A unique photosynthetic reaction center from *Heliobacterium chlorum*

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A previously unknown type of photosynthetic reaction center in the brownish-green bacterium *Heliobacterium chlorum* is described. The reaction center is tightly bound in a highly proteinaceous undifferentiated plasma membrane and contains bacteriochlorophyll *g*, which has major in vivo absorbancies at 788, 576 and 370 nm. The purified membrane shows a reversible photobleaching at 798 nm; the reaction center bacteriochlorophyll *g* is designated P798. A reversible photobleaching at 553 nm is assigned to photooxidation of a membrane-bound *c*-type cytochrome. The membrane structure, pigment composition and photochemical properties suggest that *H. chlorum* may represent a fifth family of anoxygenic photosynthetic procaryotes.

Heliobacterium chlorum *Photosynthesis* *Bacteriochlorophyll* *Reaction center* *Cytochrome* *Evolution*

1. INTRODUCTION

Photosynthetic energy storage is carried out by pigment-protein complexes associated with the cell membrane in procaryotic organisms or the chloroplast thylakoid membranes in eucaryotic forms. Study of the relatively simple membrane architecture and single photochemical reaction center of the anoxygenic (non-oxygen-evolving) photosynthetic bacteria provides a basic structural model for understanding the more complex oxygen-evolving organisms, which contain two types of reaction centers. Comparative biochemical studies suggest that oxygenic organisms must have evolved from relatively simple anoxygenic photosynthetic procaryotes living on the early, anaerobic earth [1].

Purple photosynthetic bacteria (families Rhodospirillaceae and Chromatiaceae) contain a somewhat differentiated plasma membrane, in which photosynthetic components are localized in a specialized portion of the cell membrane called the intracytoplasmic membrane [2]. When isolated, these membranes are also known as chromatophores. The green photosynthetic bacteria (families Chlorobiaceae and Chloroflexaceae) possess a single undifferentiated cytoplasmic membrane. Light-harvesting structures known as chlorosomes are attached to this membrane [3].

The recent discovery of a strictly anaerobic phototrophic bacterium, *Heliobacterium chlorum* [4], that contains a previously unknown bacteriochlorophyll (BChl *g*) [5] provides the opportunity to extend our knowledge of photosynthesis over a broader range of organisms. Here we describe the membrane structure, antenna absorption and reaction center photochemistry of this unusual organism.

Abbreviations: BChl, bacteriochlorophyll; LDS-PAGE, lithium dodecyl sulfate polyacrylamide gel electrophoresis

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2. MATERIALS AND METHODS

Washed cells (grown in medium 112 [4]) were disrupted by passage through a French pressure cell at 15000 lb/inch^2. The particle fraction was spun at 15000 × g for 10 min to remove unbroken cells and large fragments. The supernatant fluid was centrifuged at 144000 × g for 90 min. For electron microscopy, the cells were glycerinated to 35% (v/v) immediately after delivery (24 h shipping time on ice), and dip-frozen in liquid nitrogen-cooled Freon 12. Samples were fractured and replicated with Pt-C at −110°C in a Balzers 360M instrument equipped with electron guns. Replicas were cleaned in commercial bleach, 35% (v/v) sulfuric acid, and washed in several changes of distilled water before viewing in a JEOL 100C electron microscope. Magnification: 90000×.

Laser flash-induced kinetics and difference spectra were measured using the spectrophotometer described in [6]. Excitation was by 1 μs, 584 nm dye laser pulses.

Absorption spectra were taken on a Varian Cary 219 spectrophotometer. Continuous-light-induced difference spectra were obtained by focusing the output of a 100 W tungsten lamp onto a flexible light pipe which plugged into a hole drilled in a cuvette holder. Appropriate cutoff filters isolated broadband blue or red illumination, and complementary filters protected the photomultiplier from scattered light. Spectra were stored on an Apple II+ computer and subtracted numerically.

LDS-PAGE (12.5% acrylamide) was carried out at 4°C using a modification of the Laemmli procedure [7]. 2-Mercaptoethanol was added only to the M_r standards. Gels were stained for protein using Coomassie blue. Heme staining was done using the method of Thomas et al. [8].

3. RESULTS AND DISCUSSION

Cells of *Heliobacterium* do not show any of the characteristic membrane components seen in the other photosynthetic procaryotes. As shown in fig.1, freeze-fracture preparations indicate an extremely particle-rich cytoplasmic membrane. The intracytoplasmic membrane system typical of purple bacteria, phycobilisomes of the cyanobacteria and the antenna chlorosomes of the green photosynthetic bacteria are all absent. The high

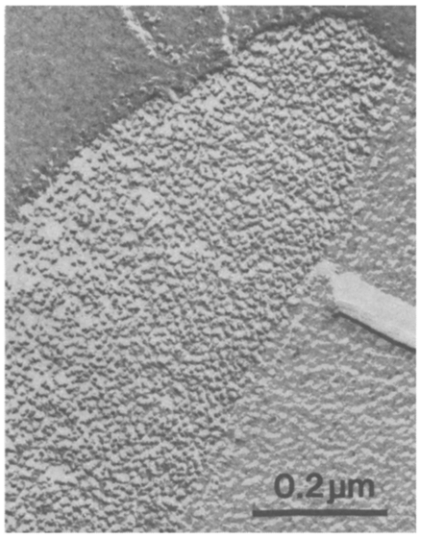

Fig.1. Protoplasmic fracture face (PF) of *Heliobacterium* showing high density of protein particles in the cytoplasmic membrane. No depressions in the PF, and none of the complementary elevations in the EF, views of the membranes that are characteristic of the chlorosomes of the green bacteria are evident. Magnification: × 90000.

density and apparent uniformity of particles seen in the cytoplasmic membrane fracture face are unusual for bacterial membranes in general, but are reminiscent of the photochemical membrane system in *Rhodopseudomonas tenue* [9]. The particle density indicates a highly proteinaceous membrane. This was confirmed by the isolation and partial purification of cytoplasmic membranes from *Heliobacterium*.

Membrane prepared as described in section 2 were resuspended in buffer and applied to a 30/45/65% sucrose density step gradient. A single green band was observed in the 65% fraction after only 2 h of centrifugation at 200000 × g. This high-density membrane band was shown to contain over 70% protein on a dry weight basis (Lowry protein analysis), in contrast to the thylakoid membranes of chloroplasts (~50% protein [2]) and the cytoplasmic membrane of the green bacterium *Chlorobium* (~27% protein [10]).

An absorption spectrum of the purified membrane fraction is shown in fig.2. The major infrared absorbance at 788 nm, the band at 670 nm, and the band at 576 nm are identical to the in vivo

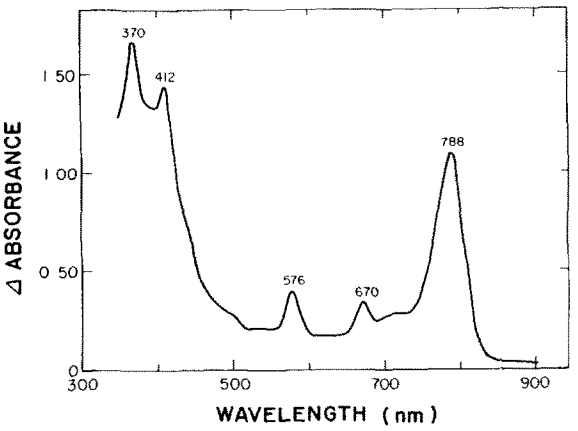

Fig.2. Absorption spectrum of purified membrane fraction from 65% sucrose density gradient band (see text). Membranes were prepared and suspended in 10 mM Tris, 10 mM ascorbate at pH 7.0.

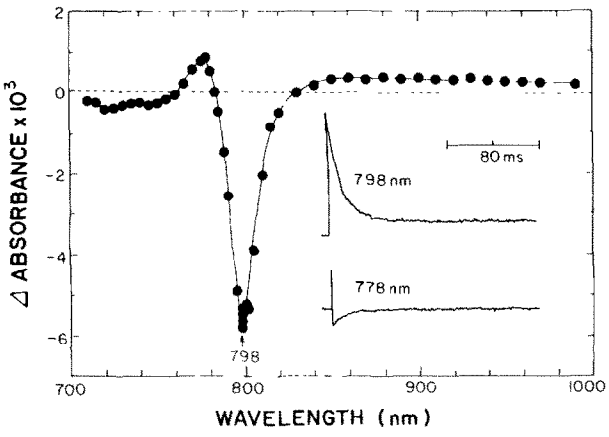

Fig.3. Laser-flash-induced difference spectrum of purified membranes in 10 mM Tris, 10 mM ascorbate buffer (pH 8.0). Inset: kinetics of transmittance changes at 798 and 778 nm (an upward deflection represents an absorbance decrease).

bands seen in whole cells [4]. The band at 370 nm is presumably the Soret absorbance of BChl *g*. The low level of highly conjugated carotenoid pigments in *Heliobacterium* (shown by the low absorption near 430 nm) may account for the extreme sensitivity of the growth of this organism to oxygen.

The BChl *g* absorbing at 788 nm was rapidly converted to a species absorbing at 670 nm when the membrane fraction was suspended in O_2-saturated buffer and illuminated with white light (not shown). The rate of this conversion was decreased 10-fold when a nitrogen atmosphere replaced the oxygen. Based on the structure of BChl *g* and the known chemical and spectroscopic properties of chlorophylls, the 670 nm pigment may represent a form of Chl *a*, the pigment used by oxygenic photosynthetic organisms (J. Fajer and J.J. Katz, personal communications).

A reversible light-induced photobleaching at 798 nm is observed in the purified membranes of *H. chlorum* upon either continuous or laser flash illumination (fig.3) reflecting the photooxidation of BChl *g* in the reaction center, which is designated P798. Concomitant with P798 photobleaching is an absorbance increase at 778 nm and a featureless absorbance increase at wavelengths greater than 850 nm. The absorbance changes reverse with an approximately exponential decay, characterized by $\tau = 12$ ms (inset fig.3). It is interesting to note that the difference spectrum is similar to that observed in photosystem I (P700) of oxygenic phototrophs but is displaced 100 nm towards longer wavelength.

Isolated membranes also exhibited a reversible, light-induced photobleaching centered at 553 nm (fig.4). This is indicative of the involvement of a membrane-bound *c*-type cytochrome in the light-induced electron transport system of *Heliobacterium*. The kinetics of photooxidation were ex-

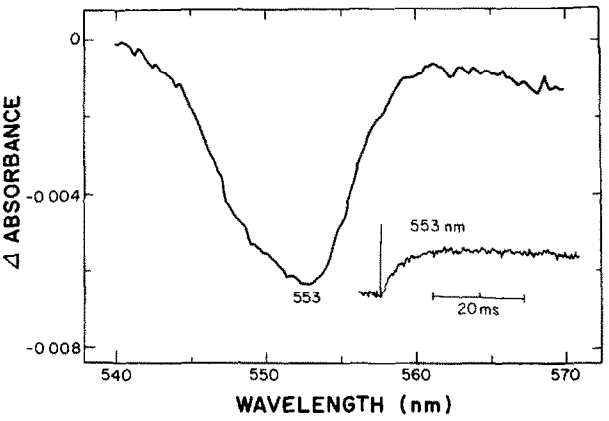

Fig.4. Continuous light-induced difference spectrum of purified membranes in the cytochrome α-band region. Inset: laser-flash-induced photooxidation kinetics measured at 553 nm (an upward deflection represents an absorbance decrease).

ponential, having τ = 4.5 ms, which is very slow compared to cytochrome photooxidation kinetics in other photosynthetic bacteria. Preliminary experiments by Dr T.E. Meyer, University of Arizona, indicate that the *c*-type cytochrome in *Heliobacterium* is almost entirely membrane-bound. In contrast, most other photosynthetic bacteria contain one or more types of soluble cytochrome *c*. In this regard, *Heliobacterium* is most similar to *Chloroflexus aurantiacus* [11,12].

Low-temperature LDS-PAGE provides further evidence for membrane-bound heme proteins. Fig.5 shows an LDS gel of broken whole cells and isolated membranes. Heme-staining bands are evident at 50 and 20 kDa, with an extremely weak band at 33 kDa. When the gel was run at room temperature using SDS-PAGE, a single heme-staining band was observed at 50 kDa. The similarity of the heme-stained lanes for the isolated membranes and broken whole cells supports the conclusion from spectral data that *Heliobacterium* has essentially no soluble cytochromes.

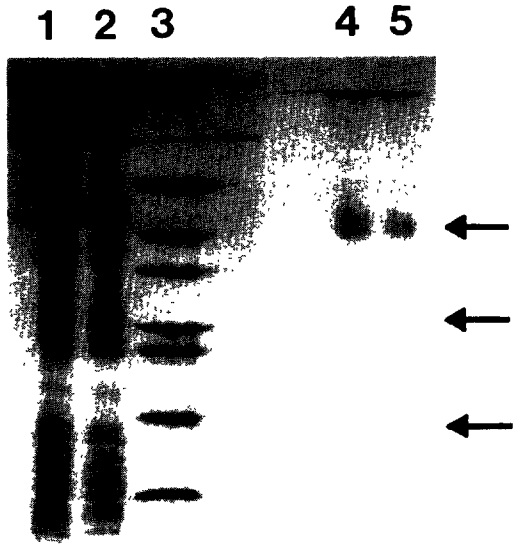

Fig.5. LDS-PAGE of broken whole cells and isolated membrane fragments of *Heliobacterium*. Lanes 1 and 4 are whole cells stained for protein (lane 1) and heme (lane 4). Lanes 2 and 5, isolated membranes stained for protein (lane 2) and heme (lane 5). Lanes 3 and 6 are M_r standards. The arrows indicate the heme-staining bands (from top to bottom) at 50, 33 (extremely weak) and 20 kDa.

One possible interpretation of the heme-protein pattern is that the 50-kDa protein corresponds to cyt *c*-553 and is analogous to the 43-kDa membrane-bound cyt *c*-554 found in *Chloroflexus* [13]. The 20- and 33-kDa peptides might then be part of a cytochrome *bc* complex similar to that found in all other photosynthetic organisms [14]. Further biochemical characterization is needed to test this hypothesis.

The physiological and structural properties of *Heliobacterium* suggest that this organism may represent a relatively primitive type of photosynthetic procaryote. Analysis of the biochemistry of organisms such as *Heliobacterium*, *Chloroflexus aurantiacus* [12,15] and other anoxygenic phototrophs will be helpful in the elucidation of the biochemical evolution of oxygenic photosynthesis.

The anoxygenic photosynthetic procaryotes have been divided into two major groups, the purple bacteria and the green bacteria. Classification of these organisms is based primarily on pigment composition, membrane architecture and metabolic capabilities [16]. Clearly, the pigment composition, the membrane structure, and the photochemical characteristics described above do not permit inclusion of *Heliobacterium chlorum* in any of the established families of photosynthetic bacteria.

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