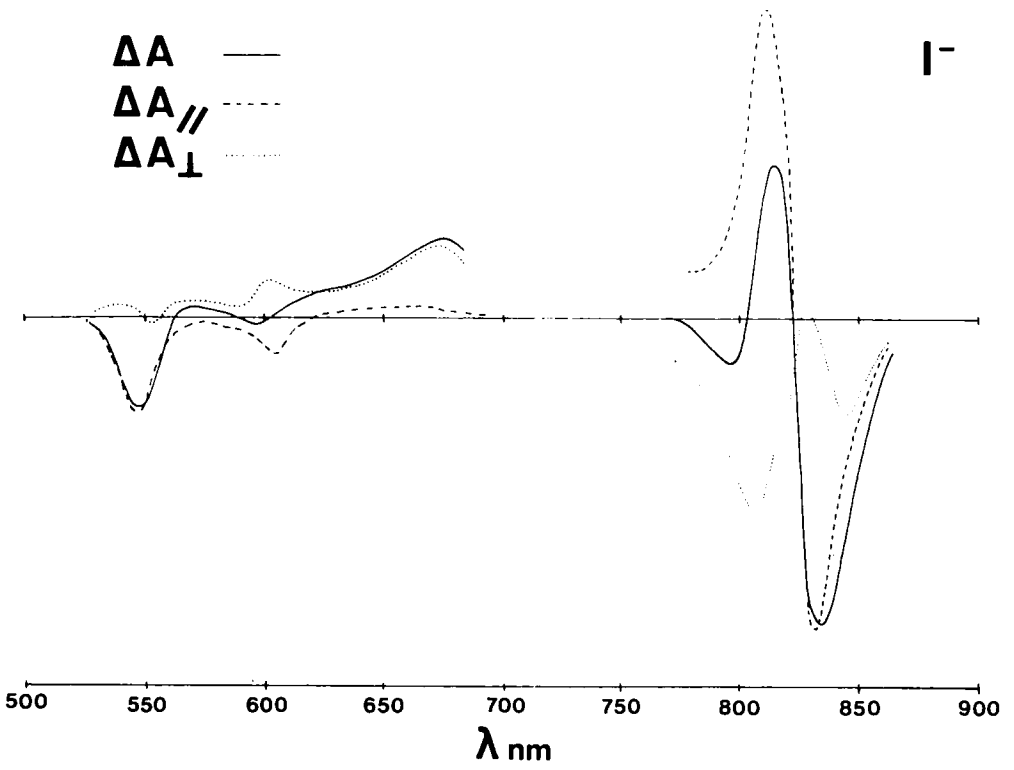

Fig. 8. Same as Fig. 7 but for absorption changes related to state PI^-.

(545 nm) transitions of one of the two bacteriopheophytin molecules, bleaching of bands centered at 845 and 605 nm are also observed (Fig. 8). The wavelength positions of the two bands suggest to us that there are transitions belonging to the primary electron donor (see preceeding paragraph in the Discussion). In the excitonic coupling theory [28], the relative area of the two bands corresponding to the two resultant transitions are dependent on the angle between the monomers's starting transitions. Comparison of the amplitude of the bleaching of the bacteriopheophytin Q_y transitions and of the 845 nm transition suggests, if we assumed that only one bacteriopheophytin molecule is involved in state PI^-, that the 845 nm transition bleaches completely. We therefore propose that the reduction of the intermediate electron carrier I induces a reorientation of the two bacteriochlorophyll molecules constituting the dimer, leading to a bacteriochlorophyll dimer where the two molecules have their planes and Q_y and Q_x transitions more parallel.

Conclusions

The antennae bacteriochlorophyll molecules are perpendicular to the membrane plane. Their Q_y and Q_x transitions make respectively an angle of approximately 20 and 70° to the membrane. The antenna carotenoid molecules make an angle of 45° to the membrane plane.

The bacteriochlorophyll dimer *P*-970 shows a second absorption band at 845 nm corresponding to a transition directed out of the membrane plane while its 970 nm transition is parallel to the membrane plane. The planes of the two bacteriochlorophyll molecules of the dimer are perpendicular to the membrane. The two Q_y transitions make an angle of 35° and are symmetrically arranged with respect to the membrane plane. Upon photooxidation of the primary electron donor, the two other bacteriochlorophyll molecules are subjected to absorption band shifts which occur in the opposite direction. In state PI^- one bacteriopheophytin molecule is photoreduced. This molecule is perpendicular to the membrane plane; its Q_x and Q_y transitions being respectively parallel and perpendicular to the membrane. The photoreduction of this bacteriopheophytin molecule induces an absorption band shift for only one of the two bacteriochlorophyll molecules not involved in the dimer. This bacteriochlorophyll molecule has its Q_y and Q_x transitions respectively parallel and perpendicular to the membrane plane. Finally, the geometry of the dimer seems to be affected by the presence of the negative change on the bacteriopheophytin molecule: the two bacteriochlorophyll molecules reorient to bring their Q_x and Q_y transitions nearly parallel.

References

1 Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597–609

2 Kaufmann, K.J., Dutton, P.L., Netzel, T.L., Leigh, J.S. and Rentzepis, P.M. (1975) Science 188, 1301–1304

3 Rockley, M.G., Windsor, M.W., Cogdell, R.J. and Parson, W.W. (1975) Proc. Natl. Acad. Sci. U.S. 72, 2251–2255

4 Dutton, P.L., Prince, R.C., Tiede, D.M., Petty, K.M., Kaufmann, K.J., Netzel, T.L. and Rentzepis, P.M. (1976) Brookhaven Symp. Biol. 28, 213–237

5 Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 387, 265—278
6 Fajer, J., Brune, D.C., Davis, M.S., Forman, A. and Spaulding, L.D. (1975) Proc. Natl. Acad. Sci. U.S. 72, 4956—4960
7 Tiede, D.M., Prince, R.C., Reed, G.H. and Dutton, P.L. (1976) FEBS Lett. 65, 301—304
8 Tiede, D.M., Prince, R.C. and Dutton, P.L. (1976) Biochim. Biophys. Acta 449, 447—469
9 Van Grondelle, R., Romyjn, J.C. and Holmes, N.G. (1976) FEBS Lett. 72 187—192
10 Shuvalov, V.A. and Klimov, V.V. (1976) Biochim. Biophys. Acta 440, 587—599
11 Netzel, T.L., Rentzepis, P.M., Tiede, D.M., Prince, R.C. and Dutton, P.L. (1977) Biochim. Biophys. Acta, 460, 467—479
12 Trosper, T.L., Benson, D.L. and Thornber, J.P. (1977) Biochim. Biophys. Acta 460, 318—330
13 Prince, R.C., Tiede, D.M., Thornber, J.P. and Dutton, P.L. (1977) Biochim. Biophys. Acta 462, 467—490
14 Norris, J.R., Prayan, M.E. and Katz, J.J. (1973) J. Am. Chem. Soc. 95, 1680—1682
15 Feher, G., Hoff, A.J., Isaacson, R.A. and Ackerson, L.D. (1975) Ann. N.Y. Acad. Sci. 244, 239—259
16 Norris, J.R., Uphaus, R.A., Crespi, H.L. and Katz, J.J. (1973) Proc. Natl. Acad. Sci. U.S. 68, 625—628
17 McElroy, J.D., Feher, G. and Mauzerall, D. (1972) Biochim. Biophys. Acta 267, 363—374
18 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500—515
19 Rafferty, C.N. and Clayton, R.K. (1978) Biochim. Biophys. Acta 502, 51—60
20 Vermeglio, A., Breton, J., Paillotin, G. and Cogdell, R. (1978) Biochim. Biophys. Acta 501, 514—530
21 Drews, G. and Giesbrecht, P. (1965) Arch. Mikrobiol. 52, 242—250
22 Breton, J. (1974) Biochem. Biophys. Res. Commun. 59, 1011—1017
23 Shuvalov, V.A., Krakhmaleva, I.N. and Klimov, V.V. (1976) Biochim. Biophys. Acta 449, 597—601
24 Gagliano, A.G., Geacintov, N.E. and Breton, J. (1977) Biochim. Biophys. Acta 461, 460—474
25 Thornber, J.P., Dutton, P.L., Fajer, J., Forman, A., Holten, D., Olson, J.M., Parson, W.W., Prince, R.C., Tiede, D.M. and Windsor, M.W. (1977) Proc. Fourth Int. Congr. Photosynthesis (Hall, D.O., Combs, J., Goodwin, T.W., eds.), pp. 55—70
26 Breton, J., Michel-Villaz, M. and Paillotin, G. (1973) Biochim. Biophys. Acta 314, 42—56
27 Holten, D., Windsor, M.W., Parson, W.W. and Thornber, J.P. (1978) Biochim. Biophys. Acta 501, 112—126
28 Kasha, M., Rawls, H.R. and El-Bayoumi, M.A. (1965) Pure Appl. Chem. 11, 371—392
29 Clarke, R.H., Connors, R.E., Frank, H.A. and Hochl, J.C. (1977) Chem. Phys. Lett. 45, 523—528
30 Hoff, A.J. and Gorter de Vries, H. (1978) Biochim. Biophys. Acta 503, 94—106