Protein sequences and redox titrations indicate that the electron acceptors in reaction centers from heliobacteria are similar to Photosystem I

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Abstract

Photosynthetic reaction centers isolated from *Heliobacillus mobilis* exhibit a single major protein on SDS–PAGE of 47 000 M r. Attempts to sequence the reaction center polypeptide indicated that the N-terminus is blocked. After enzymatic and chemical cleavage, four peptide fragments were sequenced from the *Heliobacillus mobilis* apoprotein. Only one of these sequences showed significant specific similarity to any of the protein and deduced protein sequences in the GenBank data base. This fragment is identical with 56% of the residues, including both cysteines, found in the highly conserved region that is proposed to bind iron–sulfur center Fₓ in the Photosystem I reaction center peptide that is the psaB gene product. The similarity to the psaA gene product in this region is 48%.

Redox titrations of laser-flash-induced photobleaching with millisecond decay kinetics on isolated reaction centers from *Heliobacterium gessii* indicate a midpoint potential of −414 mV with n = 2 titration behavior. In membranes, the behavior is intermediate between n = 1 and n = 2, and the apparent midpoint potential is −444 mV. This is compared to the behavior in Photosystem I, where the intermediate electron acceptor A₁, thought to be a phylloquinone molecule, has been proposed to undergo a double reduction at low redox potentials in the presence of viologen redox mediators.

These results strongly suggest that the acceptor side electron transfer system in reaction centers from heliobacteria is indeed analogous to that found in Photosystem I. The sequence similarities indicate that the divergence of the heliobacteria from the Photosystem I line occurred before the gene duplication and subsequent divergence that lead to the heterodimeric protein core of the Photosystem I reaction center.

Abbreviations: BChl – bacteriochlorophyll; %C – percent bisacrylamide as a percentage of total acrylamide; DTT – dithiothreitol; EPR – electron paramagnetic resonance; Fe–S – iron–sulfur center; H. – *Heliobacterium*; Hb. – *Heliobacillus*; k – one thousand; M r – molecular retention; PS I – Photosystem I; PS II – Photosystem II; RCs – reaction centers; SDS – sodium dodecyl sulfate; SDS–PAGE – sodium dodecyl sulfate polyacrylamide electrophoresis; %T – percent total acrylamide; Tris – tris(hydroxymethyl)aminomethane.
Introduction

Photosynthetic organisms store energy by carrying out light-induced charge separation, followed by stabilization of the primary photoproducts by a series of secondary electron transfer reactions. The initial photochemical step and the early secondary reactions all take place in a specialized pigment-protein complex known as a reaction center. These integral membrane complexes have been isolated from a variety of photosynthetic systems, both oxygen-evolving and anoxygenic.

All reaction centers (RCs) that have been isolated to date can be grouped into one of two broad classes, based on the nature of the early electron acceptors. The purple photosynthetic bacteria and the green gliding bacterium, Chloroflexus aurantiacus, contain RCs that are similar in many ways to the Photosystem II (PS II) reaction center of oxygenic organisms. These RCs all contain (bacterio) pheophytin as an intermediate electron acceptor and two quinones in association with a metal ion (in most cases iron). The spectroscopic, kinetic, magnetic resonance properties and protein composition of all these 'pheophytin-quinone type' RCs bear striking similarities to each other, and there is little doubt that they share a common ancestor (Barber 1988, Michel and Deisenhofer 1988, Rutherford 1988, Beanland 1990, Mathis 1990, Nitschke and Rutherford 1991, Blankenship 1992). Other evidence suggests that a similar functional relationship exists among the RCs of the green sulfur bacteria, Photosystem I (PS I) and the heliobacterium group. All these RCs contain membrane-bound iron–sulfur centers as early acceptors, and are therefore called 'Fe–S type' RCs. (Knaff and Malkin 1976, Prince et al. 1985, Nitschke et al. 1987, Nitschke et al. 1990a,b, Amesz 1991, Golbeck and Bryant 1991).

The heliobacteria are a recently discovered family of strictly anaerobic photosynthetic prokaryotes (Gest and Favinger 1983, Madigan 1992). Ribosomal RNA sequencing (Woese et al. 1985) and chemical analysis (Beer-Romero et al. 1988, Beck et al. 1990) indicate that the heliobacteria belong to the gram positive line of eubacteria, in contrast to all other known photosynthetic bacteria. Heliobacteria also contain the unique pigment bacteriochlorophyll g (BChl g), as the main pigment (Brockmann and Lipinski 1983). The pigment is a molecular hybrid of chlorophyll a found in higher plants and BChl b found in some purple photosynthetic bacteria (Michalski et al. 1987). Bacteriochlorophyll g contains the vinyl group on ring I characteristic of the chlorophylls from oxygenic organisms and the ethylidine group on ring II that has previously been found only in BChl b. Kobayashi et al. (1991a,b) have reported that the heliobacteria contain small quantities of BChl g', the 13'-epimer to BChl g. The same group has found chlorophyll a' in PS I reaction center preparations, but not in RCs from PS II, purple bacteria or green sulfur bacteria (Watanabe and Kobayashi 1990). Recently, 8'-hydroxychlorophyll a has been suggested as the primary electron acceptor in the heliobacteria (van de Meent et al. 1991).

In the green sulfur bacteria, efforts to obtain a minimal complex have been hampered by the large size of the chlorosome antenna system (Hurt and Hauska 1984, Kjaer et al. 1991, Feiler, Nitschke and Michel, in press). The antenna pigments are not easily removed without damaging the reaction center. Heliobacteria have only a small antenna of 30–60 antenna pigments per primary donor, P800, which are associated directly with the reaction center core (Nuijs et al. 1985). Apparently, they do not contain any other antenna pigment-proteins (Trost and Blankenship 1989, Amesz 1991) and excitations do not appear to be shared between RCs (Deinum et al. 1991). A moderate number of antenna pigments as part of the core reaction center structure is again similar to PS I and the green sulfur bacteria (Golbeck and Bryant 1991).

We recently reported the isolation of photoreactive minimal RCs from Heliobacillus (Hb.) mobilis (Trost and Blankenship 1989). A similar preparation of RCs from Hb. mobilis and H. chlorum has also been reported (van de Meent et al. 1990). In our hands, the complexes obtained using either protocol consist of a single major band on SDS–PAGE with a molecular retention of 47 000. Almost the complete antenna complement found in the membranes is also found on the purified reaction center in a single pigment-