Isolation of a Photoactive Photosynthetic Reaction Center–Core Antenna Complex from *Heliobacillus mobilis*†

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ABSTRACT: A photoactive reaction center–core antenna complex was isolated from the photosynthetic bacterium *Heliobacillus mobilis* by extraction of membranes with Deriphat 160c followed by differential centrifugation and sucrose density gradient ultracentrifugation. The purified complex contained a M_r 47 000 polypeptide(s) that bound both the primary donor (P800) and approximately 24 antenna bacteriochlorophylls *g*. Time-resolved fluorescence emission spectroscopy indicated that the antenna bacteriochlorophylls *g* are active in energy transfer to P800, exhibiting a decay time of 25 ps. The complex contained 1.4 menaquinones, 9 Fe, and 3 labile S^{2-} per P800. The complex was photoactive with an exponential decay time of 14 ms for $P800^+$ yet showed no EPR-detectable Fe–S center signal in the $g \leq 2.0$ region, either by chemical reduction to −600 mV or by illumination of reduced samples. The complex is similar to photosystem I of oxygen-evolving photosynthetic systems in that both the primary donor and a core antenna are bound to the same pigment–protein complex.

Study of the photosynthetic reaction centers from various organisms has greatly increased the understanding of the physical processes involved in the primary events of photosynthesis as well as having given insight into the evolutionary path that has led to modern photosynthetic organisms. The reaction centers found in photosynthetic bacteria, plants, and algae can be grouped into two main classes: those with a pair of quinones that serve as a two-electron gate (quinone-type reaction center) and those with very low-potential iron–sulfur proteins (Fe–S-type reaction center) as early electron acceptors. The single photosystems found in the anoxygenic photosynthetic bacteria are often useful as model systems for the analogous reaction centers found in the linked photosystems of oxygen-evolving photosynthetic organisms.

The reaction centers found in the purple photosynthetic bacteria and the green gliding bacterium *Chloroflexus aurantiacus* are of the quinone type and exhibit striking functional and moderate sequence similarity to the photosystem II reaction center from oxygenic organisms (Michel & Deisenhofer, 1988; Ovchinnikov et al., 1988; Rutherford, 1988). High-resolution X-ray structures are available for the reaction centers isolated from the purple bacteria *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* [for recent reviews, see Budil et al. (1987), Rees et al. (1989), and Deisenhofer and Michel (1989)]. Both this structural and a wealth of functional information on these simple reaction centers have contributed immeasurably to the understanding of the photosystem II reaction center.

In contrast, the Fe–S type of reaction center remains less well understood, both in photosystem I and in those anoxygenic bacteria that contain low-potential Fe–S centers as early electron acceptors. The bacterial groups that have this type of acceptor complex are the green sulfur bacteria (family Chlorobiaceae) and the Heliobacteria. The large light-gathering antenna system found in the green sulfur bacteria has made it difficult to isolate reaction centers from these organisms, and as a consequence, relatively little is known about their protein components (Hurt & Hauska, 1984).

The Heliobacteria are a recently discovered family of photosynthetic bacteria (Ormerod et al., 1990). The first representative, *Heliobacterium* (*H.*)[1] *chlorum*, was isolated in 1983 (Gest & Favinger, 1983). These are the only known organisms that contain bacteriochlorophyll *g* (BChl *g*) (Brockmann & Lipinski, 1983; Michalski et al., 1987). On the basis of 16S ribosomal RNA sequences, the Heliobacteria are the only photosynthetic bacteria that are grouped with the Gram-positive line of bacteria (Woese et al., 1985; Woese, 1987).

Isolated membranes from *H. chlorum* and the closely related organism *Heliobacillus* (*Hb.*) *mobilis* (Beer-Romero & Gest, 1987) have pigment compositions and photochemical properties distinct from those found in all other families of photosynthetic bacteria. Time-resolved optical difference spectra show a maximum of photobleaching at 800 nm (P800) with a line shape very similar to that of the photobleaching difference spectrum seen in photosystem I (Fuller et al., 1985; Nuijs et al., 1985). *H. chlorum* has a relatively small antenna size, 30–60 BChl *g* per P800 (Nuijs et al., 1985; Vos et al., 1989). EPR measurements on membranes under reducing conditions show a distinct Fe–S center signal, and redox titrations of photochemistry indicate an acceptor with a midpoint redox potential of −510 mV (Prince et al., 1985). Illumination under reducing conditions gives rise to a Fe–S center signal as well as a $g = 2.0038$ signal attributed to a quinone (Brok et al., 1986). Optical difference spectra characteristic of reduction of an Fe–S center have also been observed (Smit et al., 1987).

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[1] Abbreviations: Chl, chlorophyll; BChl, bacteriochlorophyll, *H.*, *Heliobacterium*; *Hb.*, *Heliobacillus*; P700, primary donor of photosystem I; P800, primary donor of heliobacterial reaction center; UV/vis, ultraviolet/visible; Tris, tris(hydroxymethyl)aminomethane; 20T5A8, 20 mM Tris–5 mM sodium ascorbate, pH 8.0, buffer; K_r, $-\log R_m$/% total acrylamide; M_r, gel electrophoretic relative mobility.

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The small antenna size and clear involvement of Fe–S centers as early electron acceptors suggest that the Heliobacteria might be a good source of Fe–S-type reaction centers. We report here the isolation of a reaction center complex from *Hb. mobilis* that appears to contain either a single polypeptide or very similar polypeptides yet still exhibits native light-induced charge separation.

A preliminary report of this work was presented at the VIII International Congress on Photosynthesis (Trost & Blankenship, 1990), where a report of a similar preparation was presented by van de Meent et al. (1990).

Materials and Methods

Protein Purification. Hb. mobilis was grown anaerobically in a 12-L fermenter for 12–16 h at 37 °C under 1200 W of incandescent illumination from a 30-mL inoculum in Medium 112 (Gest & Favinger, 1983) plus 20 mM sodium pyruvate. Cells were harvested by centrifugation at 10000*g* and washed once with 20 mM Tris–5 mM sodium ascorbate, pH 8.0, buffer (20T5A8). All buffers used were thoroughly degassed and stored under nitrogen. Membranes were isolated by sonication and centrifugation at 10000*g* for 10 min, followed by ultracentrifugation of the supernatant liquid at 200000*g* for 2 h. The pelleted membranes were resuspended in 20T5A8 buffer to an absorbance of 15 at 788 nm. The detergent Deriphat 160c (disodium 3-[*N*-(carboxyethyl)-*N*-laurylamino]-propionate) (Henkel Corp.) was added to a final concentration of 1%, and the solution was stirred under nitrogen for 1 h in the dark at room temperature. The solubilized membranes were ultracentrifuged at 200000*g* for 2 h. The supernatant liquid was carefully decanted and dialyzed against 10 volumes of 20T5A8 to yield a cytochrome-containing fraction. The pellet, containing most of the reaction centers, was resuspended to an absorpbance of approximately 100 at 790 nm in 20T5A8 + 0.1% Deriphat, applied to a 10–60% (w/v) sucrose gradient in 20T5A8 + 0.1% Deriphat, and centrifuged at 200000*g* for 16 h. The reaction center complex formed a dark green band at 30–40% sucrose. Degraded pigment remained on the top of the gradient, and a small amount of material formed a pellet. A light orange carotenoid-containing band formed at 45% sucrose. For the quinone and carotenoid analyses described below, a 30–50% sucrose gradient was used in order to achieve a complete resolution of the reaction center band and the light orange band.

Subunit Analysis. SDS–PAGE was done by the method of Laemmli (1970), except samples were solubilized in 2% SDS, 100 mM dithiothreitol, and 3 M urea with 0.002% pyronin Y as the tracking dye and heated at 65 °C for 30 min. Acrylamide gradient gels (10–25%) were poured in a Hoefer Mighty Small multiple-gel casting stand with sucrose to stabilize the gradient. Molecular weights were determined from single-concentration gels. Heme staining was done by the method of Thomas et al. (1976) on samples without dithiothreitol. For Ferguson plot analysis, transverse acrylamide gradient gels were cast three at a time in a homemade stand (Retamal & Babul, 1988). All gradient gels were polymerized with 0.035% ammonium persulfate and 0.03% TEMED for at least 2 h before use. SDS–PAGE-purified samples for HPLC analysis were eluted from SDS–PAGE gels by diffusion into 20 mM NH_4HCO_3, pH 8. HPLC of the purified reaction center complex was performed as in Blankenship et al. (1988).

Chemical Analysis. Protein was quantified by a modified Lowry assay (Peterson, 1977) on triplicate samples at three different sample amounts.

Total iron was analyzed by atomic absorption by the method of standard additions with deuterium arc background correction. All samples for iron analysis were passed over Chelex resin (Bio-Rad) to remove any unbound iron.

Labile sulfur was analyzed according to the method of Golbeck and San Pietro (1976) except that $CHCl_3$ was used for extraction of pigment acidification products. Unknowns and standard Na_2S were analyzed at five concentrations from 5 to 50 μM S^{2-} to control for the small amount of methylene blue extracted by the $CHCl_3$.

Quinones were extracted from wet membranes and reaction centers by addition of 10 volumes of 1:1 acetone–methanol followed by stirring in the dark for 10 min. The acetone–methanol extract was extracted four times with 10 volumes of light petroleum ether (30–60 °C bp) with 5 min of stirring for each extraction. The petroleum ether extracts were decanted, combined, and dried on a rotary evaporator at room temperature in the dark. The dried extracts were stored overnight under vacuum at −20 °C in a desiccator over $CaSO_4$. After the samples were allowed to come to room temperature, they were removed from the desiccator, dissolved in 100 μL of 1:1 chloroform–methanol, and applied as a streak along the bottom of a 10 × 10 cm 250 μm thick silica TLC plate with fluorescent indicator. The sample flask was washed with two further 100-μL volumes of the chloroform–methanol mixture, which were also applied to the TLC plate. The TLC plate was developed with 90:10 light petroleum ether–diethyl ether. Silica showing any color or quenching of the fluorescence was scraped from the TLC plate and extracted five times with diethyl ether. The diethyl ether extracts were dried under a stream of nitrogen and redissolved in absolute ethanol or hexane (carotenoid band only). Quinone was quantified by reduced-minus-oxidized difference spectra (Dadák & Krivánková 1980). Standard quinone was recovered quantitatively with this procedure. The isoprenoid tail length of the extracted quinones was determined by reverse-phase HPLC on a C18 column using 90:10 methanol–2-propanol as the solvent (Hale et al., 1983). The only carotenoid found appeared to be neurosporene and was determined by the absorbance at 440 nm with ϵ_{440} = 155 mM^{-1} cm^{-1} (Davis et al., 1966).

Spectroscopy and P800 Quantitation. Room temperature and 5 K optical spectra were recorded on a Varian Cary 219 spectrophotometer. An Oxford CF-1204 flowing-He cryostat was used for the low-temperature spectra on samples dissolved in 67% glycerol–33% 20T5A8 buffer. Steady-state photobleaching was assayed at room temperature on a modified Varian Cary 219 spectrophotometer. Identical samples with an absorbance of ≈0.5 at 790 nm in 20T5A8 were placed in both the sample and reference cell holders in capped, nitrogen-flushed cuvettes. Actinic illumination from a 100-W quartz–halogen lamp was filtered through 3.5 cm of water and a Corning 4-96 filter. A Hoya R72 filter was used to protect the photomultiplier. Chemically oxidized-minus-reduced difference spectra were recorded on a Shamadzu UV-160 spectrophotometer and performed essentially as described by Markwell et al. (1980) with the modifications that samples in 20T5A8 were adjusted to +300 mV by addition of 600 mM potassium ferricyanide to a final concentration of 11 mM and reduced to 0 mV by addition of a 10% excess of 300 mM sodium ascorbate. Phenazine methosulfate and 2,3,5,6-tetramethyl-*p*-phenylenediamine were added to a final concentration of 20 μM to ensure rapid mediation for the ferricyanide and ascorbate. Laser flash spectroscopy was done essentially as described by Mancino et al. (1984).

Time-resolved fluorescence emission was determined by time-correlated single-photon counting as described by

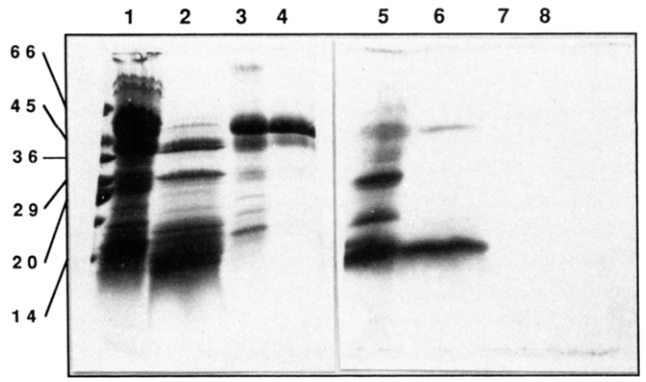

FIGURE 1: SDS–PAGE of membranes and reaction centers of *Hb. mobilis* on 10–25% linear acrylamide gradient gels. Lanes 1–4 are stained with Coomassie blue. Lanes 5–8 are stained for heme. Lanes 1 and 5 are isolated membranes, lanes 2 and 6 are the supernatant liquid, and lanes 3 and 7 are the pellet from the 1% Deriphat extraction. Lanes 4 and 8 are the sucrose gradient purified reaction centers. 20 μL of sample with 790-nm absorbance of 7.5 was loaded in each lane except for lanes 2 and 6 where 20 μL of the supernatant from the 1% Deriphat extraction was loaded after a 1:1 dilution with dissociation buffer. Gels were run at 10-mA constant current until the tracking dye just reached the bottom of the gel. The positions and masses, in kDa, of standard water-soluble proteins are shown on the left side. See Materials and Methods for other conditions.

Causgrove et al. (1989). Membranes and reaction center complexes were diluted in 20T5A8 buffer and degassed (reaction center buffer contained 0.1% Deriphat). The samples were loaded in an anaerobic chamber into a closed-loop flow system with a 0.15-cm path-length cuvette. Flowing was maintained throughout the experiments.

EPR. EPR spectra were taken on a Bruker ER200D spectrometer equipped with an Oxford ESR-900 flowing-helium cryostat attachment operating at 4 K. Pelleted membranes and reaction centers were suspended in 100 mM CAPS, pH 10, with 100 μM methylviologen. The redox potential was adjusted with 1 M dithionite and measured with an Orion 501A pH meter, a Radiometer P101 platinum electrode, and a Radiometer K401 calomel reference electrode. For light-induced EPR signals, samples poised at −600 mV were illuminated while being frozen in liquid nitrogen with a 250-W tungsten–halogen lamp focused on the sample in an optical Dewar. EPR signals induced by light at 4 K were assayed on identical samples that were frozen in the dark and then illuminated via a liquid-filled light pipe while in the EPR cavity.

Results

SDS–PAGE analysis of membranes and the reaction center complex isolated from *Hb. mobilis* is shown in Figure 1. The reaction center band appeared to be the major protein in membranes (compare lanes 1 and 4). The 200000*g* pellet from the 1% Deriphat extraction was greatly enriched in the reaction center but still contained several smaller proteins (lane 3). Purification on sucrose gradients lead to the removal of most of the smaller bands, leaving a single major band with a molecular retention (M_r) of 47000 and a minor doublet at M_r 40000 (lane 4). When samples were heated above 75 °C prior to SDS–PAGE, the major reaction center band was found at about M_r 80000, and prolonged heating at 100 °C resulted in protein bands with even higher apparent molecular masses. This suggests aggregation of the reaction center polypeptide under these conditions. When stained for heme, both membranes and the 1% Deriphat extracted supernatant liquid showed similar bands but at different relative abundances (lanes 5 and 6). Neither the 1% Deriphat extracted pellet nor the purified reaction center complex showed any heme-staining proteins (lanes 7 and 8), suggesting the absence of *c*-type cytochromes in the purified reaction center complex. In addition, in these samples neither the dithionite-reduced minus ascorbate-reduced nor the ascorbate-reduced minus ferricyanide-oxidized optical difference spectra revealed any absorption peaks attributable to cytochrome (data not shown).

Analysis of transverse acrylamide gradient gels (Ferguson, 1964; Rodbard, 1976) suggested that the major reaction center polypeptide has a larger mass than that indicated by standard SDS–PAGE (data not shown). A calibration curve generated by plotting the molecular mass of standard proteins versus the slopes of the Ferguson plots (K_r) could be fit to a second-order polynomial with a correlation coefficient of 0.99 and resulted in a mass estimate of 74 kDa for the major reaction center polypeptide (see Discussion). From SDS–PAGE analysis it appears that the reaction center is composed of a single polypeptide or extremely similar polypeptides. Except for the apparent aggregation bands, the M_r 47K polypeptide did not show any further separation on SDS–PAGE with gels from 4 to 25% acrylamide or sample treatments ranging from no heating in 20T5A8 + 0.1% Deriphat to heating at 65 °C for 1 h in 4% SDS–6 M urea, to boiling in 10% SDS (data not shown).

Reverse-phase HPLC of the reaction center preparation in the presence of high concentrations of formic acid (Blankenship et al., 1988) showed a single protein peak (data not shown). While several UV-absorbing peaks eluted when untreated reaction centers were run, only the peak that corresponded to the major reaction center polypeptide showed any protein by both Lowry and SDS–PAGE analysis. The color of the other eluted peaks suggested that they were either carotenoid or bacteriochlorophyll products. When the M_r 47K band eluted from SDS–PAGE was chromatographed, it eluted as a single, although broader, peak with the same retention as the protein peak from untreated reaction centers.

The room temperature and 4 K UV/vis absorption spectra for membranes and the reaction center complex are shown in Figure 2. The spectra were very similar at both temperatures. Reaction centers showed a smaller peak at 670 nm which is likely due to the solubilization and removal of some degraded BChl *g*. There also appeared to be a slight red shift of the long-wavelength peak in the reaction center from 788 to 790 nm. The major absorption peaks in the reaction center were at 790, 715, 671, 576, 411, and 369 nm. Removal of ascorbate by repeated washing over an ultrafilter revealed a protein peak at 272 nm (Trost & Blankenship, 1990). The 790-nm peak is the Q_y band of the BChl *g*, and the 715-nm band is likely a vibrational subband of the Q_y band. The 671-nm peak is probably a combination of a chlorophyll-like degradation product of BChl *g* and a proposed bacteriochlorophyll *c* like intermediate electron acceptor (Nuijs et al., 1985). The band at 576 nm is the Q_x band of BChl *g*. The bands at 411 and 369 nm are the Soret bands of the 670-nm pigment and BChl *g*, respectively. At 4 K, the long-wavelength absorption peak of the reaction center and membranes showed almost identical splitting. The fourth derivative of the 4 K absorption spectrum indicated peaks at 809, 794, and 779 nm in the long-wavelength region (inset of Figure 2B).

The quality of the reaction center preparation can be judged from the ratio of the absorbance at 790 nm (BChl *g*) to that at 670 nm (chlorophyll *a* like degradation product). Our best preparations of reaction centers achieved ratios as high as 8.0 but routinely were about 7. The removal of ascorbate from the sample led to a large decrease in this ratio in a matter of hours and was even more rapid if the sample was exposed to

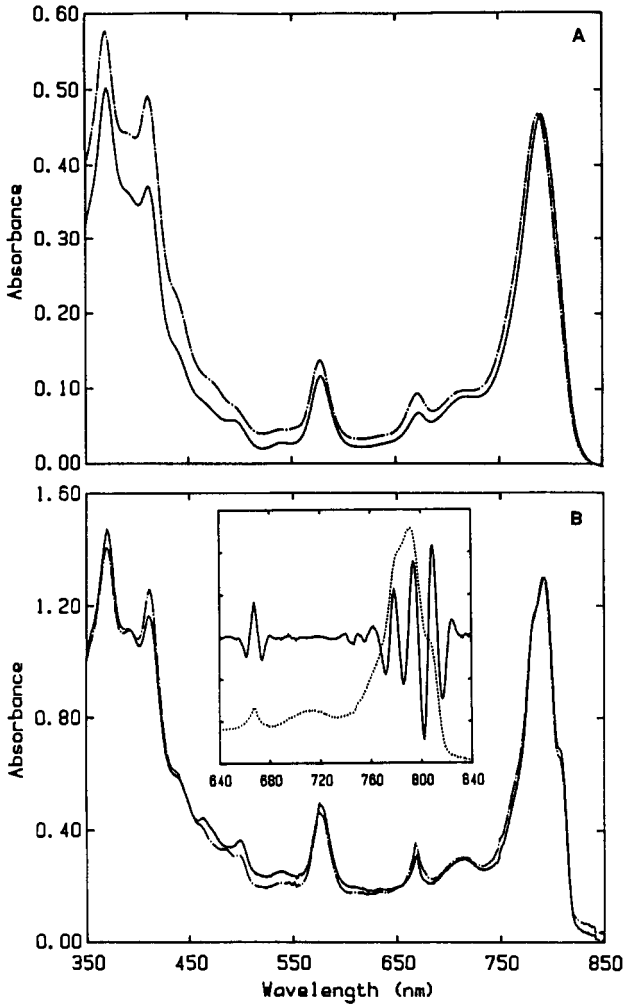

FIGURE 2: Absorption spectra of membranes and purified reaction centers. Panel A shows the room temperature spectra of membranes (dot–dash line) and reaction centers (solid line). To reduce scatter, Deriphat was added to a final concentration of 0.1% to the membrane sample immediately before recording the spectrum. Panel B shows the 4 K spectra of membranes (dot–dash line) and the 1% Deriphat extracted pellet (solid line). The inset shows the fourth derivative (solid line) of the long-wavelength region of the 4 K absorption spectrum of the pellet (dotted line).

light. The reaction center and membranes had comparable stability and showed no appreciable degradation over several weeks if stored at 4 °C in the presence of ascorbate in darkness under nitrogen. With our procedure, about 10% of the reaction centers found in the membranes were destroyed by the detergent extraction as assayed by steady-state photobleaching. Of the remaining 90% of the reaction centers, 75% were found in the 200000g pellet of the extraction mixture, and 15% remained in the supernatant liquid. In contrast, 80% of the total protein from the membranes remained in the supernatant liquid, including all of the cytochromes as assayed by heme staining of SDS–PAGE (Figure 1).

Laser flash induced photobleaching was maximum at 800 nm and decayed essentially monoexponentially with a time constant τ ($1/e$) of 15 and 14 ms in membranes and reaction centers, respectively (Figure 3 inset). The steady-state light-induced difference spectra of reaction centers and membranes were identical and essentially equivalent in the long-wavelength region to difference spectra induced by laser flashes (Fuller et al., 1985; Nuijs et al., 1985). The steady-state photobleaching spectrum also showed the major bleaching centered at 800 nm (P800) and an absorbance increase centered at 776 nm (Figure 3). At 5 mM sodium ascorbate, the

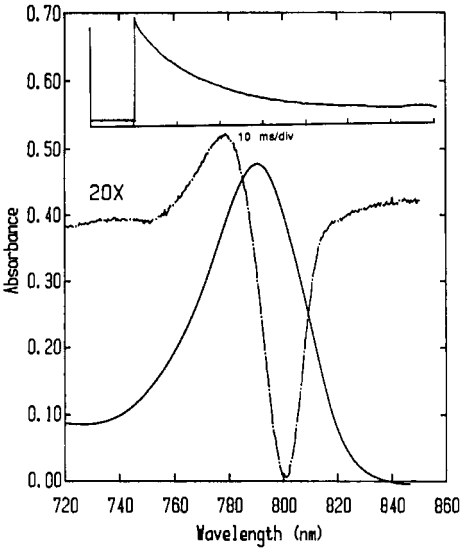

FIGURE 3: Steady-state light-induced difference spectrum (dot–dash line) and absorption spectrum (solid line) of sucrose gradient purified reaction centers. The difference spectrum is the average of three determinations on a single sample. The inset shows the decay of the laser flash induced photobleaching at 800 nm.

decay of the 800-nm bleaching in the steady-state assay exhibited $\tau \approx 20$ s for reaction centers and $\tau \approx 2$ s for membranes. The decay rate of the steady-state photobleaching signal in isolated reaction centers appeared to be proportional to the concentration of added ascorbate. The same magnitude of 800-nm photobleaching was observed from 0.5 to 20 mM sodium ascorbate. In contrast, the magnitude and recovery rate constant of laser flash induced photobleaching were independent of ascorbate concentration from 0.5 to 20 mM final concentration. Chemically induced oxidation of the reaction center gives the same magnitude of bleaching at 800 nm as laser flash induced or steady-state photobleaching but shows a more pronounced increase at 776 nm (data not shown).

If it is assumed that the extinction coefficients of the primary donor (P800) and the antenna BChl g are equal, then there are ≈24 BChl g per P800 in the reaction center complex and ≈34 BChl g per P800 in membranes. Another method to estimate the number of antenna pigments per P800 is to use the ratios of the areas of the gound-state absorption and photobleaching spectra (Vos et al., 1989). This suggests 62 and 94 BChl g per P800 for the reaction center and membranes, respectively. Precise numbers for these quantities await the determination of extinction coefficients for both P800 and BChl g.

Time-resolved fluorescence emission from reaction centers and membranes, excited at 750 nm, showed a dominant exponential decay at 810 nm of about 25 ps [97 and 94% of the amplitude, respectively (Figure 4)]. This fast decay made up 99% of the amplitude in both membranes and reaction centers when excited at 790 or 800 nm.

Table I gives the results of protein, iron, labile sulfur, quinone, and carotenoid analyses. The precision of the iron and labile sulfide analysis appeared to be limited by the quantitation of P800. Assuming a differential extinction coefficient of P800 of $\Delta\epsilon_{800} = 100$ mM^{-1} cm^{-1}, there were 56 ± 6 Fe and 17 ± 1 labile sulfides per P800 in membranes. In the reaction center complex there were 9.4 ± 0.7 Fe and 2.7 ± 0.1 labile sulfides per P800.

In agreement with Hiraishi (1989), both membranes and the reaction center complex contained only menaquinone as determined by relative mobility on TLC and reduced-minus-oxidized UV difference spectra. With the same as-

Table I: Chemical Analysis of Membranes and Reaction Centers

sample	BChl g/P800[a]	BChl g/P800[b]	Fe/P800[c]	S^{2-}/P800[c]	quinone/P800[c]	neurosporene/ P800[c]	g of protein/mmol of P800[c]
membranes	34–39	98–94	56 ± 6	17 ± 1	4.8 ± 0.4	≈2.3	850 ± 60
reaction center	24–27	67–62	9.4 ± 0.7	2.7 ± 0.1	1.4 ± 0.3	≈0.6	147 ± 8

[a] Estimated by comparison of peak heights. The range given is the value determined by steady-state photobleaching to the value from chemically induced bleaching. [b] Estimated by comparison of peak areas. The range is as in footnote *a*. [c] Assuming $\Delta\epsilon_{800} = 100\ mM^{-1}\ cm^{-1}$. Values are the mean ± the standard deviation of the propagated errors of P800 determination from the steady-state assay and chemical quantitation.

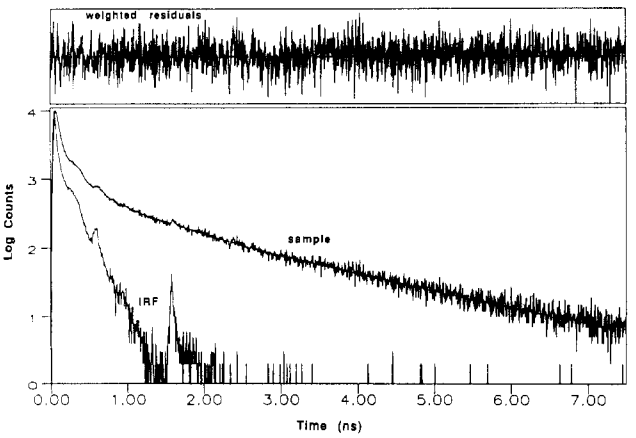

FIGURE 4: Fluorescence emission decay from purified reaction centers. Sample absorbance was 0.6 cm^{-1} at 790 nm in 20T5A8 + 0.1% Deriphat. Excitation was at 750 nm and emission at 810 nm. The top panel shows the weighted residuals of the calculated curve from the data. The lower trace in the bottom panel is the instrument response function, and the upper trace is the actual data with the fitted curve. The fitted curve has a χ^2 value of 1.017 and gives a 25-ps exponential decay component with 97% of the amplitude. Three further components of 170, 700, and 1900 ps account about equally for the remaining 3% of the amplitude.

sumptions as above, this resulted in 4.8 ± 0.4 menaquinones per P800 in membranes and 1.4 ± 0.3 menaquinones per P800 in the reaction center complex. Lower amounts of menaquinone were found if the sample was lyophilized before quinone extraction. Integrated peak areas from reverse-phase HPLC of the extracted quinone showed MK_8, MK_9, and MK_{10} in ratios of 1:8:1 in both membranes and reaction centers.

A pigment that displays the same mobility as neurosporene appeared to be the only carotenoid pigment in both reaction centers and membranes (Gest & Favinger, 1983; van Dorssen et al., 1985). A single determination of the quantity of this pigment indicated 2.3 and 0.6 mol of neurosporene/mol of P800 in membranes and reaction center complexes, respectively, assuming the same differential extinction coefficient for P800 as above. The three absorption peaks of the carotenoid pigment in hexane all showed a blue shift of 5 nm as compared to the published maxima of synthetic neurosporene (Davis et al., 1966). In addition, the absorption peak at 435 nm was 10% greater than the peak at 465 nm. In synthetic neurosporene these peaks are of equal height. These discrepancies may be due to a mixture of isomers in the isolated natural neurosporene as compared to the pure all-trans synthetic neurosporene.

EPR spectra of membranes and the reaction center complex are shown in Figure 5. At a measured potential of −590 mV vs NHE, membranes showed a strong Fe–S center signal similar to that observed with membranes from *H. chlorum* by Prince et al. (1985) and Brok et al. (1986). A broad resonance was observed at $g = 1.900$ along with a radical signal centered at approximately $g = 2.003$. Under some spectrometer conditions a resonance was also resolved at $g = 1.942$. A slight increase in the Fe–S center signal was observed in membranes

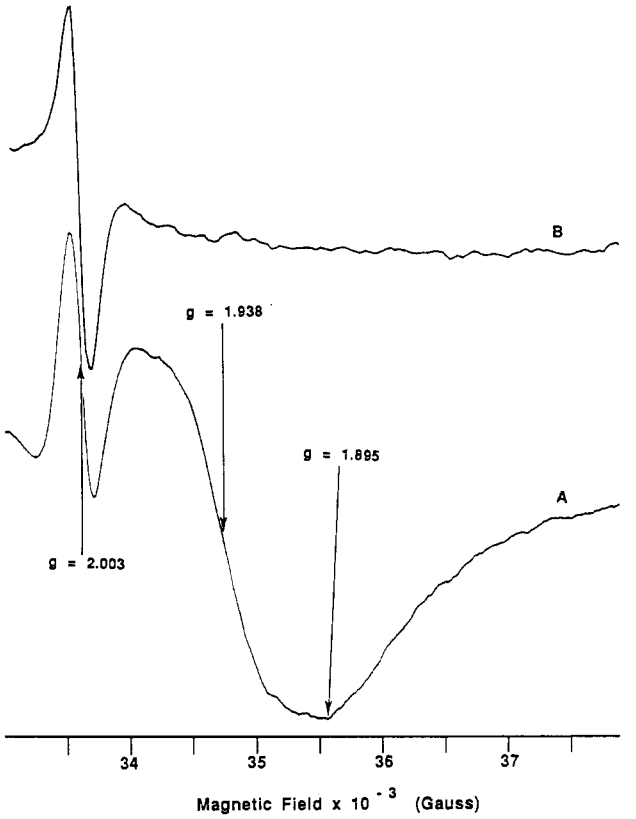

FIGURE 5: EPR spectra of (A) membranes poised at a redox potential of −590 mV and (B) reaction centers poised at a redox potential of −590 mV. Sample absorbance was 110 at 788 nm for the membranes and 90 at 790 nm for the reaction centers. The reaction center trace was plotted on a 1.3 times more sensitive scale than the membranes. Spectrometer conditions: microwave power, 20 mW; modulation amplitude, 10 G; temperature, 4 K; microwave frequency, 9.4217 GHz.

poised at −600 mV both with illumination while freezing and with illumination at 4 K of dark frozen membranes. In contrast, the reaction center complex at a potential of −590 mV vs NHE showed no observable Fe–S center signal at g values of ≤2.0. We did not observe any light-induced Fe–S center signal in purified reaction centers in the $g \leq 2.0$ region either, although there was an ≈60% increase in the radical signal (data not shown). The radical signal was reversible after the illumination was turned off at 4 K, and the remaining signal was still present even after the sample was warmed and refrozen in the dark. Membranes showed a similar reversible light-induced radical signal.

Discussion

The remarkably similar absorption spectra of reaction centers and isolated membranes at both 4 and 300 K (Figure 2) suggest that the bulk of the antenna BChl g molecules are still present in the isolated reaction center protein complex. Unlike the purple bacterial photosynthetic systems and photosystem II (Thornber, 1986; Nanba & Satoh, 1987), almost all of the antenna pigments appear to be organized on the same polypeptide(s) as the primary donor. This is a pattern similar

to that observed in photosystem I, where a core antenna of ≈100 Chl is associated with the same peptides that bind the primary electron donor, P700, and the early electron acceptors (Takahashi et al., 1982; Golbeck 1987).

The 25-ps decay of the 810-nm fluorescence indicates that the BChl *g* associated with the reaction center is functional in efficient excitation transfer to the reaction center and not just nonspecifically bound to the complex (Figure 4). The rapid fluorescence decay is almost certainly due to trapping of the excitations by photochemistry. This indicates that the antenna found in the reaction center protein complex is still in a native state. Low-temperature steady-state fluorescence excitation and emission spectra of a similar reaction center–antenna preparation are in agreement with the above results (van de Meent et al., 1990).

The rate of decay of the laser flash induced photobleaching of the reaction center was the same as that found in isolated membranes (Figure 3 inset). The absence of cytochrome in the reaction center and lack of dependence of the decay rate on ascorbate concentration suggested that the decay was due to charge recombination from some early electron acceptor in the reaction center. Cytochrome oxidation capability is apparently easily lost in the Heliobacteria (Vos et al., 1989), so in both cases the 14-ms time probably reflects recombination from an early electron acceptor (see below). The small amount of irreversible laser flash induced photobleaching is due to an as yet uncharacterized trap that is stable on greater than the tens of seconds time scale. This is supported by the slow, concentration-dependent donation by ascorbate to $P800^+$ in the steady-state photobleaching assay.

The strong Fe–S signal observed by EPR in *Hb. mobilis* membranes at a potential of −590 mV is consistent with low-potential Fe–S centers as early electron acceptors in photosynthetic electron transport (Figure 5), although it has previously been suggested that the Fe–S center EPR signal does not arise from the component that recombines with $P800^+$ on the millisecond time scale (Smit et al., 1987; Vos et al., 1989). The resonance is similar to that seen in membranes of *H. chlorum*. The width of the signal and the resolution of a distinct resonance at $g = 1.942$ under some conditions are consistent with the suggestion that the signal may be a convolution of signals from two or more centers (Prince et al., 1985; Brok et al., 1986).

The reaction center complex shows no chemically or light-induced Fe–S center EPR signals at a measured potential of −590 mV. This result is consistent with the loss of labile sulfide during the reaction center purification (Table I). This may be analogous to the situation in photosystem I where a small polypeptide binds Fe–S centers F_a/F_b (Golbeck, 1987). The detergent extraction of the membranes may solubilize and remove analogous polypeptide(s) in the isolated *Hb. mobilis* reaction center complex and lead to the loss of the Fe–S EPR signal seen in membranes. It is also possible that the detergent treatment merely disrupts the native structure of the Fe–S center(s) organized on the large reaction center polypeptide(s).

The nature of the reduced acceptor that recombines with $P800^+$ on the millisecond time scale has yet to be determined. We have not detected an acceptor analogous to Fe–S center F_x from photosystem I; however, F_x is difficult to detect, and our EPR assay conditions may have been inappropriate. The reaction center particle contains sufficient Fe and labile sulfide for such a center to exist (considering the uncertainty due to the extinction coefficient of P800), so this remains a possibility.

To explain the kinetic correspondence of the recombination in membranes to that in reaction centers, it is necessary to postulate that the electron transfer from the putative early acceptor to the Fe–S centers observed in isolated membranes occurs with low efficiency. Therefore, the 14-ms decay observed in membranes would be dominated by the decay from the earlier acceptor. A similar conclusion was reached by Smit et al. (1987) from experiments on membranes from *H. chlorum*.

An assignment of the protein subunit stoichiometry of the heliobacterial reaction center is premature. Protein analysis, along with the P800 assays, resulted in 147 000 g of protein/mol of P800 (Table I), which would suggest a dimeric complex. However, the inequality of the Lowry assay from protein to protein and the assumed value of $\Delta\epsilon_{800}$ cause this value to be somewhat uncertain. In addition to the uncertainty of the Lowry assay, there is also some uncertainty in the molecular mass of the major polypeptide. Standard SDS–PAGE and Ferguson plot analysis indicated different masses, 47 and 74 kDa, respectively. A similar result was found for photosystem I where the major polypeptides have an apparent molecular mass of 65 kDa by SDS–PAGE, but sequence analysis indicates a mass of 83 kDa (Fish & Bogorad, 1986). The Ferguson plot of the reaction center polypeptide suggests that it binds an anomalously high amount of SDS, similar to that found in photosystem I (Takahashi et al., 1982). While the molecular mass of a particle determined by Ferguson plot analysis should be independent of the mass to charge ratio, it reflects the total particle size, including bound SDS. Identically sized proteins that bind different proportional amounts of SDS will appear to have different molecular masses; the more SDS bound the higher the apparent molecular mass. The calibration curve used to obtain the M_r 74 000 estimate was constructed with water-soluble proteins that bind proportionally less SDS than the M_r 47K polypeptide. Therefore, it seems that the true molecular mass of the reaction center polypeptide is between 47 and 74 kDa.

The reaction center complex from *Hb. mobilis* appears to be located on a single polypeptide or very similar polypeptides. The variety of sample treatments and gel concentrations used for SDS–PAGE consistently resulted in a single band for the major reaction center polypeptide, unlike the results for the PS I core polypeptides. Additionally, a single protein was eluted from HPLC of the reaction center complex in the presence of formic acid.

The reaction center from *Hb. mobilis* appears to be structurally similar to photosystem I in that both the primary electron donor and a core antenna are bound to a single polypeptide complex. Some major unanswered questions are whether the major polypeptide of the isolated reaction center complex has sequence similarity to the photosystem I large polypeptides and what is the nature of the initial charge-separated state. Experiments are currently underway to answer these questions.

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