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Photoreactions of bacteriopheophytins and bacteriochlorophylls in reaction centers of *Rhodopseudomonas sphaeroides* and *Chloroflexus aurantiacus*

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A comparison of spectral properties of reaction centers from *Chloroflexus aurantiacus* and *Rhodopseudomonas sphaeroides* (R-26) is reported. Treatment of reaction centers from *Rps. sphaeroides* with $NaBH_4$ leads to a decrease of the dipole strength of the 800-nm band by factor of approx. 1.75–1.95 and to the formation of new bacteriopheophytin, BPh-715, which is almost completely removed during the purification of reaction centers. The modification of the reaction centers does not change the quantum yield of P photooxidation and the spectrum of BPh-545 (H_1) photoreduction which includes the changing of the 800-nm band. This implies the preservation of the photoactive chain P-B_1-H_1-Q_A (where B_1 is the bacteriochlorophyll (BChl)-800 molecule situated between P and H_1) and the modification of the second BChl-800 (B_2). The preparation of modified reaction centers is a mixture of at least three types of reaction centers with different contents of B_2 and of the second BPh (H_2). Some of the reaction centers (5–25%) contain the original B_2 and H_2 molecules (type I). In the CD spectrum of modified reaction centers a decrease of the 800-nm band and the appearance of a positive band at 765 nm is observed. This spectrum is similar to the CD spectrum of *Chloroflexus* reaction centers containing 3 BPh's and 3 BChl's. This implies that in some (approx. 40%) of the modified *Rps. sphaeroides* reaction centers (type II) B_2 has been replaced by BPh *a* which interacts with H_2. Probably some of the modified reaction centers (approx. 40%) have lost both B_2 and H_2 (type III). The modification of reaction centers leads to a considerable decrease of the CD bands at 800 (+) nm and 810 (−) nm and to a decrease of the absorbance changes near 800 nm in the difference absorption spectrum of the oxidation of P. The data are interpreted in terms of the interaction between P and B_1 molecules which gives two transitions at 790–800 and 810 nm with different orientations in modified *Rps. sphaeroides* as well as in *Chloroflexus* reaction centers. Similar transitions are observed for the interaction between P and B_2. The spectral analysis shows the existence of two chains P-B_1-H_1, and P-B_2-H_2 in which the distances between the centers of molecules are 1.3 nm or less.

Introduction

Reaction centers of purple bacteria contain three polypeptides, four molecules of bacteriochlorophyll (BChl), two molecules of bacteriopheophytin (BPh), one or two quinones and one

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; P, primary electron donor, BChl dimer; B_1 and B_2, BChl's absorbing at 800 nm; H_1 and H_2, BPh's absorbing at 545 nm and 530 nm, respectively; LDAO, lauryldimethylamine *N*-oxide; Q_A and Q_B, primary and secondary quinones, respectively.

non-heme iron (see for a review, Refs. 1–3). Two BChl's form the primary electron donor, P, absorbing at 870 nm (or 960 nm for BChl *b* containing bacteria), one BChl absorbing at 800 nm (or 830 nm for BChl *b*) is the primary electron acceptor B_1 [4,5], one BPh absorbing at 545 nm (Q_x transition) and 760 nm (or 800 nm for BPh *b*) (Q_y transition) is the secondary electron acceptor H_1 [4–6] and two quinones are the terminal acceptors Q_A and Q_B [1–3]. Picosecond selective spectroscopy has shown that an electron from excited P (P*) is transferred to B_1 within less than 1 ps [5] with the formation of the state $^1[P^+B_1^-]$ which is mixed with P* and lies approx. 0.01 eV below the pure state P* [7]. The observation of the state $P^+B_1^-$ was not confirmed by other laboratories (see Ref. 8) for reasons discussed elsewhere [7]. A second step of electron transfer occurs within 4–7 ps [1–3,5,7] with the formation of the state $P^+H_1^-$ and an energy loss of approx. 0.03–0.1 eV. Electron transfer from H_1^- to Q_A takes 150–200 ps [1–3]. Using photodichroism and circular dichroism data, a scheme of photoactive pigment arrangmeent in *Rhodopseudomonas viridis* reaction centers was proposed [9] where the two monomeric BChl molecules (B_1 and B_2) lie on the same side of the P dimer and are separated from P by a center-to-center distance of approx. 1.0–1.3 nm, and where B_1 is located between P and H_1 with a center-to-center distance of 1.3 nm or less between B_1 and H_1. This model is in good agreement with the recent X-ray structure analysis of *Rhodopseudomonas viridis* reaction centers [10] which gives the same arrangmeent of P, B_1, B_2 and H_1 molecules with a center-to-center distance of 1.3 nm between P and B_1, P and B_2, and of 1.1 nm between B_1 and H_1. X-ray analysis gives also information about the second BPh (H_2) which is part of the nonactive secondary chain P-B_2-H_2-Q_B symmetrical to the photoactive chain P-B_1-H_1-Q_A [10].

Quantum mechanical calculations based on the picosecond electron transfer rates and singlet-triplet splittings in the charge transfer states estimated minimal edge-to-edge distances of approx. 0.3 nm between the macrocycles of P and B_1 and of 0.41–0.53 nm between B_1 and H_1 [11] for *Rps. sphaeroides* R-26 reaction centers.

Reaction centers isolated from the gliding thermophilic green bacterium *Chloroflexus aurantiacus* are similar to those from purple bacteria and cotnain two 26 kDa polypeptides [12], three BChl's, three BPh's [13] and two menaquinones [13,14] as terminal acceptors. Picosecond masurements have shown [15] the bleaching of the BPh bands at 750 nm and 540 nm at 53 ps delay after excitation pulses at 600 nm. It was suggested that BPh is an intermediate acceptor between P and Q. It is not clear which BChl is replaced by a BPh in these reaction centers and how this replacement affects the quantum efficiency of charge separation in reaction centers.

Another modification of reaction centers from purple bacteria is reported for *Rhodopseudomonas sphaeroides* R-26 reaction centers treated with $NaBH_4$ [16]. It has been found that this treatment leads to a relative decrease of the absorption band at 800 nm. No information was given [16] about charge separation in treated reaction centers and the interaction between pigments.

This paper presents the results of an investigation of the pigment interaction and efficiency of the charge separation in intact and modified reaction centers from *Rps. sphaeroides* R-26 and *Chloroflexus* reaction centers.

Materials and Methods

Reaction centers from *Rps. sphaeroides* (the blue-green carotenoidless strain R-26) were isolated by treatment of chromatophores with lauryldimethylamine *N*-oxide (LDAO) followed by DEAE-cellulose chromatography. Isolation of purified cytoplasmic membranes from *Chloroflexus aurantiacus* was performed as described by Feick et al. [17]. Final purification was achieved by centrifugation using a three-step sucrose density gradient (10%, 20%, 30%, w/v). The membrane fraction was found between 10% and 20% sucrose concentrations. Reaction centers were isolated from the membrane fraction by treatment of cytoplasmic membranes ($A_{860} = 2$) with 1% LDAO and DEAE-cellulose chromatography.

The modification of R-26 reaction centers with $NaBH_4$ was carried out as described in [16]. When the A_{800}/A_{870} ratio reached 1.4–1.5 the reaction centers were dialyzed against 100 mM Tris-HCl (pH = 8.0) buffer containing 0.1% LDAO and were purified using DEAE-cellulose chromatography.

Light-minus-dark difference absorption spectra in unpolarized and linearly polarized light and circular dichroism (CD) spectra were measured as described earlier [9].

Results

Fig. 1 shows the absorption spectra of R-26 reaction centers before (dashed line) and after (solid line) the treatment with $NaBH_4$ and purification (see Materials and Methods) (we will call this preparation 'modified reaction centers'). One can see that in modified reaction centers the A_{800}/A_{870} ratio is decreased by a factor of approx. 1.55 at 295 K and of 1.39 at 77 K with respect to that of intact reaction centers. Furthermore, the low-temperature spectrum (Fig. 1B) shows a narrowing of the 800-nm by a factor of approx. 1.25 after the modification. If the narrowing of the 800-nm band is the same for 295 K, the dipole strength of the 800-nm band is decreased by a factor of approx. 1.95 at 295 K after the modification. The dipole strength of the 800-nm band at 77 K is decreased by a factor of approx. 1.75. Thus the limits of the dipole strength decrease of the 800-nm band are between 1.75 and 1.95. The spectra show the appearance of a small band at 715 nm in modified reaction centers. The main fraction of the 715-nm absorption [16] was eliminated after the purification. In the Q_x transitions region one can see a relative increase of the 545-nm band and the development of bands at 523 and 575 nm.

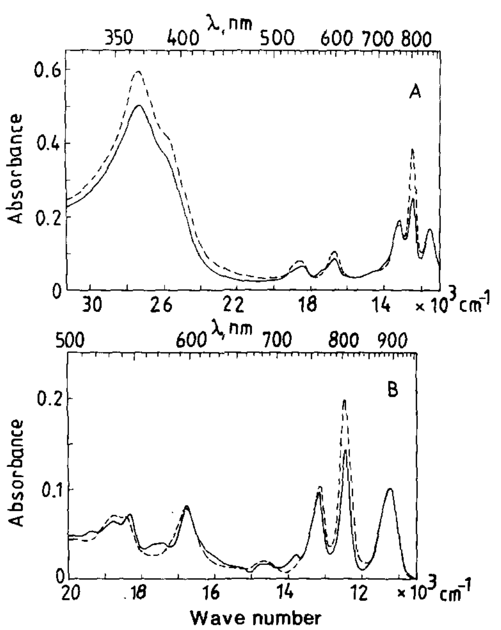

Fig. 1. Absorption spectra of intact (dashed line) and modified (solid line) reaction centers from *Rhodopseudomonas sphaeroides* R-26 at 295 K (A) and 77 K (B).

Both the intact and modified R-26 and *Chloroflexus* reaction centers are capable of photooxidation of the special pair P upon illumination. Measurements of the relative quantum yield of P photooxidation were carried out as described earlier [6] with intact and modified R-26 and *Chloroflexus* reaction centers with excitation at 855 nm of reaction centers samples with equal absorption at 855 nm. The relative quantum yield (ϕ) was determined from the comparison of rates of the photooxidation of P at the beginning of the illumination when the rate is not distorted by the recombination process and the decrease in the amount of reduced P. In this way a direct comparison of the quantum yields is possible; it was found that for modified reaction centers from *Rps. sphaeroides* R-26 and *Chloroflexus* reaction centers the yield was $100 \pm 5\%$ and $61 \pm 5\%$, respectively, as compared to that for normal R-26 reaction centers. Since the absolute value of ϕ for normal R-26 reaction centers is 1.02 ± 0.04 [18], the absolute values of ϕ are approx. 1 and 0.6 for modified R-26 and *Chloroflexus* reaction centers, respectively. This means that the modification of R-26 reaction centers does not affect the very efficient light conversion in these reaction centers. We therefore conclude that the treatment with $NaBH_4$ modifies only one BChl molecule (B_2) which does not participate in electron transfer. The relatively small ϕ for *Chloroflexus* reaction centers may be due to insufficient electron transfer to quinones, which were partially removed from the reaction centers during the purification and replaced by ubiquinone$_6$.

Redox titration of a P oxidation in intact and modified R-26 and *Chloroflexus* reaction centers (Fig. 2) showed that the modification of reaction centers from *Rps. sphaeroides* R-26 does not change the redox potential (E_m) of P (454 ± 5 mV). In agreement with the redox titration of P in

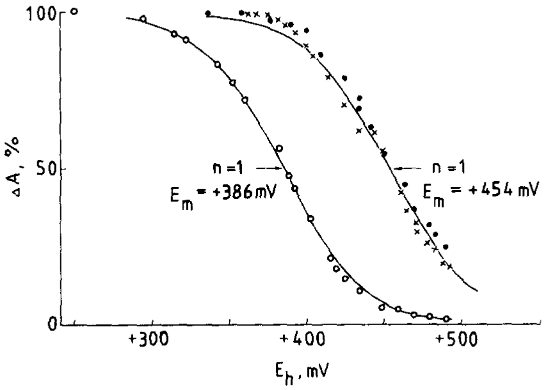

Fig. 2. Redox titration of photooxidation of P in intact (●) and modified (×) reaction centers from *Rhodopseudomonas sphaeroides* R-26 and *Chloroflexus* reaction centers (○). Redox titration was carried out by changing the relative concentration of ferro- and ferricyanide. The solid lines represent theoretical curves obtained from the Nernst equation for $n = 1$ and $E_m = +386$ mV and $E_m = +454$ mV, respectively. A_{870} was 0.1.

cytolasmic membranes of *Chloroflexus* [12] the redox potential (E_m) of P in reaction centers from *Chloroflexus* (386 ± 5 mV) is more negative than that of R-26 reaction centers.

Fig. 3 shows the excitation spectra of the P photooxidation in intact and modified R-26 and in *Chloroflexus* reaction centers. One can see that excitation in all bands in the near infrared region is effective in the photooxidation of P. The action spectrum for reaction centers from *Chloroflexus* was very close to the absorption spectrum of these reaction centers described in [15] and showed two BChl transitions near 786 and 813 nm.

Fig. 4 shows the circular dichroism spectra of intact and modified R-26 and *Chloroflexus* reaction centers in the normal (Fig. 4A) and in the oxidized (Fig. 4B) state. One can see that the modification of R-26 reaction centers leads to a decrease of the negative 815-nm band and to a decrease by a factor of approx. 2 of the positive 800-nm band of oxidized reaction centers where the 815-nm band is not observed and does not interfere with the 800-nm band. The CD spectra of modified reaction centers in the BPh absorption region are similar to those of reaction centers from *Chloroflexus*, both at room (Fig. 4A and B) and at low (Fig. 4C and Refs. 20,21) temperature, and show the splitting of the 760-nm band into a negative and a positive component. The CD spectrum of *Chloroflexus* reaction centers shows two BChl bands with opposite signs at 790 and 816

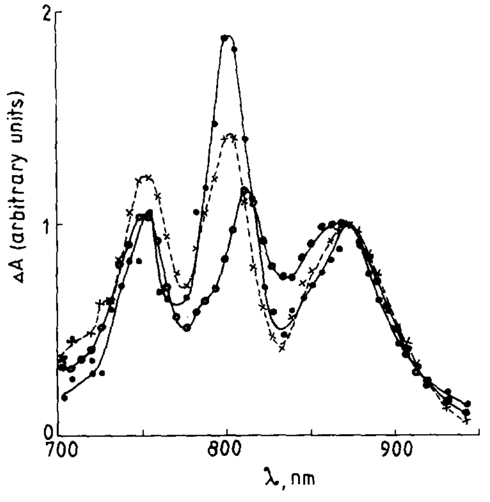

Fig. 3. Action spectrum of photooxidation of P in intact (●) and modified (×) *Rhodopseudomonas sphaeroides* R-26 reaction centers and in *Chloroflexus* reaction centers (○) at 295 K, obtained using the phosphoroscopic set-up to separate the measuring and excitation in the time. The spectral width of the excitation was 7 nm. A_{870} was 0.1.

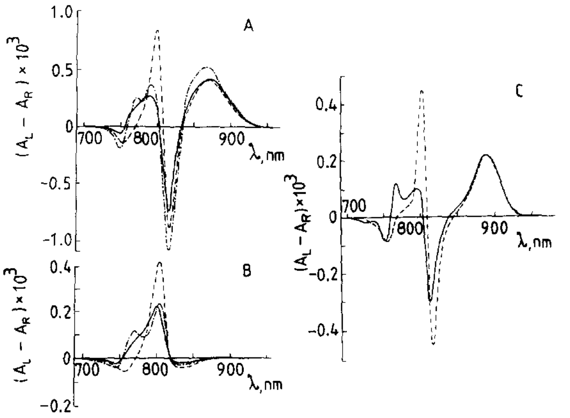

Fig. 4. Circular dichroism spectra in intact (------) and modified (———) reaction centers from *Rhodopseudomonas sphaeroides* R-26 and of reaction centers from *Chloroflexus* (·–·–·). (A) Reaction centers ($A_{870} = 0.11$) in the state $PB_1H_1Q_A$ at room temperature. (B) Reaction centers (initial A_{870} was 0.05) in the state $P^+B_1H_1Q_A$ at room temperature. (C) Reaction centers in the state $PB_1H_1Q_A$ at 77 K. In Fig. 4C the spectra were normalized at 890 nm; the vertical scale applies to the spectrum of modified reaction centers.

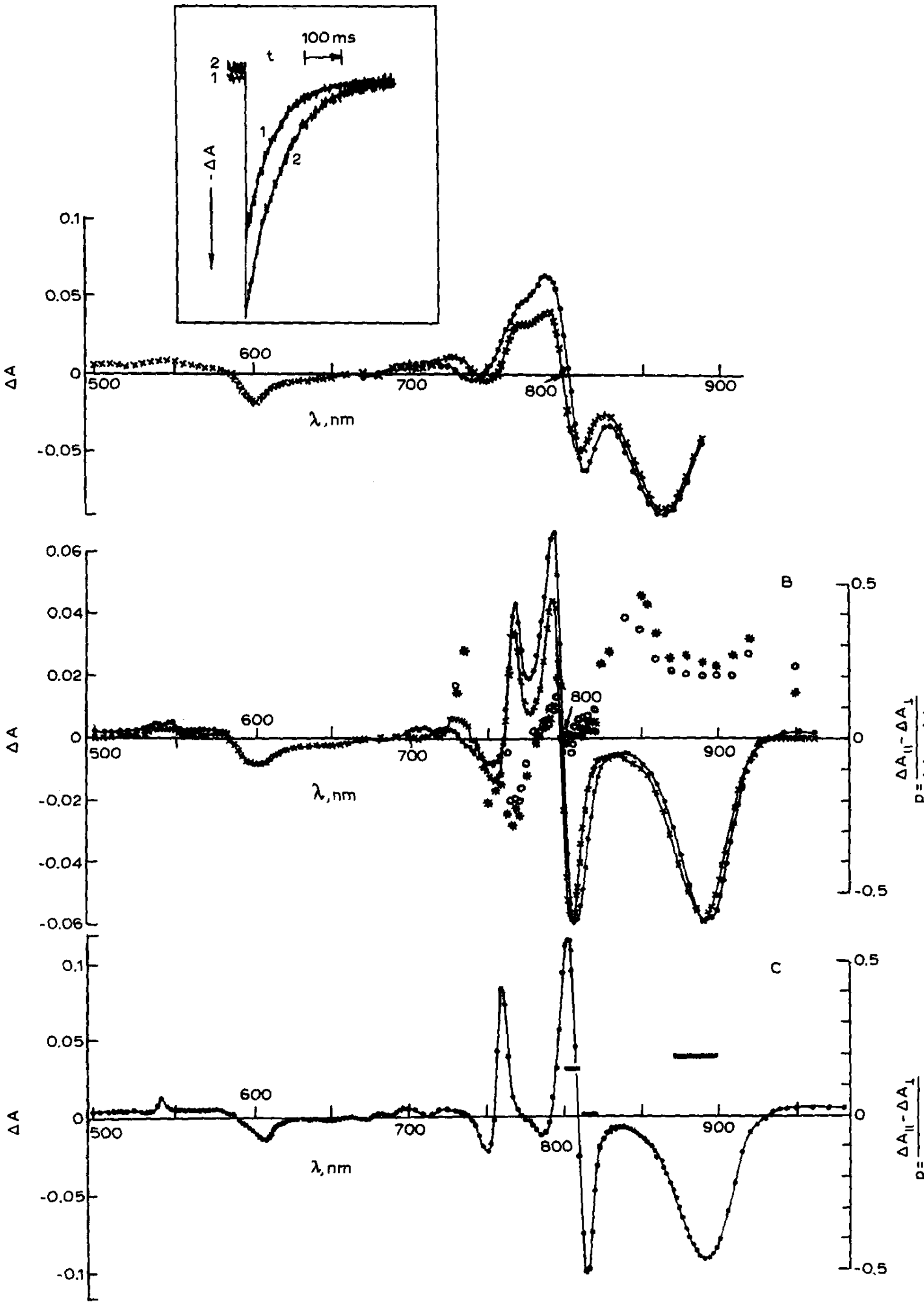

Fig. 5. Difference (light-minus-dark) absorption spectra for photooxidation of P in intact (●) and modified (×) *Rhodopseudomonas sphaeroides* R-26 reaction centers at 295 K (A) and 77 K (B) and in *Chloroflexus* reaction centers at 77 K (C). The spectrum of the polarization $p = (\Delta A_{\parallel} - \Delta A_{\perp})(\Delta A_{\parallel} + \Delta A_{\perp})$ is shown for intact (O) and modified (*) *Rps. sphaeroides* R-26 reaction centers (B) and for *Chloroflexus* reaction centers by horizontal bars (C). A_{870} was 0.11 for A and B and 0.2 for C. Inset: kinetics of ΔA at 850 nm induced by 20 ns pulses at 694 nm in intact (1) and modified (s) reaction centers from *Rps. sphaeroides* R-26 at 295 K. A_{860} was 0.06 for curve 1 and 0.09 for curve 2.

nm. The rotational strength of the 790-nm band is smaller than that of the 800-nm band for intact and approximately equal to that for modified R-26 reaction centers.

Fig. 5 shows light-minus-dark difference absorption spectra of the photooxidation of P in intact and modified R-26 (Fig. 5A and B) and *Chloroflexus* (Fig. 5C) reaction centers at room (Fig. 5A) and at low (Fig. 5B and C) temperature. The modification of one BChl molecule in R-26 reaction centers leads to a decrease of absorbance changes around 800 nm and the appearance of a new band at 730 nm upon the oxidation of P. The absorbance decrease at 750 nm becomes more pronounced. The spectrum of P^+ formation in *Chloroflexus* reaction centers in the near-infrared is similar to that described earlier [20,21] and shows the bleaching of two BChl transitions at 790 and 815 nm and the development of a new transition at 804 nm.

Fig. 5B and C also show the spectra of the polarization $p = (\Delta A_{\parallel} - \Delta A_{\perp})(\Delta A_{\parallel} + \Delta A_{\perp})$ for the absorbance changes (ΔA) (see Ref. 9) induced by the photooxidation of P upon continuous illumination at 890 nm at 77 K. The intact and modified R-26 (Fig. 5B) and *Chloroflexus* (Fig. 5C) reaction centers reveal the same spectrum of p. The polarization value of the Q_y band of P of R-26 reaction centers is smaller by a factor of approx. 2 than that reported for millisecond measurements [22] due to reasons which are not completely clear. This should be taken into account when the angles between the transitions are estimated: the measured value of p and this value multiplied by a factor of 2 correspond to the minimal and maximal value of the angle, respectively. However, the negative polarization of the shifting BPh band at 750–765 nm approaches the maximal value (approx. −0.3). The angles between the dipole transition moment (μ) at 880 nm and that of the developing transitions at 770 and 790 nm were estimated to be $85 \pm 10°$ and $30 \pm 10°$, respectively, for all measured reaction centers. The bleaching band near 810 nm has low polarization values for all measured reaction centers and the developing band at 730 nm has the positive polarization value for modified R-26 reaction centers.

The kinetics of recombination of P^+ and Q_A^- in intact and modified R-26 reaction centers are shown in Fig. 5A (inset). One can see that the modification of these reaction centers do not change the rate of recombination of P^+ and Q_I^-.

Fig. 6 shows difference absorption spectra for the photoaccumulation of reduced BPh-545 at low redox potential in intact and modified R-26 (Fig. 6A) and *Chloroflexus* (Fig. 6B) reaction centers. One can see that the difference absorption spectra for intact and modified R-26 reaction centers are almost identical. This implies the preservation of the electron-transfer chain $P\text{-}B_1\text{-}H_1$ and of the interaction between B_1 and H_1 during modification of the reaction centers by $NaBH_4$. The spectra are characterized by the bleaching of BPh bands at 545 and 760 nm, by a blue shift of the B band at 800 nm and a decrease of its dipole strength, and by the development of the radical anion band at 660 nm.

The difference spectrum for the photoreduction of BPh-542 in reaction centers from *Chloroflexus* (Fig. 6B) is characterized by the bleaching of BPh bands at 542 and 760 nm, by a blue shift of the BChl band at 813 nm and a decrease of its dipole strength, by an increase of the dipole strength of

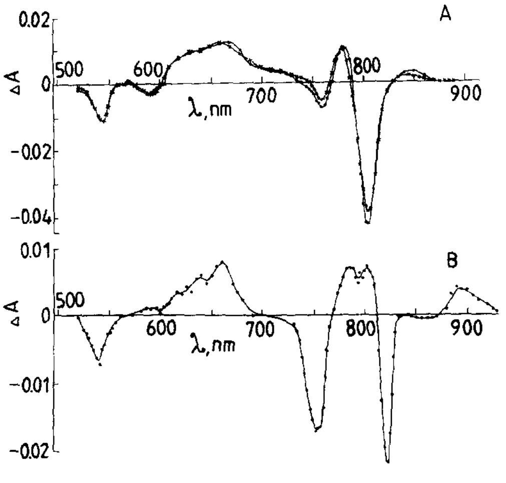

Fig. 6. Difference (light-minus-dark) spectrum of photoreduction of BPh (H_1) in intact (●) and modified (×) *Rhodopseudomonas sphaeroides* R-26 reaction centers (A) and in *Chloroflexus* reaction centers (B) at 295 K. The reaction mixture contained indigo tetrasulfonate (10^{-4} M), neutral red (10^{-4} M) and dithionite (1 mg/ml). A_{870} was 0.2. The spectra in Fig. 6A were normalized at 545 nm; the vertical scale applies to the spectrum of modified reaction centers.

the transition at 790 nm and by the development of the BPh radical anion band at 660 nm.

Discussion

Treatment of reaction centers from *Rps. sphaeroides* R-26 with $NaBH_4$ [16] leads to a relative decrease of the absorption at 800 nm. A new molecule absorbing at 715 nm is formed by this treatment [16] which is practically completely removed after the purification of reaction centers (Fig. 1).

The modification of reaction centers does not change the yield of the effective charge separation in reaction centers. This implies that the modification does not change the chain of molecules participating in the charge separation: $P\text{-}B_1\text{-}H_1\text{-}Q_A$ [4]. This suggestion is supported by the preservation of the interaction between B_1 and H_1 indicated by the similarity of the difference absorption spectra of H_1 photoreduction in modified and intact reaction centers (Fig. 6).

This conclusion suggests that the modification of pigments in reaction centers is mainly related to the modification of the B_2 and H_2 molecules. Since the dipole strength of the band at 800 nm is decreased by factor of 1.75–1.95 which is less than 2, some reaction centers (5–25%) appear to contain the normal B_2 and H_2 molecules. The decrease of the BChl CD band at 800 nm and the appearance of the conservative spectrum in the BPh region with a new positive band at 765 nm may indicate that in some reaction centers (approx. 40%) B_2 is replaced by a new BPh *a* (H_3) molecule, which interacts with H_2 according to the expression for the rotational strength (R_A) of interacting transitions [9]:

$$R_A = -\frac{2\pi}{c}\sum_{j \neq i}\sum_{b \neq a}\frac{V_{ioa;job}\nu_a\nu_b(\boldsymbol{R}_j - \boldsymbol{R}_i)\cdot(\boldsymbol{\mu}_{job} \times \boldsymbol{\mu}_{ioa})}{h(\nu_b^2 - \nu_a^2)} \tag{1}$$

where

$$V_{ioa;job} = \frac{\boldsymbol{\mu}_{ioa}\boldsymbol{\mu}_{job}}{R_{ij}^3} - 3\frac{(\boldsymbol{\mu}_{ioa}\cdot\boldsymbol{R}_{ij})\cdot(\boldsymbol{\mu}_{job}\cdot\boldsymbol{R}_{ij})}{R_{ij}^5} \tag{2}$$

and $\boldsymbol{R}_j - \boldsymbol{R}_i = \boldsymbol{R}_{ij}$ is the distance between the centers of the chromophores i and j, in which the transitions a ← o and b ← o are observed, respectively; μ is the electric dipole transition moment.

Indeed, the CD spectrum of modified reaction centers in the 730–800 nm region is very similar to that of *Chloroflexus* reaction centers (Fig. 4) which have been shown to contain 3 BChl and 3 BPh [13]. These data suggest that H_3 is situated near H_2. The positive sign of the new CD band in modified R-26 reaction centers might indicate that the Q_y transition of H_3 is oriented in the same way as that of B_2. This agrees with a new blue shift of the BPh band in the P^+ spectrum (Fig. 5) accompanied by the appearance of a new band at 730 nm with a positive polarization, in contrast to the normal BPh band shift to the red with a negative polarization. The replacement of B_2 by H_3 should also be seen in the absorption spectrum. A small increase of the dipole strength of the band at 760 nm is indeed observed in the absorption and action spectra at 295 K, but not in the absorption spectrum at 77 K. We therefore conclude that some of the modified reaction centers do not contain H_2, B_2 and H_3 molecules at all, in agreement with the smaller rotational strengths for modified R-26 as compared to those for *Chloroflexus* reaction centers in the region of 730–775 nm (Fig. 4A). It thus appears that approx. 40% of the reaction centers contain PH_3H_2, approx. 15% PB_2H_2 and approx. 40% do not contain B_2, H_2 and H_3. The action spectra of the photooxidation shows (Fig. 3) that all pigments seen in the absorption spectra transfer their excitation energy to P and therefore are close to P inside the modified reaction centers. The band at 715 nm which appears during the treatment with $NaBH_4$ [16] is almost completely removed from reaction centers after purification. However, a small contribution from the band at 715 nm can be seen in the absorption (Fig. 1), CD (Fig. 4) and action (Fig. 3) spectra for modified reaction centers. This implies that the remaining fraction of reaction centers (approx. 5–10%) contains BPh-715 participating in excitation transfer.

Now we discuss the origin of the transitions at 800 and 810 nm in R-26 and at 790 and 813 nm in *Chloroflexus* reaction centers. These transitions give two bands with opposite sign in the CD spectra and two absorption bands with different polarizations and amplitudes: the extinction coefficient for the 813-nm band is bigger than the 790-nm band by a factor of 6–7 in *Chloroflexus* reaction centers [20,21]. The modification of R-26 reaction centers leads to the decrease by a factor of 1.5 of the 810-nm CD band and by a factor of approx. 4 of the 800-nm CD band at 77 K (Fig. 4). The room temperature measurements are more difficult to interpret quantitatively because of the stronger overlap of the two bands. In oxidized R-26 reaction centers the 810-nm band is eliminated and the 800-nm band is decreased by a factor of 2 after the modification. If one takes into account that only B_2 is modified, it can be suggested that B_1 and B_2 give rise to two transitions at 800 and 810 nm due to interaction with P, but the contribution of the B_1 molecule in the CD band at 810 nm seems to be bigger (approx. 60%, suggesting a fraction of B_2 equal to 0.2 in modified reaction centers). The exciton interaction of the B and P transitions gives rise to the conservative CD spectrum in the region of 810–900 nm which disappears when P is oxidized according to Eqn. 1. The similarity in the interaction between B_1 and P and between B_2 and P suggests a symmetrical arrangement of B_1 and B_2 with respect to P. If both transitions at 810 and 860 nm would belong to P itself as has been suggested [23], a preservation of the CD bands at 810 and 860 nm should be expected but this is not the case. The spectrum of the triplet state formation for normal [1–3] and modified R-26 reaction centers [7] has a bleaching at 860–880 nm, but not at 810 nm, indicating again that the origin at the 810-nm transition is not related to interaction within the P dimer. The more pronounced CD band at 813 nm in *Chloroflexus* reaction centers can be related to the larger extinction coefficient of the 813-nm band in these reaction centers (see Eqn. 1). This implies some difference in the interactions between B_1 and P molecules in two types of reaction center. The oxidation of P leads to the disappearance of two transitions at 813 and 790 nm and the appearance of a new transition at 805 nm in *Chloroflexus* reaction centers (Fig. 5C). This suggests that the last transition belongs to B_1 itself. The same can be concluded for B_1 and B_2 in R-26 reaction centers. Then the polarization value of the 800-nm band for oxidized reaction centers is directly related to the angle between the long axes of P and B_1 molecules when P is excited at 880 nm. The value of the angle is estimated to be 30 ± 10° for both B_1 (R-26 and *Chloroflexus*) and B_2 molecules (R-26).

The spectrum of the oxidation of P at 77 K (Fig. 5) shows a decrease by a factor of 1.4 of the dipole strength (due mostly to sharpening) of the bleaching band at 810 nm after the modification of reaction centers. The decrease of the dipole strength by a factor of 1.83 of the appearing band at 790 nm is more pronounced. This suggests that the transitions of both B_1 and B_2 are involved in the spectrum of P^+ formation, but the bleaching of the B_1 transition at 810 nm is more pronounced (approx. 60%), in agreement with CD spectra. The spectrum of the polarization value of the region of 790–900 nm is similar for normal and modified R-26 as well as for *Chloroflexus* reaction centers. These data imply the same mutual orientation of the transitions of B_1 and B_2 and a symmetrical arrangement of these molecules with respect to P (see above).

The spectra of the P^+ and H^- formation in modified R-26 reaction centers show changes of the dipole strength of the transitions around 800 nm. It was suggested [9] that similar changes in *Rps. viridis* reaction centers are due to the distortion of the excitonic interaction between P, B and H_1, according to the expression for the dipole strength of interacting transitions [9]:

$$\nu_A \mu_{oA}^2 = \boldsymbol{\mu}_{ioa} \cdot \boldsymbol{\mu}_{ioa} \nu_a - 4 \sum_{j \neq i} \sum_{b \neq a} \frac{V_{ioa;job} \boldsymbol{\mu}_{ioa} \cdot \boldsymbol{\mu}_{job} \nu_b \nu_a}{h\left(\nu_b^2 - \nu_a^2\right)} \tag{3}$$

In modified R-26 reaction centers there is a decrease of the dipole strength of the transitions at 800–810 nm by approx. 4.5 debye^2 ($5 \cdot 10^{-59}$ $C^2 \cdot m^2$) and 5 debye^2 ($5.5 \cdot 10^{-59}$ $C^2 \cdot m^2$) induced by formation of P^+ (77 K) and H^- (293 K), respectively, if one takes the overlap between the bleaching BPh band and the developing BChl band into account for the H^- spectrum. The change of the dipole strength of the transition at 860 nm during H_1^- formation is negligible. This implies that H_1 and P do not interact with each other, but that each of them interacts with B_1 which thus seems to be situated between P and H_1. This is in agreement with the arrangement proposed for *Rps.*

viridis reaction centers [9] and confirmed by X-ray analysis [10]. Using the experimental data and the expressions [1–3] for the dipole and rotational strength and for the energy of the excitonic interaction (see for details Ref. 9), one can estimate the mutual arrangmeent of P_1, B_1 and H_1 (Fig. 7). The center-to-center distances between P and B_1 and between B_1 and H_1 are not more than 1.3 nm. The polarization value of the BPh transition at 760 nm shifted by Q_A^- formation (Fig. 5) approaches the large negative value for normal and modified reaction centers. The angle between the dipole moments of the 880 and 760 nm transitions is estimated to be 85 ± 10°.

As was discussed above, B_2 is situated near H_2 and the mutual arrangement of B_2 and P is similar to that of B_1 and P. This indicates that the secondary chain has a similar arrangement and is symmetrical to the photoactive one if the structure of *Rps. sphaeroides* reaction centers is similar to that of *Rps. viridis* reaction centers [10]. Fig. 7 shows the pigment arrangement for R-26 reaction centers. The orientation of the 880 nm transition moment in Fig. 7 has been chosen according to the data [23] showing that this moment is parallel to the membrane plane and therefore perpendicular to the electron transfer direction.

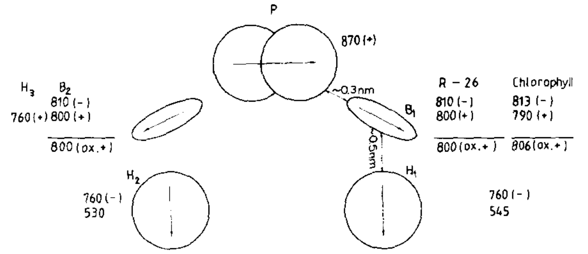

Fig. 7. Arrangement of pigment molecules in *Rhodopseudomonas sphaeroides* reaction centers derived from optical data for the chains $P\text{-}B_1\text{-}H_1\text{-}Q_A$ and $P\text{-}B_2\text{-}H_2$. The symmetrical arrangement of the two chains is based on the assumption of a similarity in the structure of *Rps. sphaeroides* and *Rps. viridis* reaction centers [10]. The numbers indicate the position in nm of transition moments for BChl (P and B) and BPh (H) in the reaction centers (− and + show the sign of rotation in CD spectra). Arrows show the orientation of the long axis of B and H and the long-wavelength transition of P. The B_2 molecule can be replaced by a BPh *a* (H_3) molecule in modified R-26 reaction centers. The distances indicated are edge-to-edge distances. Optical data also indicate that the pigment arrangmeent for *Chloroflexus* reaction centers appears to be similar to that for modified *Rps. sphaeroides* reaction centers.

The interaction of B_1, H_1 and P in *Chloroflexus* reaction centers seems to be slightly different from that in R-26 ones although the mutual orientation of the transition moments is similar. First of all the dipole strength of the transition at 813 nm is larger than that at 790 nm by a factor of 6–7 (Fig. 5 and Refs. 20 and 21). The sum of the dipole strengths of the two transitions is preserved during P^+ formation (Fig. 5). However, this effect may be related to an increase of the 806-nm band, due to the appearance of a monomeric BChl transition in P^+ which might be stronger for *Chloroflexus* reaction centers [20]. The interaction between P and B_1 is clearly seen in CD spectra (Fig. 4). The interaction between B_1 and H_1 is observed in the spectrum of the H_1^- formation, which shows a decrease of the dipole strength of the 813-nm transition. Thus, B_1 seems to be situated between P and H_1 in these reaction centers also. The conservative CD spectrum in the region of the BPh absorption near 760 nm shows the interaction of two BPh molecules which are close to each other. As was discussed above this CD spectrum appears to be due to the interaction between H_2 and H_3 of the secondary chain $P\text{-}H_3\text{-}H_2$. Thus we conclude that the pigment arrangement for *Chloroflexus* reaction centers appears to be similar to that for *Rps. sphaeroides* reaction centers.

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