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Magnetic Circular Dichroism and Linear Dichroism for Elucidation of Electronic Transitions and Their Orientations in a Reaction Center Isolated from a Thermophilic Purple Sulfur Photosynthetic Bacterium Chromatium tepidum

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Electronic transitions and orientations of pigments in reaction center complex isolated from a purple sulfur bacterium Chromatium tepidum were determined by magnetic circular dichroism and linear dichroism. They are very similar to those of non-sulfur purple bacteria, indicating a convergent nature of this complex in the photosynthetic organisms.

A primary reaction for photochemical energy conversion in reaction center (RC) complex is a charge separation followed by stabilization processes. These are carried out in a highly organized complex where a spatial arrangement of prosthetic groups is the primary prerequisite for an efficient reaction. This is known in non-sulfur purple bacteria by X-ray crystallography[1,2] and suggests the convergent nature of the RC through bacteria to higher plants.[3] However this convergence may not be complete unless the information on sulfur purple bacteria becomes available.

We recently established a new method for the isolation of RC from a novel thermophilic sulfur purple bacterium Chromatium (C.) tepidum.[4] The RC complex showed distinct differences in the ground state absorption spectrum from those of non-sulfur purple bacteria; red-shift of the absorption maximum by 20 nm and a smaller extinction coefficient by one half. These might arise from difference in either the molecular arrangement of prosthetic groups or interactions between prosthetic groups and amino acid residues. Thus we investigated these points by two kinds of spectroscopy; magnetic circular dichroism (MCD) and linear dichroism (LD). The former is known to be highly sensitive in detection of electronic transition as shown for various heme complexes[5] and the latter very useful for elucidation of the orientation of the transition moment as proved for the RC complexes of

non-sulfur purple bacteria R. viridis[6] and R. sphaeroides.[7]

Culture of C. tepidum and isolation of its RC complex were carried out by the procedures reported previously.[8,9] MCD was measured with a Jasco J-500 circular dichrometer equipped with a data processor (Jasco DP-500) and an electromagnet of 13.5 T in the sample room. LD was measured with a Hitachi 330 spectrophotometer using a prism polarizer. RC's were oriented by so-called gel squeeze method;[10] they were embedded in a polyacrylamide gel and the gel was compressed to half in thickness using a home built holder. Measurements were done at room temperature (about 22 °C).

Figure 1 shows absorption (A) and MCD spectra (B, C) of the RC's of C. tepidum in the visible region (450-700 nm, 1B) and in the near infrared region (700-950 nm, 1C). To our knowledge, this is the first report on the MCD spectra of RC complex of any kinds of photosynthetic organisms. When the primary electron donor (so-called special pair, P) was reduced, three absorption maxima were observed at 885, 800, and 755 nm (Fig. 1A); those were assigned to the special pair P, accessory bacteriochlorophyll (BChl) and bacteriopheophytin (BPh), respectively. Location of the absorption maximum of the P was almost the same as that of another sulfur purple bacterium C. vinosum.[11] It was longer by 20 nm than that of the non-sulfur purple bacteria.[12] The MCD spectrum exhibited a similar band shape to that of the absorption spectrum, but relative magnitudes and peak positions of respective bands substantially differed; blue-shifts of the BChl

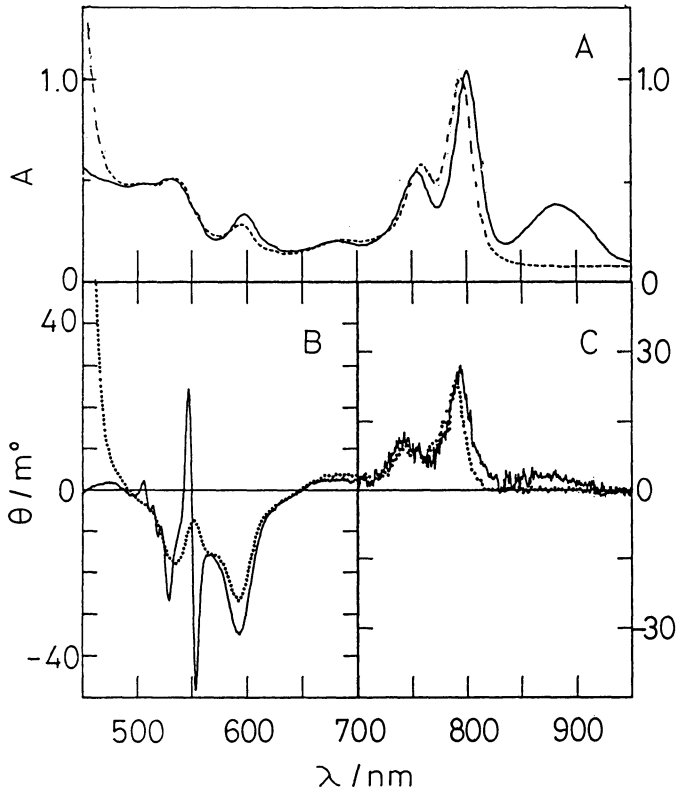

Fig. 1. Absorption (A) and magnetic circular dichroism (MCD, B, and C) spectra for RC's from C. tepidum, at room temperature in the reduced (————) and oxidized (----------) states.

and BPh bands, and a smaller magnitude of the band of P. Note that a shoulder was discernible around 815 nm in the MCD spectrum, which could not be detected in the absorption spectrum (see later).

By oxidation of P with tripotassium hexacyanoferrate, spectra were remarkably changed as shown by dotted lines in Fig. 1. The 885-nm band disappeared and a clear blue shift was observed around 800 nm. In addition, the BPh band (755 nm) shifted to the red by about 5 nm. These changes were also detected in the MCD spectra (Fig. 1C). Furthermore the 815-nm band disappeared in the P^+ state. Thus it was concluded that the 885- and 815-nm bands were ascribed to the P.

The MCD spectrum in the wavelength region between 500 to 570 nm was very complicated due to the superposition of the band of a bound cytochrome c centering at 550 nm. Large negative bands around 600 nm and 535 nm originated from the Q_x transitions of BChl a and BPh a, respectively. Since the MCD spectrum resembled the absorption spectrum in all the wavelength region investigated, the MCD spectrum was interpreted to be composed of so-called Faraday B term[5] which derives from the magnetic mixing of one state (corresponding to the lowest or the second excited states, Q_y or Q_x, respectively) with other states (especially, the third excited state, B (Soret)). It is apparent that the magnitude of Q_x band of BChl a or BPh a was larger than respective Q_y bands. This is ascribable to a stronger mixing between Q_x and B_y bands than that between the Q_y and B_x bands.

Figure 2 shows electronic absorption (A), LD (B) and anisotropic ratio (B) spectra of the reduced RC. The LD spectrum is the difference of the absorption between the horizontally polarized light and the vertically polarized light. Corresponding to the absorption of P, BChl and BPh, two positive LD bands and one negative LD band were clearly observed. The spectral feature was very similar to that of the RC's isolated from non-

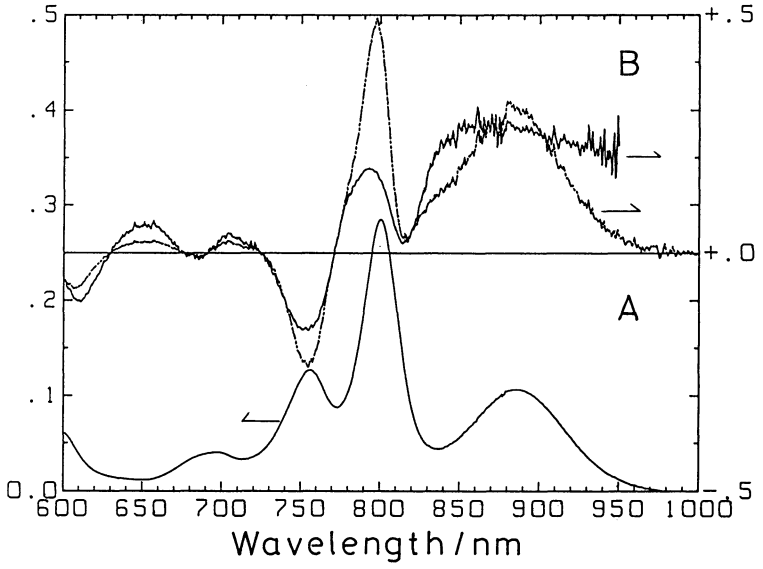

Fig. 2. Absorption (A:————), linear dichroism (LD:——·——), and anisortopy (LD/A:————, top) spectra for RC isolated from C. tepidum that is embedded in a squeezed polyacrylamide gel at room temperature. The absorbance at 800 nm was 0.3.

sulfur purple bacteria.[6,7] Moreover, the 815 nm band was again clearly detected as a trough (Fig. 2). This band corresponds most likely to the 815-nm band detected by the MCD spectrum (Fig. 1C). The presence of a transition around 815 nm was originally reported by Breton et al.[6,7] based on the low temperature (10 K) LD spectrum on the RC's isolated from R. sphaeroides.[6] This band corresponds mainly to the higher energy component of the two transitions derived from the dimer structure of the P. Disappearance of the 815-nm band in the oxidized state supports this assignment.

Orientation of the pigments to the membrane normal (anticipated C_2 axis) were calculated by the method described in Ref. 13. Assuming that P orients parallel to the membrane normal, the angles of the other pigment orientation relative to the membrane normal were calculated to be 71° and 43° for the accessory BChl a and the BPh a, respectively. These results are very similar to those obtained for non-sulfur purple bacteria.[6,7] The 815-nm band was calculated to be at 57° to the membrane normal.

In conclusion, MCD spectrum disclosed the higher energy band of the special pair around 815 nm in the RC isolated from a thermophilic sulfur purple bacterium. The LD spectrum was consistent with the MCD spectrum, and also showed a very similar orientation of the prosthetic groups in the RC complex. This leads to an idea that the RC complex is highly convergent through bacteria to higher plants. The difference in the location of the absorption maximum of P (885 nm) in C. tepidum may be due to another reason, e.g., an interaction between the P and the amino acid residues.

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