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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 (BChl-P) and a complex which also contained photochemically-active reaction centers (BChl-RC) were isolated from *Chlorobium thiosulfatophilum*. The procedures were similar to those used previously on *Chloropseudomonas ethylica* (FOWLER, NUGENT AND FULLER, 1971), 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 BChl-RC complex is distinctly different from that in the BChl-protein.

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INTRODUCTION

Green photosynthetic bacteria contain a small amount of bacteriochlorophyll a (BChl a) in addition to the principal light collecting pigment, chlorobium chlorophyll.[1] In vivo, chlorobium chlorophyll transfers electronic excitation energy to BChl a,[2] which in turn transfers the energy to a specialized reaction center chlorophyll known as P840.[3] The photosynthetic unit appears to be very large in green bacteria with approximately 80 BChl 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 400,000 x g, FOWLER et al.[4] separated the material into a "light" fraction (1.12 g-cm^{-3}) which contained chlorobium chlorophyll and a "heavy" fraction (1.18 g-cm^{-3}) which contained BChl a,

carotenoid, P840 and cytochrome. The "heavy" fraction was photochemically active as shown by the light-induced oxidation of P840 ($E_{m,7.5}$ = +0.24V) and cytochrome 553 ($E_{m,7.5}$ = +0.17V). This heavy fraction, which will be designated henceforth as a BChl-Reaction-Center complex (BChl-RC), has a molecular weight in excess of 1.5 x 10^6 dalton.[4]

It had previously been shown that BChl a in the form of a water-soluble BChl-protein (M.W. = 152,000) could be obtained from green bacteria by alkaline extraction followed by conventional methods of protein purification.[1,2,10] The BChl-protein from Cps. ethylica has been characterized most extensively. It is a globular protein consisting of four probably identical subunits, each containing five BChl molecules which are bound inside the protein by non-covalent forces.[9,10] The BChl-protein is completely free of chlorobium chlorophyll, carotenoid, cytochrome, P840 or any photochemical activity. The absorption spectrum at 80°K,[10] and the circular dichroism (CD) spectra at either room temperature[10,11] or 80°K,[12] give strong evidence for exciton splitting of both the Q_x- and Q_y-transitions of BChl in the protein. For the Q_y-transition the resolution of 4 components in the absorption spectrum and of 5 components in the CD spectrum suggests that interactions between the 5 BChl molecules in each subunit accounts for the exciton splittings.[12]

In order to compare the pigment-pigment interaction in the BChl-RC complex to those in the purified BChl-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 BChl-protein and BChl-RC complex for comparison with the previous preparations from Cps. ethylica.

MATERIALS AND METHODS

BChl-protein from Cps. ethylica* was prepared at Brookhaven National Laboratory[10] and stored in the refrigerator as a precipitate in 30 g ammonium sulfate per 100 ml of original solution. Solutions of BChl-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-watt 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 liter of complete medium: KH_2PO_4 (0.27 g), KCl (0.35 g), $CaCl_2$ (55 mg), $MgSO_4$-7 H_2O (0.49 g), NH_4Cl (0.80 g), 37% HCl (4.5 ml), Fe-EDTA (prepared from 20 mg of $FeSO_4$-7 H_2O), and

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*Cultures of "Cps. ethylica" grown at Brookhaven National Laboratory and elsewhere have been shown recently to consist of syntrophic mixtures of Chlorobium limicola and one or more colorless bacteria which function as sulfate-reducers [Gray, B. H., Fowler, C. F., Nugent, N. A., Rigopoulos, N., and Fuller, R. C. (1972) Int. J. Syst. Bacteriol., in press]. The BChl-protein and the BChl-RC complex obtained from these mixed cultures should properly be attributed to C. limicola.

solution E_7 (1.0 ml) which provided trace elements H_3BO_4 (2.9 mg), $MnCl_2$-4 H_2O (1.8 mg), $ZnSO_4$-7 H_2O (0.22 mg), $CuSO_4$-5 H_2O (79 μg), $NaMoO_4$-2 H_2O (0.25 mg), NH_4VO_3 (23 μg), and $Co(NO_3)_2$-6 H_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-H_2O (5.0 g), Na_2S-9 H_2O (0.12 g) and $Na_2S_2O_3$-5 H_2O (1.55 g). Bottles were harvested 4 or 5 days after inoculation by the addition of alum (1.4 $g\text{-}l^{-1}$ 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 to 150 g of packed wet cells.

BChl-protein from C. thiosulfatophilum

BChl-protein from C. thiosulfatophilum was prepared according to the procedure[10] described for Cps. ethylica with some steps omitted and others modified slightly. The green precipitate from step 2 was resuspended in 10 mM Tris, pH 8.0 and dialyzed against buffer to remove ammonium sulfate. Step 3 was omitted. The salt-free suspension from the dialysis bag was centrifuged at 30,000 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 BChl-protein thus obtained was completely free of chlorobium chlorophyll, but was contaminated with some colorless protein from the bacteria. Partially purified BChl-protein from two DEAE columns (2.5 cm x 40 cm and 2.5 cm x 90) was concentrated by ultrafiltration in a collodion bag suction apparatus

(Schleicher and Schuell), dialyzed against 10 mM Tris, pH 8.0, and rechromatographed on DEAE-cellulose. A constant gradient of increasing NaCl (0 to 0.3 M) in 10 mM Tris was used to elute the BChl-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.

BChl-RC complex from C. thiosulfatophilum

Crude preparations of BChl-RC 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 Na/K phosphate (pH 7.5). The suspension was centrifuged at 10,000 x 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 wash d cells were again resuspended in 10 mM phosphate. Solid Na ascorbate was added to a concentration of 10 mM, and the cell suspension was passed through a French pressure cell at 20,000 psi 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 40,000 x g to sediment unbroken cells and intact vesicles. The supernatant, which is enriched in BChl 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 27,000 rpm to concentrate the BChl-RC complex in the 40% sucrose. Fractions (1 ml) were

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