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OPTICAL PROPERTIES OF THE PHOTOSYNTHETIC REACTION CENTER OF *CHLOROFLEXUS AURANTIACUS* AT LOW TEMPERATURE

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A study was made of the optical properties of isolated reaction centers of the gliding green bacterium *Chloroflexus aurantiacus*. The absorption spectrum measured at 4 K indicates that these reaction centers contain bacteriochlorophyll *a* and bacteriopheophytin *a*, but no other pigments. The fluorescence excitation spectrum at 4 K showed efficient energy transfer from all pigment molecules to P-865, the primary electron donor. Absorption, linear and circular dichroism spectra of reduced and oxidized samples were measured at 77 K in order to obtain information about the dipole strengths, orientations and interactions of the transition dipoles. The results agree with a model for the reaction center that involves three bacteriopheophytin and four closely interacting bacteriochlorophyll molecules. The three bacteriopheophytins do not seem to be closely coupled to the primary donor. The primary electron donor consists of a bacteriochlorophyll dimer absorbing at 887 nm at 77 K, while the other two bacteriochlorophylls are responsible for the absorption around 800 nm and are also strongly coupled as indicated by their exciton splitting.

Introduction

Recent studies of the gliding green bacterium *Chloroflexus aurantiacus* have shown that its photochemistry resembles that of purple bacteria (Refs. 1 and 2 and Holton, D., Blankenship, R.E., Kirmayer, C. and Feick, R.G., personal communication). This notion is supported by the similarities in the absorption spectra of isolated reaction centers [3] and in the absorption difference spectra for the oxidation of the primary electron donors [1–4]. However, as was pointed out by Pierson and Thornber [3], the pigment composition of the reaction center of *C. aurantiacus* seems to be different from that of purple bacteria. It was suggested that the reaction center contains three molecules of BPh *a* and three molecules of BChl *a*, rather than two bacteriopheophytins and four bacteriochlorophylls as in purple bacteria.

In the present paper we present an analysis of the spectral properties of *Chloroflexus* reaction centers at low temperature. In order to investigate the molecular arrangement of the pigment molecules we have measured the linear and circular dichroism spectra, both with the primary electron donor in the reduced and in the oxidized form. Comparison of these spectra with results obtained earlier for several species of purple bacteria suggests that the optical properties can be explained by a similar model for the two types of reaction centers.

Materials and Methods

Purified reaction centers of *C. aurantiacus* strain J-10-fl were obtained as described in Ref. 2. The

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; P-865, P-870, P-960, bacterial primary electron donors.

final preparation was dissolved in buffer containing 10 mM Tris, pH 8.0, and 0.025% lauryldimethylamine *N*-oxide. To obtain optically clear samples for absorption, fluorescence and CD measurements at low temperature, 40% sucrose (w/w) was added, and the solution was mixed with an equal volume of glycerol.

For LD measurements reaction centers were oriented in a polyacrylamide gel [5]. The gel was prepared by mixing one sample volume with 7 vol. of a mixture consisting of 19% (w/v) acrylamide, 0.28% (w/v) *N*,*N*′-methylenebisacrylamide, 47% (v/v) glycerol, 0.26% (v/v) *N*,*N*,*N*′,*N*′-tetramethylethyldiamine and 0.05% (w/v) ammonium persulfate. Oriented samples with an optical path length of 10 mm were obtained by squeezing the gel simultaneously in two perpendicular directions. In this way, axially symmetric samples were obtained. The increase in length along the orientation axis was by a factor of 1.5. When gels were prepared in the dark, approx. 25% of the reaction centers were irreversibly oxidized, presumably by the persulfate catalyst. Samples that were for about 75% in the oxidized form were obtained by preparing and cooling the gel to 77 K under continuous illumination.

Fluorescence excitation spectra were measured as described in ref. 6. LD and CD measurements were performed employing a photoelastic modulator (Morvue FS-3) in a setup essentially similar to that used by Breeze and Ke [7]. LD was measured using a 100 kHz modulation with a half-wave retardation amplitude, while a 50 kHz modulation with a quarter-wave retardation amplitude yielded the CD signal. A microcomputer was used for operational control, including the generation of a wavelength-independent retardation amplitude and spectral scanning. The photomultiplier d.c. current level was maintained at a constant value by a feedback circuit. The a.c. component of the photocurrent was directly proportional to either the LD or CD signal of the sample. In some cases baseline corrections were applied by subtracting a second-order polynomial, connecting points of minimal absorbance.

Results

The absorption spectrum of a reaction center preparation of *C. aurantiacus,* measured at 4 K, is shown in Fig. 1. The primary electron donor P-865 was fully reduced in this preparation. Q_y bands of BChl *a* are seen at 887, 813 and 787 nm, whereas the Q_x band shows a maximum at 607 nm and a shoulder near 596 nm. Absorption by BPh *a* gives rise to a strong absorption in the Q_y region with maximum at 757 nm and a double Q_x band at 533 and 540 nm. The relative amplitudes of these bands are approx. 2 : 1, as compared to about 1 : 1 for reaction centers of purple bacteria [8,9], which supports the notion [3] that the reaction center of *C. aurantiacus* contains three, rather than two molecules of BPh *a*. The origin of the bands at 678 and 688 nm is not clear. A band around 680 nm of possibly similar nature is observed in reaction center preparations from various purple bacteria, the band being attributed to an oxidized product of BChl *a* [10]. The weak bands at 498 and 716 nm are probably vibrational subbands of the Q_x and Q_y bands of BPh *a*, respectively. Although a non-crystallizing medium was used the spectrum was distorted by scattering, especially at shorter wavelengths. Such a distortion was absent from the fluorescence excitation spectrum (see below), because this technique is less sensitive to scattering artefacts.

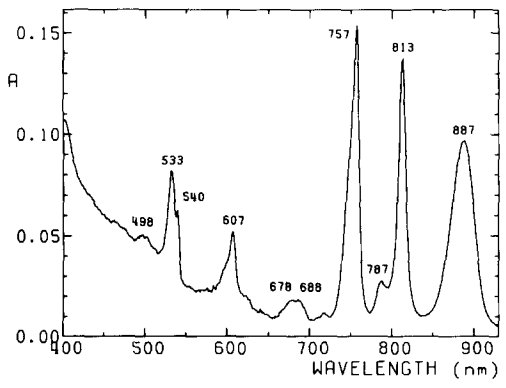

Fig. 1. Absorption spectrum at 4 K of a reaction center preparation of *C. aurantiacus* in noncrystallizing medium (see Materials and Methods). Spectral resolution, 1.0 nm.

Fig. 2 shows the results of a Gaussian deconvolution of the absorption spectra at 77 K of reduced and oxidized reaction centers in the near-infrared region. In the spectrum of the reduced reaction centers (Fig. 2A) bands were obtained at 746 (0.44), 757 (0.57), 792 (0.21), 813 (0.65) and

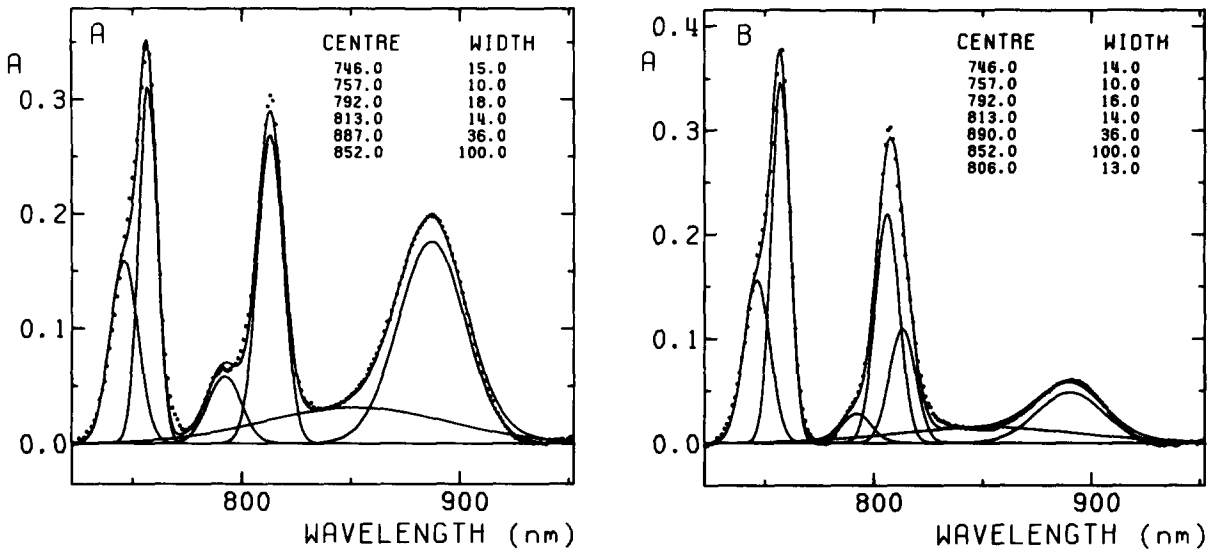

Fig. 2. Gaussian deconvolution of absorption spectra of *C. aurantiacus* reaction centers at 77 K. (A) Frozen in the dark, (B) frozen under continuous illumination. Before deconvolution a straight baseline through the absorbance values at 720 and 950 nm had been subtracted. Peak positions and half-bandwidths of the bands are listed in the figures. The points represent the measured absorption spectra.

887 nm (1.00). The values in parentheses indicate the relative dipole strengths of the bands obtained by plotting A/ν vs. ν (where ν is the frequency) and integrating over the absorption bands. The band at 887 nm is due to P-865; the first two bands probably represent spectral forms of BPh *a*. The other bands are due to BChl *a*. The very broad band at 852 nm, which was needed to obtain a reasonable fit, is of doubtful physical significance, and may reflect a deviation from the Gaussian shape in the wings of the other bands, mainly in the short-wavelength wing of the band at 887 nm. In the oxidized preparation (Fig. 2B) the BChl *a* bands are replaced by a single band at 806 nm. The relative dipole strengths of the BPh *a* bands at 746 and 757 nm are slightly changed, to 0.37 and 0.66 relative units, respectively.

The preparation showed several fluorescence emission bands, the relative intensities of which were dependent on the wavelength of excitation. The most prominent bands were located at 765 and 917 nm. Addition of dithionite enhanced the relative intensity of the band at 917 nm, which is presumably due to P-865, and decreased that of most of the other bands. The excitation spectrum for the long-wavelength emission is shown in Fig. 3. Except for the absence of the scattering artefact the spectrum is very similar to the absorption spectrum, indicating that all pigments, including both spectral forms of BPh *a* and the pigments absorbing at 678 and 688 nm, transfer their excitation energy to P-865 with an efficiency of close to 100%.

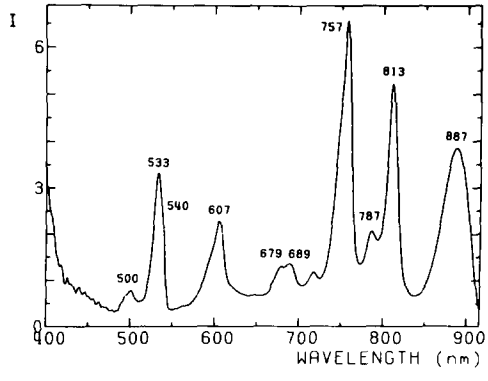

Fig. 3. Fluorescence excitation spectrum at 4 K of *C. aurantiacus* reaction centers in noncrystallizing medium in the presence of 10 mM dithionite. Detection wavelength, 925 nm. $A_{887} = 0.21$. Spectral resolution, 3.5 nm. *I* denotes the relative fluorescence intensity per incident quantum.

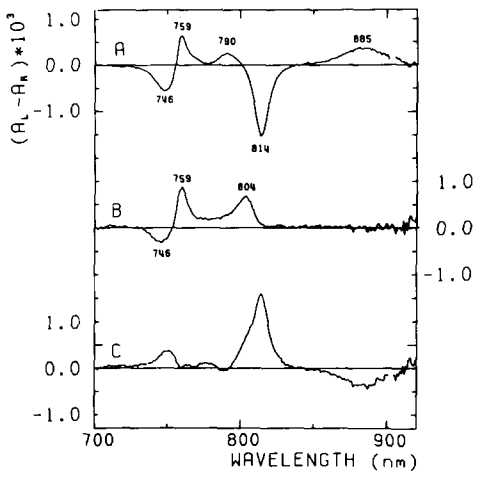

Fig. 4. CD spectra at 77 K of reaction centers of *C. aurantiacus*. $A_{865} = 0.25$ at room temperature. (A) No additions, (B) in the presence of 2 mM potassium ferricyanide to oxidize P-865, (C) difference CD spectrum of the oxidized-minus-reduced form.

The low-temperature CD spectrum of reduced reaction centers is shown in Fig. 4A. The position of the CD bands agrees with that of the absorption bands as determined by Gaussian deconvolution (Fig. 2A), with positive bands at 885 and 790 nm and a negative band at 814 nm for BChl *a*, and BPh *a* bands at 746 and 759 nm with opposite sign. In the BChl *a* region the spectrum resembles those reported earlier for *Rhodospirillum rubrum* [11] and for *Rhodopseudomonas sphaeroides* [12], measured at room temperature; its general structure is similar to that of the BChl *b*-containing *Rps. viridis* [13]. Significant differences occur, however, in the region around 750 nm. In reaction centers of *R. rubrum* and of *Rps. sphaeroides* a single broad negative BPh *a* band is found near 750 nm, whereas in *Chloroflexus* reaction centers two bands of different sign are observed. These results suggest that the macromolecular arrangement of the BChl *a* molecules in the reaction center of *Chloroflexus* resembles that of purple bacteria, while the arrangement of the BPh *a* molecules is different and presumably reflects the presence of a third molecule of BPh *a*. The CD spectrum was drastically changed by chemical oxidation of the sample, as shown in Fig. 4B. Again the position of the CD bands agrees with that found in the absorption spectrum of oxidized reaction centers (Fig. 2B). The spectrum shows a single positive BChl *a* band at 804 nm. Changes occur in the intensity of the BPh *a* bands, which probably reflect the changes in dipole strength upon oxidation mentioned above. The same features were observed upon photooxidation of the sample and subsequent cooling to 77 K. The difference CD spectrum of the oxidized minus reduced form (Fig. 4C) exhibits essentially the same characteristics as those found for purple bacteria [11,13].

To obtain more detailed information about the pigment arrangement in the reaction center we measured the LD of the absorption bands both of reduced and oxidized samples. Fig. 5 shows the LD spectra at 77 K of reaction centers oriented in polyacrylamide gel, (A) of a sample prepared in the dark, and (B) of a sample prepared under continuous illumination. $A_{\parallel}$ is defined as the absorption parallel to the orientation axis, i.e., perpendicular to both pressing directions (see Materials and Methods) and $A_{\perp}$ the absorption perpendicular to the orientation axis. By taking linear combinations of the experimentally determined spectra of Fig. 5, the spectra were deconvoluted in the LD spectra of a fully reduced and a fully oxidized preparation. The results are shown in Fig. 6. Since no information is available on the mode of orientation of the reaction center with respect to the membrane, all considerations

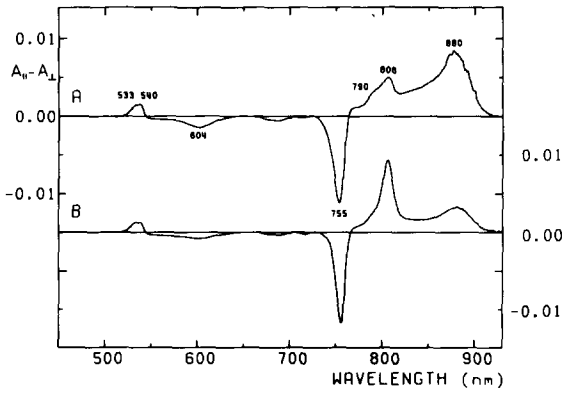

Fig. 5. LD spectra at 77 K of reaction centers of *C. aurantiacus* in pressed polyacrylamide gel (see Materials and Methods). (A) Sample prepared and frozen in the dark, (B) sample prepared and frozen under continuous illumination. $A_{865} = 0.045$ at room temperature in the fully reduced form. $A_{\parallel}$ and $A_{\perp}$ are defined in the text.

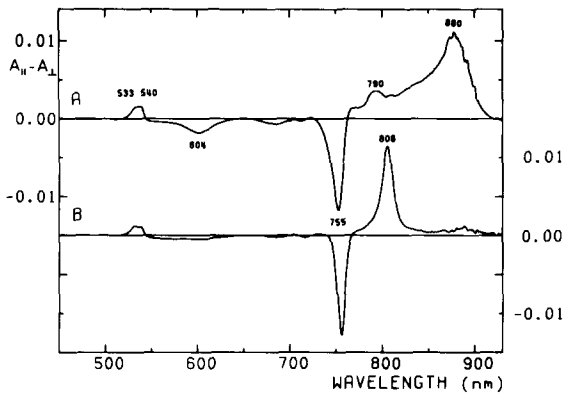

Fig. 6. LD spectra at 77 K of (A) fully reduced and (B) fully oxidized reaction centers of *C. aurantiacus*. The spectra were obtained by linear combination of the spectra of Fig. 5.

given below will apply relative to the orientation axis for gel pressing, which probably coincides approximately with the longest axis of the particle. Nevertheless, important information can be gathered from the spectrum.

We shall first discuss the spectrum of the fully reduced sample (Fig. 6A). The Q_y transition dipole of the BPh band at 757 nm is oriented more or less perpendicular to the orientation axis, whereas the positive Q_x transitions suggest a more or less parallel orientation. Since the Q_x and Q_y transition dipoles are oriented perpendicularly in the macrocycle plane [14], this suggests that the tetrapyrrole plane lies more or less in one plane with the orientation axis. The BChl *a* transitions at 792 and 887 nm are more or less parallel to the orientation axis, which seems to rule out the hypothesis [3] that the 792 nm transition is due to exciton splitting of P-865. The Q_y transition of BChl *a* at 813 nm appears to be close to the magic angle (54.7°).

The orientations of BPh *a* are not significantly changed by oxidation of P-865, although the narrowing of the 755 nm band suggests a small change in the orientation of the 746 nm transition. However, in the BChl *a* region the spectrum is drastically altered. The orientation of the newly formed Q_y band at 806 nm is approximately parallel to the orientation axis, whereas the absence of a band near 600 nm indicates that the Q_x transition of this BChl *a* species is close to the magic angle. Thus, surprisingly, the orientation of the BChl *a* transition is clearly different from that absorbing at 813 nm in the reduced sample, which indicates that the 806 nm band does not result from a simple electrochromic band shift of the 813 nm band.

Discussion

The low-temperature absorption, CD and LD spectra of *Chloroflexus* reaction centers yield detailed structural information that should be accounted for in one molecular model. We first turn to the discussion of the arrangement of the BChl *a* molecules in the reaction center. The linewidth of the ESR signal of $P\text{-}865^+$ [1] suggests that the primary donor of *Chloroflexus* consists of a BChl *a* dimer as is also the case for purple bacteria [15]. Also, the absorption difference spectrum for P-865 oxidation is very similar to that found for P-870 in several species of purple bacteria [4]. We thus assume that the primary donor of *Chloroflexus* consists of a dimer of strongly interacting BChl *a* molecules, absorbing at 865 nm at room temperature, the band shifting to 887 nm at low temperature.

At 77 K two other absorption bands are observed in reduced samples that may be assigned to BChl *a*, one at 792 nm and one at 813 nm. Upon oxidation these bands disappear and a band at 806 nm appears. The orientation of the 792 nm band indicates that it is not due to exciton interaction of the primary donor. This also applies to the CD spectrum, where bands of the same sign are observed at 790 and 885 nm, and to the triplet-minus-singlet difference spectrum of P-865 determined by absorption-detected magnetic resonance (ADMR) which does not show a decrease near 792 nm (Den Blanken, H.J. and Vasmel, H., unpublished observations). These experiments likewise seem to exclude the possibility that the 792 nm band is a vibrational subband of P-865.

Interpretation of the BChl *a* bands is hampered by lack of knowledge of the exact number of BChl *a* molecules in the reaction center. It has been suggested that this number is three [3] but the preliminary results of extraction experiments indicate a ratio of BChl *a* to BPh *a* of 1.2–1.3 which suggests that four is a more likely number. The CD and LD spectra as well as the oxidized-

minus-reduced difference CD spectrum of *C. aurantiacus* show a clear resemblance to the corresponding spectra obtained with reaction centers of various species of purple bacteria (Refs. 11–13, 16 and 17; and for a review, see Ref. 15), which suggests a similar molecular arrangement of the pigment molecules. The most detailed analysis of such spectra was given by Shuvalov and Asadov [13] for reaction centers of the BChl *b*-containing purple bacterium *Rps. viridis*. It was concluded that the most plausible way to explain these spectra was by the assumption of a supramolecular complex with mutual interaction between P-960, the dimeric electron donor and two other bacteriochlorophylls absorbing at 833 and 850 nm, respectively. If it is assumed that the reaction center of *C. aurantiacus* contains four BChl *a* molecules, a similar analysis can be applied to explain our spectra. Such an analysis leads to the assumption that the bands at 792 and 813 nm are to a first approximation due to exciton coupling between two BChl *a* monomers. The ratio of the dipole strengths would then yield an angle of approx. 59° between the transitions of the monomers; the extent of splitting, 163 cm^{-1}, is compatible with an in-plane head-to-tail arrangement of the chromophores with a distance of 10–11 Å. However, the spectrum of the oxidized preparation indicates that the two pigments absorbing around 800 nm are also closely interacting with P-865, as their mutual exciton splitting is drastically decreased by the oxidation of the primary donor. Thus, the figures given above can only serve as a first indication of the possible pigment arrangement. First-order time-independent perturbation theory [18] states that, while exciton interaction leads to a redistribution of oscillator strength between the transitions, the total oscillator strength is preserved. The total oscillator strength of the bands in the region 775–830 nm is indeed preserved upon oxidation, as can be seen from Fig. 2A and B.

The model can also account for the reorientation of the electronic transitions around 806 nm as observed in the LD spectrum of fully oxidized reaction centers, because disruption of exciton coupling leads to a reorientation of the optical transition involved. Interestingly, such a reorientation has also been reported recently by Vermeglio and Paillotin [19] for the transition at 833 nm in *Rps. viridis* reaction centers upon oxidation of the primary donor, although their model for interpretation differs from that used by Shuvalov and Asadov [13]. The strong negative CD band around 814 nm that disappears completely after photooxidation of P-865 can be attributed to the interaction of the exciton band located at 813 nm with the long-wavelength transition of the primary donor, similar to the interaction of the 850 nm transition with that of the primary donor in reaction centers of *Rps. viridis*. The conservative nature of these bands supports this hypothesis. As with *Rps. viridis*, only weak interaction appears to exist between bacteriopheophytin and the primary electron donor dimer, as indicated by the absence of a significant effect of P-865 oxidation upon the LD and CD bands of BPh *a*.

The absorption spectra of the reaction center preparation agree with the notion that the reaction center of *C. aurantiacus* contains three [3], rather than two BPh molecules as in purple bacteria. The CD and absorption spectra in the BPh *a* region would be compatible with a trimer model for these molecules as has been suggested for chlorophyll *b* in the light-harvesting complex of higher plants [20]. However, this arrangement does not agree with the observed LD spectrum, since the trimer will in general possess three mutually perpendicular transition dipoles.

Summarizing, we conclude that our data agree with a model for the reaction center of *Chloroflexus* in which the arrangement of the BChl molecules resembles that in *Rps. viridis*, and possibly other species of purple bacteria. The reaction center would then contain four closely interacting BChl *a* molecules. Two of these form the primary donor, while the other two are responsible for the absorption around 800 nm and are also strongly coupled as witnessed by their exciton splitting. Only weak interactions exist between the BPh *a* molecules and the primary electron donor. Experiments with reaction centers in which the primary acceptor is in the reduced state may help to resolve the arrangement of the bacteriopheophytins and their interaction with bacteriochlorophyll.

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