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CIRCULAR DICHROISM AND ABSORPTION SPECTRA OF BACTERIOCHLOROPHYLL–PROTEIN AND REACTION CENTER COMPLEXES FROM *CHLOROBIUM THIOSULFATOPHILUM*

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SUMMARY

A water-soluble bacteriochlorophyll–protein and a complex which also contained photochemically-active reaction centers were isolated from *Chlorobium thiosulfatophilum*. The procedures were similar to those used previously on *Chloropseudomonas ethylica* (Fowler, C. F., Nugent, N. A. and Fuller, R. C. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 2278–2282), and the corresponding complexes from the two organisms exhibit marked similarities. They are characterized in terms of their absorption spectra at 100 and 300 °K, circular dichroism spectra at 300 °K, and, in the case of the reaction center complexes, by the light- or oxidation-induced changes in the absorption and circular dichroism spectra. On the basis of exciton interactions observed in the circular dichroism and low-temperature absorption spectra, we conclude that the predominant pigment arrangement in the bacteriochlorophyll–reaction center complex is distinctly different from that in the bacteriochlorophyll–protein.

INTRODUCTION

Green photosynthetic bacteria contain a small amount of bacteriochlorophyll *a* in addition to the principal light collecting pigment, chlorobium chlorophyll[1]. *In vivo*, chlorobium chlorophyll transfers electronic excitation energy to bacteriochlorophyll *a*, (ref. 2), which in turn transfers the energy to a specialized reaction center chlorophyll known as P840 (ref. 3). The photosynthetic unit appears to be very large in green bacteria with approx. 80 bacteriochlorophyll *a* molecules and of the order of 1000 chlorobium chlorophyll molecules per reaction center[4].

The photosynthetic apparatus of green bacteria is contained within egg-shaped vesicles[5], 30–50 nm wide and 100–160 nm long, which are arranged in a cortical layer adjacent to the cytoplasmic membrane[6–8]. These vesicles are bounded by a non-unit (single-layered) membrane 20–30 Å thick. Thus, the green bacteria possess a unique photosynthetic apparatus which is not contained in the usual unit-membrane bilayer as in all other known photosynthetic organisms.

The forces which keep the vesicles of green bacteria intact appear to differ

qualitatively from those holding together the photosynthetic apparatus in unit-membrane systems. In the latter case detergents are required to "dissolve" the unit membranes and to liberate chlorophyll–proteins and reaction center complexes[9], whereas in the former case the photosynthetic units can be fractionated without the use of detergent[1,4]. By centrifugation of a broken vesicle preparation from *Chloropseudomonas ethylica* in 35% sucrose at $400000 \times g$, Fowler *et al.*[4] separated the material into a "light" fraction (1.12 $g \cdot cm^{-3}$) which contained chlorobium chlorophyll and a "heavy" fraction (1.18 $g \cdot cm^{-3}$) which contained bacteriochlorophyll *a*, carotenoid, P840 and cytochrome. The "heavy" fraction was photochemically active as shown by the light-induced oxidation of P840 ($E_{m,7.5} = +0.24$ V) and cytochrome 553 ($E_{m,7.5} = +0.17$). This heavy fraction, which will be designated henceforth as a bacteriochlorophyll–reaction center complex, has a molecular weight in excess of $1.5 \cdot 10^6$ (ref. 4).

It had previously been shown that bacteriochlorophyll *a* in the form of water-soluble bacteriochlorophyll–protein (mol. wt 152000) could be obtained from green bacteria by alkaline extraction followed by conventional methods of protein purification[1,2,10]. The bacteriochlorophyll–protein from *Cps ethylica* has been characterized most extensively. It is a globular protein consisting of four probably identical subunits, each containing five bacteriochlorophyll molecules which are bound inside the protein by non-covalent forces[9,10]. The bacteriochlorophyll–protein is completely free of chlorobium chlorophyll, carotenoid, cytochrome, P840 or any photochemical activity. The absorption spectrum at 80 °K (ref. 10), and the circular dichroism (CD) spectra at either room temperature[10,11] or 80 °K (ref. 12), give strong evidence for exciton splitting of both the Q_x and Q_y transitions of bacteriochlorophyll in the protein. For the Q_y transition the resolution of four components in the absorption spectrum and of 5 components in the CD spectrum suggests that interactions among the 5 bacteriochlorophyll molecules in each subunit accounts for the exciton splittings[12].

In order to compare the pigment–pigment interaction in the bacteriochlorophyll–reaction center complex to those in the purified bacteriochlorophyll–protein, the present study of CD and low temperature absorption characteristics was undertaken. To broaden the biological scope of the investigation a different green bacterium was chosen as the source of bacteriochlorophyll–protein and bacteriochlorophyll–reaction center complex for comparison with the previous preparations from *Cps ethylica.*

MATERIALS AND METHODS

Bacteriochlorophyll–protein from *Cps ethylica** was prepared at Brookhaven National Laboratory[10] and stored in the refrigerator as a precipitate in 30 g $(NH_4)_2SO_4$ per 100 ml of original solution. Solutions of bacteriochlorophyll–

* Cultures of "*Cps. ethylica*" grown at Brookhaven National Laboratory and in several other laboratories have been shown recently to consist of syntrophic mixtures of *Chlorobium limicola* and one or more colorless bacteria which function as sulfate reducers[16]. The bacteriochlorophyll-protein and the bacteriochlorophyll–reaction center complex obtained from these mixed cultures should properly be attributed to *C. limicola*; however, we shall relate the present work to previous studies by using the term *Cps ethylica* to refer to the consortium of organisms formerly associated with that name.

protein were prepared by centrifuging a few ml of stock suspension in a clinical centrifuge, discarding the supernatant, and dissolving the precipitate in an appropriate buffer.

Chlorobium thiosulfatophilum, strain 6230 (Tassajara) was obtained from Dr Bob Buchanan (Department of Cell Physiology, University of California, Berkeley) and grown anaerobically in 20-l bottles at 23 °C. Each bottle was illuminated by a 75-W reflector flood lamp filtered through approx. 4 cm of water. After inoculation with approx. 5 l of cell suspension, each bottle was allowed to stand overnight in dim light (50 V) without stirring. Then the lamp voltage was increased to 100 V, and the magnetic stirrer under each bottle was turned on to activate the Teflon-coated stirring bar inside the bottle. Growth medium was prepared from stock solutions which were autoclaved separately, cooled, and then mixed with the final pH adjusted to 6.8 ± 0.2. Stock Solution I (50 ml) supplied the following ingredients for 1 l of complete medium: KH_2PO_4 (0.27 g), KCl (0.35 g), $CaCl_2$ (55 mg), $MgSO_4 \cdot 7H_2O$ (0.49 g), NH_4Cl (0.80 g), 37% HCl (4.5 ml), Fe-EDTA (prepared from 20 mg of $FeSO_4 \cdot 7H_2O$), and Solution E_7 (1.0 ml) which provided trace elements H_3BO_4 (2.9 mg), $MnCl_2 \cdot 4H_2O$ (1.8 mg), $ZnSO_4 \cdot 7H_2O$ (0.22 mg), $CuSO_4 \cdot 5H_2O$ (79 μg), $NaMoO_4 \cdot 2H_2O$ (0.25 mg), NH_4VO_3 (23 μg), and $Co(NO_3)_2 \cdot 6H_2O$ (49 μg). Sodium acetate (0.5 g) and water (920 ml) were also mixed with Solution I before autoclaving. Stock Solution II (30 ml) supplied the remaining ingredients: $Na_2CO_3 \cdot H_2O$ (5.0 g), $Na_2S \cdot 9\ H_2O$ (0.12 g) and $Na_2S_2O_3 \cdot 5\ H_2O$ (1.55 g). Bottles were harvested 4 or 5 days after inoculation by the addition of alum (1.4 $g \cdot l^{-1}$ of culture) to aggregate the cells. The aggregated cells were packed by low-speed centrifugation and then frozen. Under this regime a single 20-l culture yielded approx. 100–150 g of packed wet cells.

Bacteriochlorophyll–protein from C. thiosulfatophilum

Bacteriochlorophyll–protein from *C. thiosulfatophilum* was prepared according to the procedure[10] described for *Cps ethylica* with some steps omitted and others modified slightly. Following sonication and the addition of $(NH_4)_2SO_4$, the green precipitate from Step 2 was resuspended in 10 mM Tris buffer (pH 8.0) and dialyzed against buffer to remove $(NH_4)_2SO_4$. Step 3 was omitted. The salt-free suspension from the dialysis bag was centrifuged at $30000 \times g$ to remove undissolved material, and the dark green supernatant was run onto a DEAE-cellulose column. The loaded column was washed with 10 mM Tris, and then eluted with 0.25 M NaCl, 10 mM Tris (pH 8.0). A blue band was eluted from the column and the peak fractions were combined. The bacteriochlorophyll–protein thus obtained was completely free of chlorobium chlorophyll, but was contaminated with some colorless protein from the bacteria. Partially purified bacteriochlorophyll–protein from two DEAE-cellulose columns (2.5 cm × 40 cm and 2.5 cm × 90 cm) was concentrated by ultrafiltration in a collodion bag suction apparatus (Schleicher and Schuell), dialyzed against 10 mM Tris buffer (pH 8.0) and rechromatographed on DEAE-cellulose. A constant gradient of increasing NaCl (0–0.3 M) in 10 mM Tris was used to elute the bacteriochlorophyll–protein. The peak tubes were combined and concentrated approx. 4-fold with polyethylene glycol, and then dialyzed against 0.25 M NaCl in 10 mM Tris. The tail fractions were likewise combined, concentrated, and dialyzed.

Bacteriochlorophyll–reaction center complex from C. thiosulfatophilum

Crude preparations of bacteriochlorophyll–reaction center complex were prepared from *C. thiosulfatophilum* by a modification of the procedure devised by Fowler *et al.*[4] for *Cps ethylica*. Frozen cells (approx. 100 g) of *C. thiosulfatophilum* were thawed and then resuspended (homogenized) in 200 ml of 10 mM sodium/potassium phosphate buffer (pH 7.5). The suspension was centrifuged at $10000 \times g$ for 10 min to sediment the cells, and the supernatant (wash) was discarded. In this manner the cells were washed 2 or 3 times to get rid of the alum used to harvest the cells. The supernatants from these washes were dark green and indicated considerable cell breakage in the processes of freezing, thawing, and washing the cells. The washed cells were again resuspended in 10 mM phosphate buffer. Solid sodium ascorbate was added to a concentration of 10 mM, and the cell suspension was passed through a French pressure cell at 20000 lb/inch^2 2 or 3 times to break the cells and also to break up the vesicles. The effluent from the pressure cell was centrifuged for 90 min at $40000 \times g$ to sediment unbroken cells and intact vesicles. The supernatant, which is enriched in bacteriochlorophyll relative to chlorobium chlorophyll, was then centrifuged on a layer of 40% sucrose (10 ml) in a Spinco swinging bucket rotor SW27 for 22 h at 27000 rev./min to concentrate the bacteriochlorophyll–reaction center complex in the 40% sucrose. Fractions (1 ml) were collected from the bottom of the centrifuge tubes and examined in the spectrophotometer to determine the ratio of bacteriochlorophyll (810 nm) to aggregated chlorobium chlorophyll (745 nm) in each fraction. The first fractions containing the highest ratio of bacteriochlorophyll to aggregated chlorobium chlorophyll were pooled and stored in the freezer. In the first preparation of crude bacteriochlorophyll–reaction center complex the ratio of $A_{745\,nm}$ (chlorobium chlorophyll) to $A_{810\,nm}$ (bacteriochlorophyll) was 0.73; in the second preparation the ratio was negligibly small. All preparations contained large amounts of chlorobium pheophytin which has an absorption peak at 670 nm. (Fowler *et al.*[4] recentrifuged their preparations in 35% sucrose on a layer of 40% sucrose in a Beckman 65K rotor (approx. $400000 \times g$) in order to achieve a preparation which was free of chlorobium chlorophyll, but contained a relatively small amount of pheophytin.) The presence of chlorobium pheophytin, however, did not interfere with either absorption or CD measurements in the region of the Q_y band of bacteriochlorophyll.

Absorption and CD spectra

Absorption spectra were recorded on a Cary 14R spectrophotometer and CD spectra were recorded on a Jasco–Durrum spectropolarimeter Model J-20, with extended red sensitivity. For low temperature absorption spectra ($T = 100$ °K) samples were mixed with glycerol to give a 50:50 mixture by volume. The path length was either 2.5 or 3 mm in a sandwich type cell constructed of copper with plexiglass windows. The sample in the cell was maintained at approx. 100 °K in a copper block in contact with liquid N_2.

Absorption difference spectra (light *minus* dark) in the long-wavelength region were obtained in the Cary 14R using side illumination at 530 nm by a 150-W tungsten lamp through an interference filter and a Corning 4-94 filter, with the photomultiplier protected by a 2-64 filter. A similar illumination was used for the light-

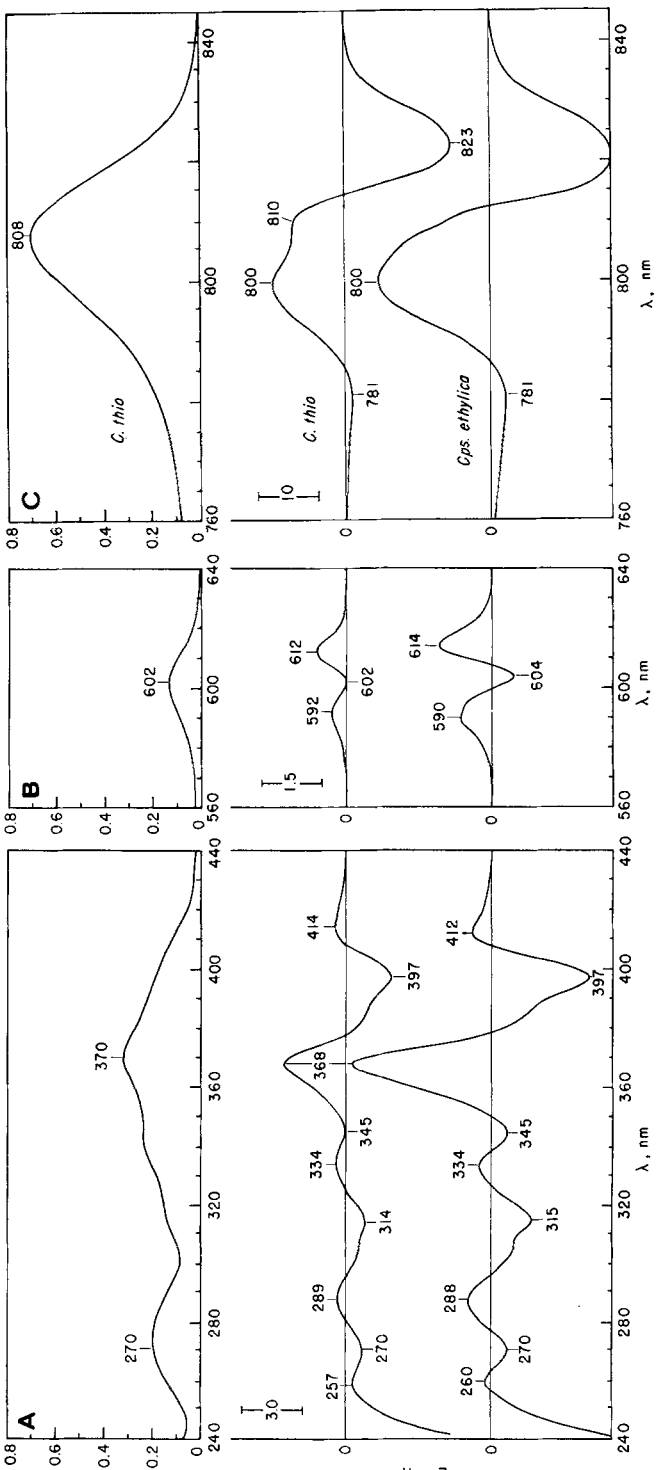

Fig. 1. Absorption (upper curve) and CD (middle curve) spectra of bacteriochlorophyll–protein from *C. thiosulfatophilum*, and CD spectrum (lower curve) of bacteriochlorophyll–protein from *Cps ethylica*. Samples dissolved in 10 mM Tris (pH 8.0), 0.25 M NaCl; pathlength, 1.0 cm; temperature, 24 °C. The figure is a composite of the spectra of several samples: absorption spectrum (upper curve) of bacteriochlorophyll–protein (*C. thiosulfatophilum*) is that of a single solution ($A_{808\ nm} = 0.695$); CD spectrum (middle curve) of bacteriochlorophyll–protein (*C. thiosulfatophilum*), in ultraviolet and visible is for a sample with $A_{371\ nm} = 0.965$ and $A_{602\ nm} \cong 0.4$ ($A_{808nm} \approx 2.0$), in far red for a sample with $A_{808\ nm} = 1.30$; CD spectrum (lower curve) of bacteriochlorophyll–protein (*Cps ethylica*) in ultraviolet and visible is for a sample with $A_{371\ nm} = 1.66$ and $A_{603\ nm} = 0.72$ ($A_{809\ nm} \approx 3.7$), in far red for a sample with $A_{809\ nm} = 1.51$.

minus-dark CD spectra, but measurements were made at a series of fixed wavelengths owing to the small magnitude of the change induced in the CD spectra. Absorption difference spectra in the 390–570-nm region were measured using a special double-beam spectrophotometer (G. Johnson, Baltimore, Md, modified for scanning by G. Hind, Brookhaven National Laboratory).

RESULTS

Bacteriochlorophyll–protein absorption and CD spectra

The room temperature absorption spectrum of the bacteriochlorophyll–protein from *C. thiosulfatophilum* (Fig. 1, upper curves) is very similar to that determined previously for the bacteriochlorophyll–protein from *Cps ethylica*. Table I presents a comparison of the peak positions and relative absorbances for the bacteriochlorophyll–proteins from the two organisms. Although the far-red peaks differ by 1 nm, the band shapes and other features are very similar for the two bacteriochlorophyll–proteins at room temperature. The far-red band ratios given in Table I are the highest values observed; more typical values ranged between 2.1 and 2.2 for *Cps ethylica* and between 2.0 and 2.1 for *C. thiosulfatophilum*.

TABLE I

ABSORPTION CHARACTERISTICS OF BACTERIOCHLOROPHYLL–PROTEINS AT ROOM TEMPERATURE

Samples dissolved in 10 mM Tris (pH 8.0), 0.25 M NaCl; path lengths, 1.0 cm.

Cps. ethylica		*C. thiosulfatophilum*	
λ (*nm*)	$\varepsilon_\lambda/\varepsilon_{370.5}$	λ (*nm*)	$\varepsilon_\lambda/\varepsilon_{370.5}$
267	0.55	270	0.61
343	0.73	342.5	0.76
370.5	1.00	370.5	1.00
603	0.42	602.5	0.42
745	0.20	745	0.20
809	2.30	808	2.28

The room temperature CD spectra of the two proteins are compared in Fig. 1 (lower curves). As described previously by Philipson and Sauer[12], the CD spectrum of bacteriochlorophyll–protein shows complex features resulting from exciton interactions involving each of the electronic absorption bands. Careful comparison of the CD spectra of the two bacteriochlorophyll–proteins shows that the same components appear to be present in both, but the absolute and relative amplitudes are somewhat different.

Absorption spectra measured at 77–100 °K are compared in Fig. 2. At the lower temperature, a greatly improved resolution of the long wavelength absorption bands of the bacteriochlorophyll–proteins enables one to observe that each of the bacteriochlorophyll–protein molecules exhibits exciton components in its absorption spectrum, as already indicated by the CD spectrum. There is a close

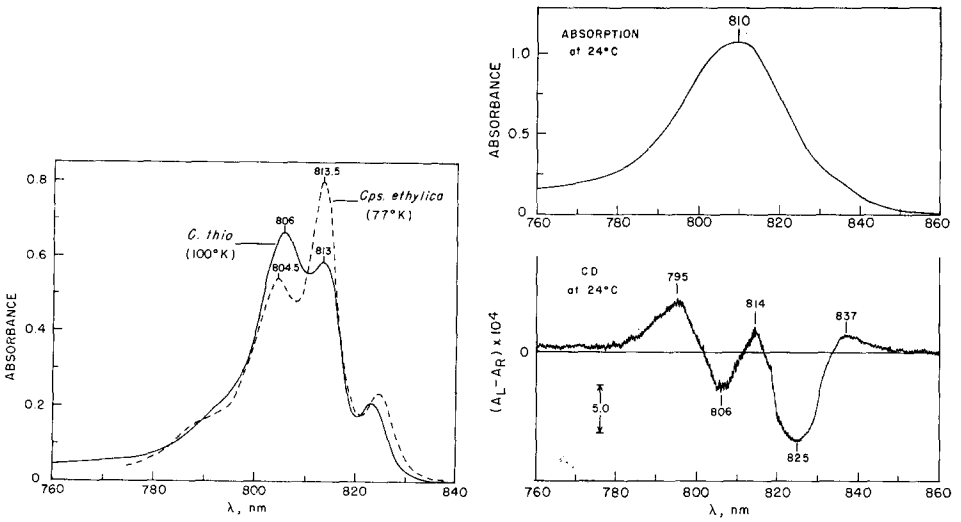

Fig. 2. Low-temperature absorption spectra of bacteriochlorophyll–proteins from *C. thiosulfatophilum* (——) and from *Cps ethylica* (- - -). *C. thiosulfatophilum* bacteriochlorophyll–protein dissolved in 10 mM Tris buffer (pH 7.5) + 0.2 M NaCl glycerol (1:1, v/v) matrix at approx. 100 °K. *Cps ethylica* bacteriochlorophyll–protein dissolved in 75% potassium glycerophosphate, 10 mM Tris (pH 7.5)+0.2 M NaCl glycerol (4:2:1, v/v/v) matrix at 77 °K (see ref. 12 for details). Pathlengths, approx. 3 mm.

Fig. 3. Absorption (upper curve) and CD (lower curve) spectra of bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum*. Pathlength, 1.0 cm; temperature, 24 °C. Sample prepared by diluting the centrifuge fraction (in 40% sucrose) 8-fold with 10 mM phosphate (pH 7.5), 2 mM KCl solution.

TABLE II

ABSORPTION CHARACTERISTICS OF Q_y BAND OF BACTERIOCHLOROPHYLL–PROTEINS AT LOW TEMPERATURE

Solution of bacteriochlorophyll–protein from *Cps ethylica* in 75% potassium glycerophosphate, 10 mM Tris (pH 7.5)+0.2 M NaCl, glycerol (4:2:1, v/v/v) (see ref. 12); path length 3.0 mm. Solutions of bacteriochlorophyll–protein and bacteriochlorophyll–reaction center complex in 10 mM Tris (pH 7.5)+0.2 M NaCl, glycerol (1:1, v/v); path length, 2.5 mm.

Cps ethylica bacteriochlorophyll–protein		*C. thiosulfatophilum bacteriochlorophyll–protein*		*C. thiosulfatophilum bacteriochlorophyll–reaction center complex*	
λ (*nm*)	$A_\lambda/A_{813.5}$	λ (*nm*)	A_λ/A_{806}	λ (*nm*)	A_λ/A_{814}
804.5	0.70	806	1.00	804.5	0.94
813.5	1.00	813	0.88	814	1.00
824.5	0.30	823	0.31	823	0.45
—	—	—	—	833	0.24

correspondence in the positions of the low temperature absorption peaks and a general similarity in the amplitude pattern, with the exception that the two major exciton components are reversed in intensity in the two bacteriochlorophyll–proteins. A comparison of peak wavelengths and relative amplitudes is given in Table II.

Bacteriochlorophyll–reaction center complex

Absorption and CD spectra. The absorption spectrum of the Q_y band at room temperature (Fig. 3, upper curve) for the bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum* is very similar to the corresponding spectrum for the complex from *Cps ethylica*[4]. Each exhibits an absorption maximum at 810 nm and a shoulder at about 835 nm; the latter is not present in the spectra of the purified bacteriochlorophyll–proteins.

The room temperature CD spectrum for the Q_y band of the *C. thiosulfatophilum* complex shown in Fig. 3 (lower curve) provides evidence to support the presence of multiple exciton components in this region. A distinct contrast is seen with the CD spectrum of the bacteriochlorophyll–protein (Fig. 1) from the same organism, particularly the presence of a trough at 806 and a small peak at 837 nm in the CD spectrum of the bacteriochlorophyll–reaction center complex.

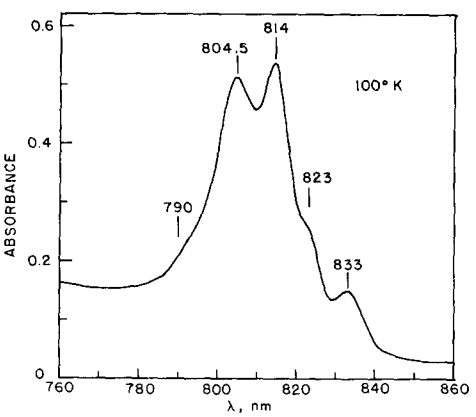

Fig. 4. Low-temperature absorption spectrum of bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum.* Sample dissolved in a glycerol, 10 mM Tris (pH 7.5)+0.2 M NaCl (1:1, v/v) matrix; spectrum measured at approx. 100 °K; pathlength, 2.5 mm.

The low temperature absorption spectrum (Fig. 4) of the complex also exhibits clear evidence of multiple components. Taken together, the room-temperature CD and the low-temperature absorption spectra suggest the presence of at least five distinct spectral components in the bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum.* These are summarized in Table III. It should be noted that the relative height of the 814-nm peak in the CD spectra varied somewhat in the preparations examined. This may be related to a slow oxidation of the complex, because the addition of potassium ferricyanide to the preparation completely eliminated the CD trough at 806 nm and the peak at 814 nm without significant effect on the low-temperature absorption spectrum.

TABLE III

COMPONENTS OF EXCITON SPLITTING OF Q_y BAND OF BACTERIOCHLOROPHYLL–REACTION CENTER COMPLEX FROM *C. THIOSULFATOPHILUM*

Absorption spectrum, 100 °K λ (nm)	*CD spectrum, 24 °C λ (nm)*	*CD sign*
(790)	795	+
804.5	806	—
814	814	+
(823)	825	—
833	837	+

On the basis of the CD and low temperature absorption spectra of preparations containing various amounts of *chlorobium* chlorophyll (745 nm), it was apparent that its presence or absence had no influence on the spectral characteristics of the bacteriochlorophyll in the bacteriochlorophyll–reaction center complex.

Reaction center absorption changes. Evidence for photochemical activity in the bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum* was obtained by measuring light-induced absorbance changes. The results in the 400–450- and 510–570-nm wavelength regions are shown in Fig. 5. Far-red actinic light (750–1000 nm) was obtained from a 500-W tungsten lamp with optical filters Corning 2-64, Wratten 88A and Corning 2-59 in series, and a lucite light pipe to

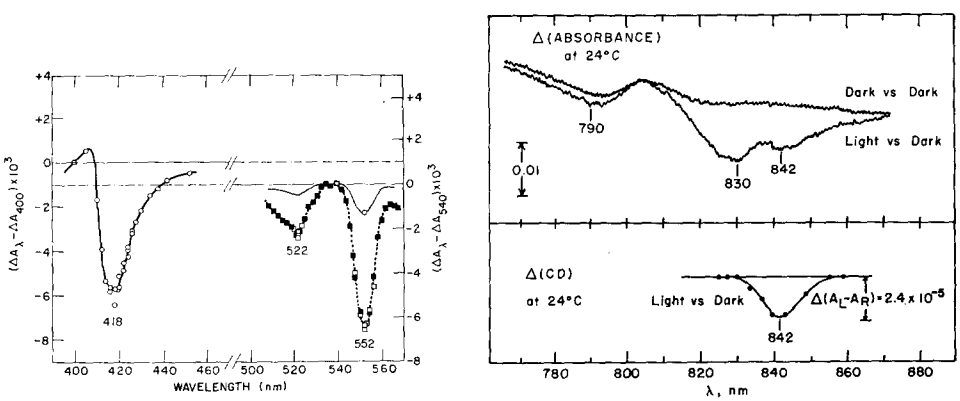

Fig. 5. Light-induced absorbance changes of the bacteriochlorophyll–reaction complex from *C. thiosulfatophilum* at room temperature. Changes referred to 400 nm in the 400–450-nm region and 540 nm in the 500–570-nm region. Data for two dilutions of the same sample are shown: $A_{809\ nm} = 0.19$ for the circles; $A_{809\ nm} = 0.90$ for the squares. Illumination patterns: intermittent light–dark for the open symbols; continuous illumination *vs* continuous dark for the filled squares. See text for additional experimental details.

Fig. 6. Light-induced absorbance (upper curves) and CD (lower curves) changes of the bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum* at room temperature. Samples used exhibited $A_{809\ nm} = 1.07$; pathlengths, 1.0 cm. A small amount of solid sodium ascorbate was added prior to the measurements. See text for additional experimental details.

conduct the resulting beam to the sample cuvette. The intensity (34 $mW \cdot cm^{-2}$) was sufficient to saturate the absorption changes. Half times of 1.0–1.5 s for the light-on response and 7–10 s for the light-off response were observed.

No light-induced absorbance changes were observed until a small amount of (solid) sodium ascorbate was added to the sample. Then either pulsed (15–30 s light followed by 90 s dark) or continuous illumination produced the oxidation of cytochrome 552. Assuming an oxidized-*minus*-reduced extinction difference ($\Delta\varepsilon_{552\ nm}-\Delta\varepsilon_{540\ nm}$) = 20 $mM^{-1} \cdot cm^{-1}$ for cytochrome 552, and $\varepsilon_{809\ nm}$ = 100 $mM^{-1} \cdot cm^{-1}$ for bacteriochlorophyll, the ratio of bacteriochlorophyll to photo-oxidizable cytochrome 552 is 28:1.

Light-*minus*-dark difference spectra in the region of the Q_y band of bacteriochlorophyll are shown in Fig. 6. The light-induced decrease in absorption (Fig. 6, upper curves) is maximal at 830 and 842 nm, and only a weak decrease is observed at wavelengths around 790 nm. This is very similar to the shape of the light-*minus*-dark difference spectrum observed by Fowler *et al.*[4] for *Cps ethylica* bacteriochlorophyll–reaction center complex. Their value of bacteriochlorophyll/P840 = 80 compares with a value of $A_{810\ nm}/\Delta A_{840\ nm} = 120$ from the data shown for *C. thiosulfatophilum* in Fig. 6. Although these ratios are probably valid for comparison purposes, they should probably not be used to count reaction center bacteriochlorophyll molecules because they ignore the larger reversible bleaching at 830 nm.

Oxidized-*minus*-reduced absorption changes (ferricyanide *versus* ascorbate) for the bacteriochlorophyll–reaction center complex complex from *C. thiosulfatophilum* exhibited essentially the same wavelength dependence in the far-red spectral region as is shown in Fig. 6 for the light-induced absorption changes. We did not observe a relatively greater oxidized-*minus*-reduced absorption change at 790 nm, as reported by Fowler *et al.*[4] for their preparation from *Cps ethylica*.

On the basis of the absorbance changes described above, we calculate a ratio of cytochrome 552 to P840 of about 4, which is the same as the value observed for the corresponding light-oxidizable components of the bacteriochlorophyll–reaction center complex from *Cps ethylica*[4]. Our value must be considered provisional, however, for the measurements of the cytochrome and P840 changes were made about a month apart and in different laboratories, although aliquots of the same frozen sample were used for the two studies.

Reaction center CD changes

The CD spectra of ferricyanide-oxidized and ascorbate-reduced bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum* were carefully measured and compared, but no significant difference between them was detectable. In particular, the long wavelength CD peak at 837 nm did not disappear upon oxidation. It was possible, however, to observe very small photoinduced CD changes by illuminating the ascorbate-reduced sample from the side with short wavelength actinic light. The CD changes were observed as dark–light–dark signals while the instrument monochromator was fixed at a particular wavelength in the region of interest. From a series of measurements carried out in this fashion, the data plotted in Fig. 6 (lower curve) were obtained. A distinct decrease in the CD centered at 842 nm was observed; this decrease was reversed upon turning off the actinic light. No measurable CD changes were observed at 830 nm. The magnitude of the CD change is

$A(A_L - A_R)_{842\ nm} = 2.4 \cdot 10^{-5}$ for a sample concentration giving $A_{810\ nm} = 1.07$; the relative change in the CD at 842 nm is calculated to be approx. 4%. It is not surprising, therefore, that this difference was not detected in the successive spectral scans of an oxidized and a reduced solution.

DISCUSSION

A comparison of the spectral properties of bacteriochlorophyll–proteins isolated from *Cps ethylica* and *C. thiosulfatophilum* suggests that the molecular structures of these two proteins are very similar to one another. The clearest evidence comes from the room temperature CD spectra (Fig. 1) and the low temperature absorption spectra (Fig. 2), which are both much richer in information content than the room temperature absorption spectra. The CD spectra throughout the ultraviolet, visible and near infrared regions, contain peaks, troughs and shoulders that are present for both bacteriochlorophyll–proteins, and there are no bands that appear in one spectrum and not in the other. The differences in CD signal amplitudes and in the relative peak heights and positions in the low temperature absorption spectra probably reflect only minor differences in the molecular arrangements. Because of the great sensitivity of the CD features that result from exciton interactions among associated pigment molecules to the geometry of the aggregates, we conclude that the bacteriochlorophyll arrangements are very similar in these two bacteriochlorophyll–proteins.

Our previous study of the low temperature CD spectrum of the bacteriochlorophyll–protein from *Cps ethylica*[12] was interpreted in terms of an exciton splitting of the Q_y band of the bacteriochlorophyll into five states in the bacteriochlorophyll–protein. This is rationalized as probably resulting from coupling among five bacteriochlorophyll molecules in each of the four subunits of the protein. The subunits are very probably identical to one another, and the bacteriochlorophyll molecules in one protein subunit are apparently encapsulated within the protein in such a manner that they do not couple strongly with bacteriochlorophyll molecules in any adjacent subunit. Although this model needs confirmation from further experiments, we can now extend it as offering the simplest interpretation of the internal molecular arrangement of the bacteriochlorophyll–protein from *C. thiosulfatophilum* as well.

The bacteriochlorophyll–reaction center complex from *C. thiosulfatophilum* exhibits its own characteristic CD and low-temperature absorption spectra. We had anticipated the possibility that this complex was a simple association of 4 to 6 normal bacteriochlorophyll–protein molecules (containing 80–120 bacteriochlorophyll molecules) with a small number of reaction center bacteriochlorophyll molecules. The CD spectra show clearly that this simple picture is not correct (*cf.* Fig. 1 with Fig. 3). While there appear to be five distinct peaks and troughs in the CD spectrum of the bacteriochlorophyll–reaction center complex in the long-wavelength region, the shape and positions are quite different from those of the bacteriochlorophyll–protein. Furthermore, there appear to be several distinct inflections and shoulders present in addition to the five major CD components.

On the basis of our light-*minus*-dark experiments, the reaction center bacteriochlorophyll molecule(s) appear to make an even smaller relative contribution to

the CD spectrum than they do to the absorption spectrum. By contrast with isolated, purified reaction centers from purple photosynthetic bacteria[13–15] there seems to be no spectral region where the absorption (or CD) is due only to a reaction center pigment of the bacteriochlorophyll–reaction center complexes from green bacteria. For this reason it is difficult to consider the possible similarities of the reaction center bacteriochlorophyll geometries in these different bacteria. The bleaching of absorption centered at 842 nm in the bacteriochlorophyll–reaction center complex is associated with a small decrease in the CD centered at the same wavelength, whereas the larger bleaching at 830 nm is not accompanied by any detectable change in the CD at that wavelength. This behavior is qualitatively different from that observed for purified reaction centers from purple bacteria[14] and suggests that the reaction center geometries are significantly different in green and purple photosynthetic bacteria.

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