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Structure of *Chloroflexus aurantiacus* reaction center: photoselection at low temperature

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Photoselection experiments have been performed on isolated Chloroflexus aurantiacus reaction centers at 20 K. Our data show that the average angle between the ground state BPh Q_y transitions and the 890 nm transition is approx. 50°. Only two BPh Q_y transitions are affected by the charge separation. These two transitions are perpendicular to the long-wavelength band of the primary donor. The ground state of the 813 nm transition makes an angle of 35° with the dimer absorption band. The polarization ratio of the light-induced absorption decrease at 815 nm is not consistent with that decrease being due solely to an electrochromic bandshift of the 813 nm transition.

The photochemistry of the green gliding thermophilic bacterium *Chloroflexus aurantiacus* is more similar to that found in the purple photosynthetic bacteria than in the green bacteria [1–4]. The similarities include the chemical nature of the early electron acceptor, a BPh molecule [4], the nature of the primary electron acceptor, a quinone molecule [5] and the dimeric structure of the primary electron donor [6]. However, two important differences have been found between the reaction centers isolated from *C. aurantiacus* and those obtained from different purple bacteria:

(1) *C. aurantiacus* reaction centers contain at most two polypeptides [7,8], while reaction centers from purple bacteria usually contain three [9].

(2) From unusual features in the absorption spectrum [2] and from measurements of the BPh/BChl ratio [8], it was concluded that *C. aurantiacus* reaction center contains three BPh and three BChl molecules (see, however, Ref. 10) rather than two and four molecules, respectively, as for all reaction centers of purple bacteria analysed thus far [9]. Taking advantages of the special features of the absorption spectrum of *C. aurantiacus* reaction centers, we have performed low-temperature photoselection experiments in order to determine the relative orientation of the different chromophores.

Reaction centers were isolated from *C. aurantiacus*, strain OK 70-Fl cells, grown anaerobically at 52°C, essentially as described by Pierson and Thornber [2]. The final purification step involved an HPLC chromatography on a MERCK RT 250.4 column eluted with lauryldimethylamine *N*-oxide 0.75% in 10 mM Tris buffer (pH 7.8), at a flow rate of 0.5 ml/mn. Dichroism of the absorbance changes linked to state P^+Q^- has been measured at 20 K upon excitation with vertically polarized light as described in Ref. 11. In the visible region (532–630 nm), excitation was provided by a Yag laser (Quantel 481 A) coupled to a dye laser (Quantel TD2 III) (pulse duration, 10 ns). For

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Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin

excitation in the near-infrared part (duration of excitation, 33 ms), a quartz iodine lamp was filtered through interference filters and appropriate polarizing sheet. In both cases, the ligth intensity was low enough to oxidize only 10% of the total reaction centers.

The values of the polarization ratio p, measured at 20 K, for the bleaching at 890 nm, upon excitation with vertically polarized light within the absorption bands characteristic of the primary donor (890 nm), the BChl (813 nm) and the BPh (757 nm) Q_y transitions, and the BChl (606 nm) and BPh (533 nm) Q_x transitions (Fig. 1A), are plotted on Fig. 1B. Here, p is defined as

$$\frac{\Delta A_V - \Delta A_H}{\Delta A_V + \Delta A_H} = \frac{3\cos^2\alpha - 1}{\cos^2\alpha + 3}$$

where ΔA_V and ΔA_H are the absorption changes detected with an analysing light polarized vertically or horizontally, respectively, and α is the angle between the excited and detected optical transitions.

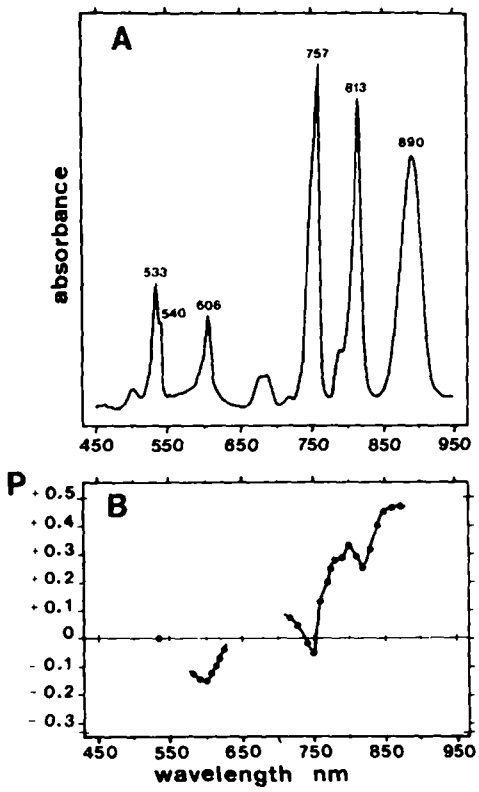

Fig. 1. (A) Low-temperature (20 K) absorption spectrum of isolated reaction centers of *C. aurantiacus*. The reaction centers were suspended in a medium containing 2/3 glycerol and 1/3 10 mM Tris buffer (pH 8), lauryldimehtylamine *N*-oxide 0.1%. 10 mM sodium ascorbate was added to insure complete reduction of primary electron donor. (B) Excitation polarization spectrum. The polarization ratio $p = (\Delta A_V - \Delta A_H)/(\Delta A_V + \Delta A_H)$ for the bleaching at 890 nm was calculated for excitation at different wavelengths with vertical polarized light. T, 20 K.

The polarization excitation spectrum obtained (Fig. 1B) for *C. aurantiacus* reaction centers is very similar to the one reported for *Rhodopseudomonas sphaeroides* [11] or *Rhodopseudomonas viridis* [12,13] in many respects. It presents, however, one important particular not observed for other reaction centers: the p value, when exciting within the BPh Q_y transitions at 755 nm, varies significantly, and never exceeds -0.05 (compared to -0.17, as for *Rps. sphaeroides* reaction centers [11]). This point will be discussed later. One can calculate from the p values (Fig. 1B) that the average angle between the 3 BPh Q_y transitions and the long-wavelength transition of P-890 is equal to 50°, whereas the angle between the 813 nm absorption band and the 890 nm transition is less than 35°. These angle values appear to be different from the one obtained by Vasmel et al. [10] in their linear dichroism study on oriented *C. aurantiacus* reaction centers in squeezed polyacrylamide gel. In their work, they found that the BPh (755 nm) and BChl (813 nm) Q_y transitions were respectively more or less perpendicular, and at the magic angle (55°) with the orientation axis. The apparent discrepancy between the two types of experiment may result from the non-coincidence of the directions of the long-wavelength transition and of the orientation axis of the protein in the gel.

Complementary information could be obtained by analysing the dichroism of the light-induced absorption changes upon excitation within the 890 nm absorption band with vertically polarized light. The spectra of the absorption changes occurring either parallel ($\Delta A_{\parallel}$) or perpendicular ($\Delta A_{\perp}$) to the long-wavelength transition of P-890, calculated from the data of Fig. 2A, are plotted in Fig. 2B. Although done at much lower temperature (20K) and extended to the visible part, our results are in

good agreement with the picosecond photodichroism study of Kirmaier et al. [14]. We can, however, go much further in the attribution and interpretation of the absorption bands than these authors went [14] by comparing the polarization values of the ground-state absorption bands (Fig. 1B) and of the transitions involved in the light-induced difference spectrum (Fig. 2B). The average angle between the BPh absorption bands and the 890 nm transition is equal to 50° ($p \approx +0.01$; Fig. 1B), whereas the BPh Q_y transitions subjected to absorption band shift are perpendicular ($p \approx -0.33$; Fig. 2A) to this last transition. The simplest way to interpret these findings is to suppose that, like in purple bacteria reaction centers [11–13,15], two BPh molecules, whose Q_y transi-

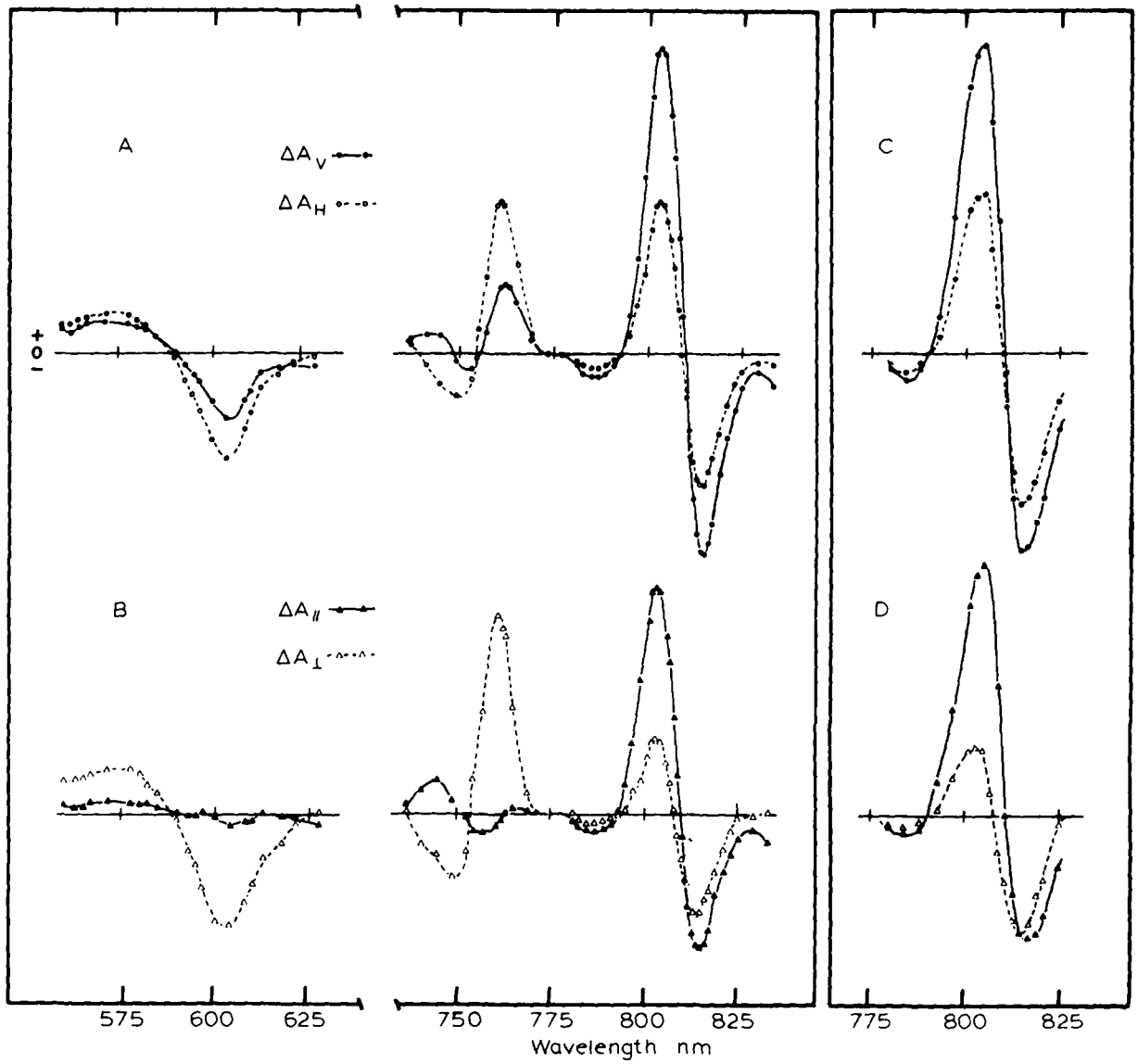

Fig. 2. (A) Light-induced difference absorption changes detected either with vertical (ΔA_V) or horizontal (ΔA_H) polarized light upon excitation, with 880 nm vertical polarized light T, 20K. (B) Absorption changes occurring either parallel ($\Delta A_{II} = 2\Delta A_V - \Delta A_H$) or perpendicular ($\Delta A_\perp = 3\Delta A_H - \Delta A_V$) to the long-wavelength transition (890 nm) calculated from the data of part A. T, 20 K. (C) Same as (A), but at 170 K. (D) Same as (B), but at 170 K.

tions are perpendicular to the long-wavelength transition of P-890, are affected by the primary photochemical processes, whereas the 'extra' BPh molecule of *C. aurantiacus* is not. Assuming now that each of the three BPh molecules has an equal contribution in the absorption spectrum around 755 nm, and that two of them are perpendicular to the 890 nm transition, it can be computed that the third one makes an angle smaller than 35°C with the 890 nm transition. This strongly suggests that the 'extra' BPh molecule has the same orientation as the 'voyeur' BChl molecule. In other terms *C. aurantiacus* reaction centers have the same structural arrangement as reaction centers isolated from purple bacteria, but one of the two 'voyeur' molecules is in the case of *C. aurantiacus* a BPh molecule, instead of a BChl molecule as in purple bacteria reaction centers.

From the pigment composition of *C. aurantiacus* reaction centers [2,8], one has to consider that mainly one BChl molecule absorbs between 785 and 820 nm. Several authors have, however, noticed the complex nature of the ground-state absorption band and absorption changes in this region [2,14]. For example, a small band centered at 790 nm, which disappears upon photooxidation, is clearly seen at low temperature [2,13]. From the polarization values ($p = +0.3$) measured when exciting within the 790 nm band (Fig. 1B) and for the bleaching centered at 790 nm (Fig. 2A), we deduce that the 790 nm is neither parallel nor perpendicular to the 890 nm band, but makes an angle of 33° with it. This implies that the 790 nm transition is neither a vibrational band of the 890 nm transition [13] nor the higher-energy transition of the primary donor [2]. The variation of the polarization value within the 813 nm band (from $p = +0.32$ at 805 nm to $p = +0.255$ at 820 nm, Fig. 1B) implies that this absorption band is composite. Also in agreement with the complex nature of the 813 nm band is the much lower value of p observed for the bleaching centered at 815 nm ($p = +0.20$ Fig. 2A; $p = +0.13$ at 170 K, Fig. 2C), compared to the average polarization ratio measured when exciting within the 813 nm band ($p = +0.30$; Fig. 1B). This marked difference in the polarization ratios implies that the absorption decrease at 815 nm is not due solely to an electrochromic bandshift of the main 813 nm absorption band. We therefore attribute part of the absorption decrease centered at 815 nm to the disappearance of a minor component of the 813 nm band upon photooxidation of the primary donor. Such a component, which has to make an angle greater than 45° with the long-wavelength band, has already been observed in *Rps. sphaeroides* [11] and *Rps. viridis* reactions [12]. It has been attributed to the higher-energy excitonic transition of the primary electron donor [11,12,15–17]. Several objections [18,19] have, however, been raised against that proposition.

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