ESR Signal of the Iron−Sulfur Center F_X and Its Function in the Homodimeric Reaction Center of *Heliobacterium modesticaldum*[†,‡]

Ryo Miyamoto,[§] Masayo Iwaki,[||] Hiroyuki Mino,[§] Jiro Harada,[⊥] Shigeru Itoh,[§] and Hirozo Oh-oka*[,@]

Division of Material Science, Graduate School of Science, Nagoya University, Nagoya 456-8602, Japan, Department of Biology, University College London, London, U.K., Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Shiga 525-8577, Japan, and Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka 560-0043, Japan

Received September 29, 2005; Revised Manuscript Received February 2, 2006

ABSTRACT: Electron transfer in the membranes and the type I reaction center (RC) core protein complex isolated from *Heliobacterium modesticaldum* was studied by optical and ESR spectroscopy. The RC is a homodimer of PshA proteins. In the isolated membranes, illumination at 14 K led to accumulation of a stable ESR signal of the reduced iron−sulfur center F_B^- in the presence of dithiothreitol, and an additional 20 min illumination at 230 K induced the spin-interacting F_A^-/F_B^- signal at 14 K. During illumination at 5 K in the presence of dithionite, we detected a new transient signal with the following values: $g_z = 2.040$, $g_y = 1.911$, and $g_x = 1.896$. The signal decayed rapidly with a 10 ms time constant after the flash excitation at 5 K and was attributed to the F_X^--type center, although the signal shape was more symmetrical than that of F_X^- in photosystem I. In the purified RC core protein, laser excitation induced the absorption change of a special pair, P800. The flash-induced $P800^+$ signal recovered with a fast 2−5 ms time constant below 150 K, suggesting charge recombination with F_X^-. Partial destruction of the RC core protein complex by a brief exposure to air increased the level of the $P800^+A_0^-$ state that gave a lifetime ($t_{1/2}$) of 100 ns at 77 K. The reactions of F_X and quinone were discussed on the basis of the three-dimensional structural model of RC that predicts the conserved F_X-binding site and the quinone-binding site, which is more hydrophilic than that in the photosystem I RC.

Heliobacteria are anoxygenic phototrophs in a group of low-GC Gram-positive bacteria (*1*). The bacteria grow only under strictly anaerobic conditions and contain bacteriochlorophyll (BChl)[1] *g* as the major photosynthetic pigment (*2*). BChl *g* is easily degraded to Chl *a*-like derivatives in air and changes its color from brown to bright green. Heliobacteria have the simplest photosynthetic system, i.e., one that consists of only a type I (Fe−S-type) RC complex in the cytoplasmic membranes (*2*, *3*). The RC, which is a homodimer of two identical PshA core proteins (*4*), as is the RC of green sulfur bacteria made of PscA (*5*), has a small antenna size of 35−40 BChl *g* molecules and is associated with no light-harvesting proteins or pigment aggregates. Homodimeric RCs are supposed to have structures that are essentially similar to those of the photosystem I (PS I) RC of cyanobacteria and plants.

An X-ray crystallography study of the PS I RC of a thermophilic cyanobacterium, *Synechococcus elongatus*, revealed the RC structure at 2.5 Å resolution (*6*). The folding motifs of the membrane-spanning α-helices and the arrangements of the cofactors in the core moiety of PS I were found to resemble those in the structure of type 2 (quinone-type) RCs of purple photosynthetic bacteria and PS II in cyanobacteria and plants (*7*, *8*). All these RCs are made of two almost homologous but partially different proteins and designated as heterodimeric RCs. The electron transfer cofactors are arranged in two almost symmetrical branches within the RCs. In type 2 RCs, electrons move only along the cofactors in one branch (*9*). In PS I, it is still controversial whether the electron moves along only one branch or both branches (*10*−*14*). On the other hand, both branches are supposed to be active in the homodimeric heliobacterial RC. An ENDOR study of a special pair $P800^+$ has shown almost symmetrical spin densities on the two constituent BChl *g* molecules (*15*). An FTIR study also suggested a symmetrical RC structure by demonstrating a single peak of the S−H signal for the two cysteine residues that are expected to be located at symmetrical positions (*16*).

The heliobacterial RC has been assumed to have the following photosynthetic electron transfer pathway in analogy

[†] This work was supported by Grants-in-Aid for Scientific Research (C) (15570135 and 17570131) (to H.O.) and (B) (15370067 and 1737055) (to S.I.) from the Japan Society for the Promotion of Science (JSPS) and by the 21st Century Center of Excellence program for "The Origin of the Universe and Matter" (to S.I., R.M., and H.M.).

[‡] The nucleotide sequences of the *pshA* genes reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with accession numbers AB233220 and AB233221 for *Heliobacterium modesticaldum* and *Heliobacterium gestii*, respectively.

* To whom correspondence should be addressed. E-mail: ohoka@bio.sci.osaka-u.ac.jp. Phone: +81-6-6850-5423. Fax: +81-6-6850-5425.

[§] Nagoya University.

[||] University College London.

[⊥] Ritsumeikan University.

[@] Osaka University.

[1] Abbreviations: BChl, bacteriochlorophyll; Chl, chlorophyll; DTT, dithiothreitol; MK, menaquinone; N-, amino-; PS, photosystem; P800, primary electron donor in heliobacteria; $P800^T$, P800 triplet; RC, reaction center.

10.1021/bi0519710 CCC: $33.50
Published on Web 04/28/2006

to the RCs of PS I and green sulfur bacteria (*17*, *18*).

$$\text{cytochrome } c \rightarrow \text{P800} \rightarrow A_0 \rightarrow (A_1?) \rightarrow F_X \rightarrow F_A/F_B$$

The primary donor P800 is a dimer of BChl *g* (or a heterodimer of Bchl *g* and *g*′) (*19*). The primary acceptor A_0 is a chlorophyll (Chl) *a* derivative, 8^1-hydroxy-Chl *a* (8^1-OH-Chl *a*) (*20*−*23*). Cytochrome *c*, which is an immediate electron donor to P800, is a protein (PetJ) that is anchored to the membranes probably through a fatty acid covalently bound to its N-terminus (*24*, *25*). The cytochrome mediates the electron transfer between the cytochrome *bc* complex and the RC (*26*), as in the case of cytochrome c_z in green sulfur bacteria, which binds to the RC with its N-terminal hydrophobic helices (*27*). The ESR signals of the reduced iron−sulfur centers, F_A and F_B, have been detected in the membranes of *Heliobacterium* (*Hbt.*) *chlorum* (*3*). However, the F_A/F_B-holding protein, which corresponds to the PsaC protein in PS I or the PscB protein in the green sulfur bacterial RC, has not yet been identified in heliobacteria. Therefore, the PshA protein is the only protein identified so far in the RC complex of heliobacteria.

The existence and function of menaquinone, which may play a role analogous to that of phylloquinone A_1 in the PS I RC, are still controversial in the heliobacterial RC. Laser spectroscopy in the membranes of *Heliobacillus* (*Hba.*) *mobilis* has shown the 600 ps electron transfer from A_0 to the unknown acceptor and almost no absorption change of semiquinone in the region of 400−470 nm (*28*). The reaction time was significantly longer than that from A_0 to phylloquinone in PS I (20−30 ps); thus, it was assumed to represent the time for electron transfer from A_0^- to F_X. Even after the almost total extraction of quinones with diethyl ether, the membranes of *Hbt. chlorum* exhibited similar A_0^- kinetics (*2*9). In the membrane preparation of *Hba. mobilis* with pre-reduced F_A/F_B centers, the charge recombination with a half-time ($t_{1/2}$) of ∼15 ns was enhanced after two successive laser flash excitations, indicating that there is only one stable electron acceptor in the RC, namely, F_X (*30*). The photoelectric response in the oriented membranes also suggested a direct electron transfer from A_0 to F_X by showing the two fast rising phases attributed to the formation of the $P800^+A_0^-$ state and the subsequent formation of the $P800^+F_X^-$ state (*30*). All these studies, thus, failed to detect the activity of functional quinone.

The case of green sulfur bacteria is similar. In a RC preparation of *Chlorobium tepidum* that contained 0.6−1.2 menaquinone-7 (MK-7) molecules per P840, Kusumoto et al. (*31*) detected enhanced charge recombination between $P840^+$ and A_0^- after four successive flashes and concluded that only the three F_A, F_B, and F_X centers, but not the quinone, function as stable electron acceptors. A similar RC preparation contained ∼0.9 MK-7 per P840 (*32*). However, stable charge separation reactions were detected in the RC preparations isolated from *Chl. tepidum* and *Prosthecochloris aestuarii* that were almost completely depleted of quinones (*33*, *34*).

The results of optical spectroscopy have been rather negative, as noted above, for the function of the quinone-type electron acceptor (A_1) in the homodimeric RCs. On the other hand, ENDOR and triple-resonance spectroscopy revealed a signal that can be ascribed to the photoaccumulated menasemiquinone radical in the membranes of *Chlorobium llimicola* and *Hbt. chlorum*, as revealed in PS I (*35*). Furthermore, in the RC complex isolated from *Chlorobium vibrioforme*, which contained 1.7 molecules of MK-7 per RC, a photoaccumulated quinone-type radical was also detected (*36*). These investigations support the presence of functional quinone.

This study focuses on the two missing links between the RCs of heliobacteria and PS I. One is the detection of F_X^- by ESR, which has never been successful to date. The F_X-type [4Fe-4S] cluster has been presumed to exist in heliobacteria from the sequence similarity between PshA and PsaA/PsaB in PS I and PscA in green sulfur bacteria (*18*). The other is the elucidation of the mechanism for the fast re-reduction of $P800^+$ at cryogenic temperatures. Although the 2−4 ms kinetic component optically observed at 77 K was ascribed to the reduction of $P800^+$ by A_1 (menaquinone) in analogy to PS I (*37*), $P800^+$ has been assumed to decay mainly in the charge recombination reaction with F_X^- or $(F_A/F_B)^-$ on the basis of ESR measurements (*38*).

We report a new transient ESR signal, which can be attributed to the iron−sulfur center F_X, detected at 4 K in the membranes of *Heliobacterium modesticaldum* and discuss the electron transfer mechanism in the homodimeric type I RC.

MATERIALS AND METHODS

Sample Preparations. Stock cultures of *Hbt. modesticaldum* were provided by M. T. Madigan (Southern Illinois University, Carbondale, IL). Cells were grown anaerobically in a PYE medium in a 1 L bottle under continuous illumination with tungsten lamps (*1*) at 47 °C for 18−20 h using 1% inocula to prevent the accumulation of lysed cells in the late-logarithmic growth phase. Longer cultivation times were found to produce a partially damaged P800 RC complex that exhibited a higher yield of the triplet state of P800 ($P800^T$) (∼20−30%) upon laser excitation (not shown).

All procedures for the preparations of membranes and the RC core complex as well as the spectroscopic measurements were carried out under anaerobic conditions, as previously described (*39*). All the media were fully degassed and flushed with N_2 gas before being used.

The cells were harvested by centrifugation at 12000*g* for 10 min, suspended in 7−8 mL of buffer A [50 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 2 mM DTT], and disrupted by being passed through a French pressure cell three times at 20 000 psi. After removal of the cell debris by centrifugation at 12000*g* for 10 min, the membranes were collected by ultracentrifugation at 180000*g* for 1 h and suspended again in buffer A.

The RC core complex was purified after solubilization of the membranes with sucrose monolaurate (SM-1200) basically as previously reported (*16*). Stepwise sucrose gradient centrifugation was carried out at 450000*g* for 2 h. The dark green fraction was collected and then subjected to hydrophobic chromatography with a Toyopearl HW-65F column (1.5 cm × 4.0 cm). The RC fraction thus obtained exhibited only a single PshA core protein band on SDS−PAGE.

Determination of the DNA Sequence of the pshA Gene. The partial amino acid sequences of the PshA protein of *Hbt. modesticaldum* were determined essentially according to the

manual for microsequencing. The denatured PshA transferred onto a PVDF membrane after SDS−PAGE analysis of the RC core complex was subjected to cyanogen bromide (CNBr) cleavage. Several polypeptides were recovered with HPLC, and their N-terminal amino acid sequences were determined with a gas-phase sequencer (Applied Biosystems, model 473A). Using several sets of oligonucleotides synthesized on the basis of the partial amino acid sequences, DNA fragments partially encoding the PshA gene were amplified from the *Hbt. modesticaldum* genome by PCR and labeled with digoxigenin-11-dUTP as probes (DNA Labeling and Detection Kit Non-radioactive, Boehringer Mannheim). The whole-length PshA gene was cloned by the conventional Southern blotting methods from the genomic DNA libraries of *Hbt. modesticaldum*.

PshA gene fragments were amplified from the *Heliobacterium gestii* genome by PCR using several sets of oligonucleotides that were designed according to the sequence similarity between *Hba. mobilis* and *Hbt. modesticaldum*. Additional DNA sequences flanking their fragments were obtained by the inverse PCR method as described in the manual. DNA sequencing was performed using the Sanger dideoxy chain termination method using a DNA sequencer (Applied Biosystems, models 373A, 377, and 3100).

Optical Measurements. A transient absorption spectrum with a time resolution of 3.5 ns was measured by a spectrograph spectrophotometer with a Jobin-Ybon 320 mm monochromator and a gatable image intensifier-photodiode array detector (Princeton Instruments). Samples were excited by a 532 nm, 10 ns (fwhm) flash from an Nd:YAG laser (Quanta Ray, DCR-2-10) at a repetition rate of 0.05 Hz and measured with a probing light with a 3 ms xenon flash through suitable band-pass glass filters. The time sequence of the excitation flash, probing flash, and the gating of the image intensifier was controlled by a delay pulse generator (Stanford Research, DG5351). Signals from the diode array were collected 64−256 times as required. The fluorescence from the sample excited by the actinic laser flash was also detected and subtracted. The absorption and fluorescence emission spectra under the steady state were measured using the same apparatus described above with tungsten−iodine lamps as the probing and excitation lights.

The time courses of the absorption change in the microsecond to millisecond time range were measured with a split-beam spectrometer with a probing light provided by a combination of a mechanical shutter and a 100 W tungsten−iodine lamp and the excitation laser flash as described above.

The sample in a cuvette with a light path of 10 mm was placed either in a cryostat (Oxford DN904) at 77 K or in a temperature-controlled holder at 283 K during the measurements. Samples were diluted with buffer A containing 2 mM SM-1200 to give an absorbance of 0.75−1.5 at the Q_y absorption peak (787−789 nm). For low-temperature measurements, glycerol was also added to give a final concentration of 60% (v/v).

ESR Measurements. ESR spectra were recorded using a Bruker ESP-300 spectrometer equipped with Oxford Instruments cryostats and a temperature-control system (CF935, Oxford Instruments). Membranes for the measurements were suspended with buffer A to give an absorbance of 250−450 at the Q_y absorption peak (787−789 nm). Magnetic field and *g* values were calibrated with DPPH signals.

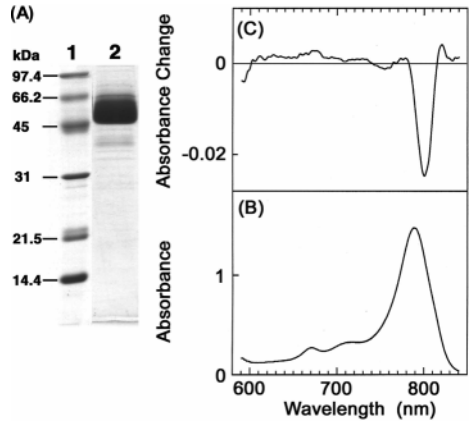

FIGURE 1: (A) SDS−PAGE analysis of the RC core complex isolated from *Hbt. modesticaldum*. Lane 1 contained molecular mass protein markers: phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa). Lane 2 contained the RC core complex. The absorption spectrum (B) and flash-induced difference spectrum of P800 (C) of the core complex were measured at 77 K. The difference spectrum 1 ms after the flash excitation is presented.

RESULTS

Isolation of the RC Core Complex. The membranes and RC core complex were isolated from cells of *Hbt. modesticaldum*. The membranes were solubilized by a treatment with sucrose monolaurate (SM-1200) followed by stepwise sucrose density gradient centrifugation and subsequent hydrophobic chromatography, as described in Materials and Methods. The photoactive RC complex thus obtained consisted of a single core protein, PshA, with an apparent molecular mass of 50 kDa via SDS−PAGE (Figure 1A).

The absorption spectrum and the flash-induced difference spectrum of the purified RC core complex at 77 K are shown in panels B and C of Figure 1, respectively. The difference spectrum represents the photo-oxidation of P800 with a negative peak at 800 nm and a positive one at 820 nm with the shift-type absorption change of the chlorophyll *a*-like pigment, 8^1-OH-Chl *a*, at 660−670 nm.

The RC core preparation obtained in this study seemed to be more intact than that previously reported by Noguchi et al. (*16*), since we detected a single-phase decay of flash-induced $P800^+$ in this study as shown below. Previously, we detected a two-phase decay that contained the faster decay of $P800^T$ formed in the charge recombination reaction between $P800^+$ and A_0^- due to depletion of the secondary electron acceptor (data not shown). The improvement of the RC purification procedure was achieved by harvesting the cells of *Hbt. modesticaldum* at the middle-logarithmic growth phase to prevent the lysis of cells.

Light-Induced ESR Signals of Iron−Sulfur Centers. In the presence of 2 mM dithiothreitol at 14 K, the dark-adapted membranes exhibited weak signals of iron−sulfur centers presumably including a Rieske-type one (Figure 2A,B, trace a, dotted line), as reported by Liebl et al. (*40*). Illumination for 5 min at 14 K induced an additional signal of the reduced iron−sulfur center [$g_z = 2.069$, $g_y = 1.938$, and $g_x = 1.891$ (Figure 1A, trace b)], showing the characteristics of center F_B^-, as demonstrated in *Hbt. chlorum* membranes by Nitschke et al. (*3*). After illumination for 20 min at 230 K followed by subsequent cooling to 14 K during illumination,

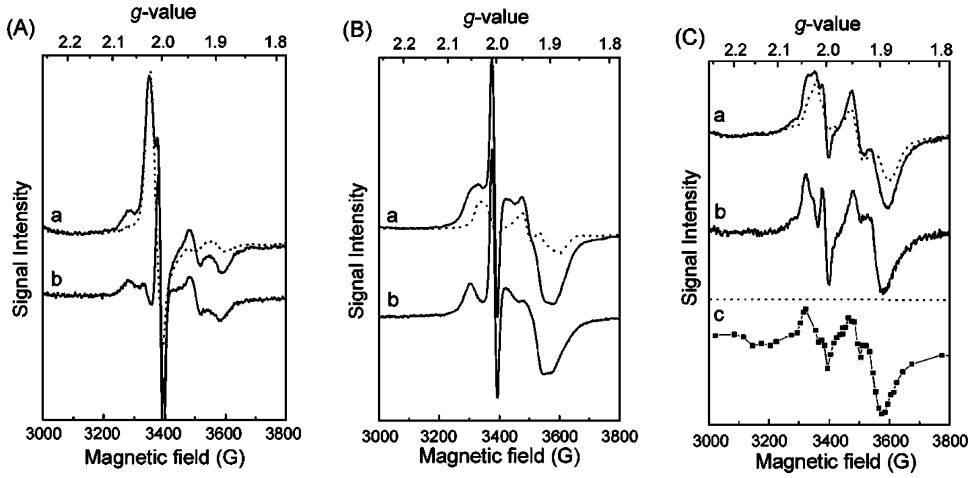

FIGURE 2: ESR spectra in the membranes isolated from *Hbt. modesticaldum* at 14 K (A and B) and 5 K (C). (A) (a) In the dark (···) and after illumination for 5 min at 14 K (—). (b) Light-minus-dark difference spectrum. (B) (a) In the dark (···) and after illumination for 20 min at 230 K followed by cooling to 14 K during illumination (—). (b) Light-minus-dark difference spectrum. (C) (a) In the dark (···) and during illumination at 5 K (—). (b) Light-minus-dark difference spectrum. (c) A transient ESR spectrum measured 20 ms after laser excitation (see the legend of Figure 3). Samples were suspended in a buffer containing 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 2 mM DTT (A and B) or in the same buffer with an excess of dithionite to fully reduce the F_A/F_B centers (C). Conditions for ESR measurements: microwave power, 10 mW; microwave frequency, 9.49 GHz; modulation amplitude, 20 G; and modulation frequency, 100 kHz.

a new signal with a 5-fold larger magnitude was revealed (Figure 2B, trace a, solid line). The difference between the photoaccumulated and dark-adapted signals gave a spectrum with apparent *g* values of 2.052, 1.927, and 1.912 (Figure 2B, trace b), which was assigned to the spin-interacting state of F_A^-/F_B^- (*3*).

The addition of excess amounts of dithionite almost fully induced the F_A^-/F_B^- signal in the dark (Figure 2C, trace a, dotted line). The illumination of this sample at 5 K induced another iron sulfur center with the following *g* values: $g_z = 2.040$, $g_y = 1.911$, and $g_x = 1.896$ (Figure 2C, trace b). The signal completely disappeared after the illumination and was fully reversible. We, therefore, assumed this signal to represent a component that mediates the electron transfer between A_0 and F_A/F_B, namely, the F_X-type center, which has never been detected in a heliobacterial RC. The signal can be detected only below 10 K at very high microwave powers, indicating a very rapid spin relaxation rate similar to that for F_X in PS I (*41*). The signal shape, however, was somewhat different from that of F_X observed in PS I (*41*).

We further studied the flash-induced kinetics at the negative peaks of F_X^- and $P800^+$ at 3570 and 3391 G, respectively, with a time constant of 5 ms (Figure 3). The F_X^- signal exhibited a two-phase decay with half-times ($t_{1/2}$) of 12 and 320 ms and $P800^+$ with a major decay phase with a $t_{1/2}$ of 10 ms. The faster decay phase of F_X^-, therefore, was nearly the same as the $P800^+$ phase within an error range at the present time constant of 5 ms, although the $P800^+$ decay seemed to contain an additional faster decay component as well. The transient ESR spectrum obtained by plotting the intensity at 20 ms was almost identical to the one obtained under continuous illumination at 5 K (see Figure 2C, traces b and c). The 320 ms decay phase of F_X^- had almost no counterpart in the $P800^+$ decay. These small discrepancies might involve minor reactions of some other components. It was concluded that F_X^- underwent fast charge recombination with $P800^+$ within 10 ms (or more rapidly) and could not accumulate even at 5 K.

In the isolated core complex, we failed to detect any signals of the reduced iron−sulfur centers upon continuous illumination or laser flash excitation. The reason for the loss of the F_X^- signal remains unknown at present. On the other hand, we detected the flash-induced kinetics of $P800^+$, which could be ascribable to the charge recombination with F_X^- (see the next section).

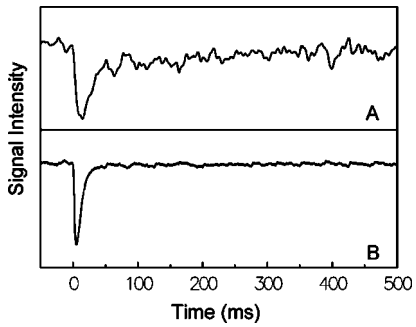

FIGURE 3: Time courses of flash-induced ESR signals in membranes of *Hbt. modesticaldum* at 8 K. Transient ESR signals were measured at 3570 (A) and 3391 G (B) to monitor F_X^- and $P800^+$. Samples were suspended in a buffer containing 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 2 mM DTT, and an excess of dithionite to fully reduce the F_A/F_B centers. Conditions for ESR measurements: microwave power, 10 mW (A) and 1 mW (B); microwave frequency, 9.49 GHz; modulation amplitude, 20 G; modulation frequency, 100 kHz; and time constant, 5 ms.

Temperature-Dependent Change of the Charge Recombination Rate. The $P800^+$ decay after the laser excitation was optically measured at various temperatures in the membranes (Figure 4A, left panel), and the rate and extent of each decay component were calculated (Figure 4B). The decay time course at 295 K in the membrane was fitted with two decay components with 3 and 30 ms time constants at amplitudes of 20 and 80%, respectively. The 3 ms component could be ascribed to the reduction of $P800^+$ by cytochrome *c*, as shown in *Hbt. gestii* membranes (*26*), and was accelerated by the addition of Mg^{2+} (not shown). The 30 ms component seemed to represent the reduction of $P800^+$ by $(F_A/F_B)^-$ and was slightly slower than the corresponding one in *Hbt. gestii* ($t_{1/2} = 7$ ms) (*26*). The magnitude of the 3 ms component decreased on cooling and completely

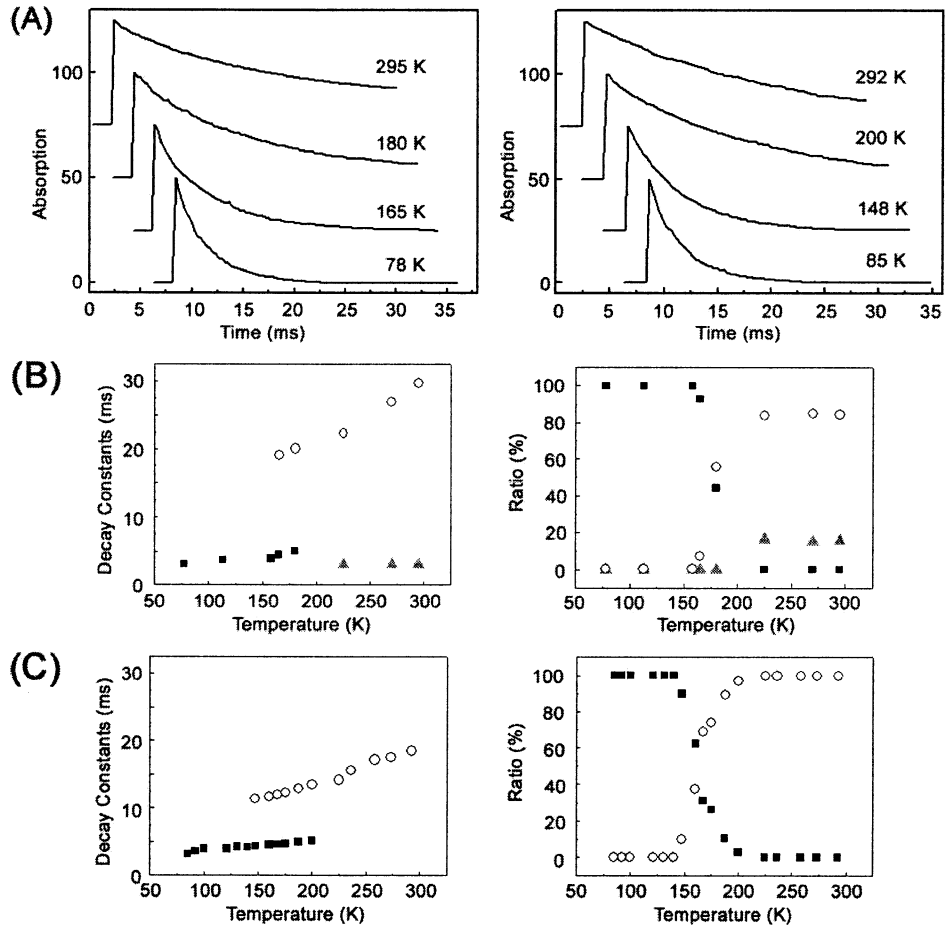

FIGURE 4: Temperature dependence of the flash-induced absorption change of $P800^+$. (A) Time courses at various temperatures in the membranes (left) and the RC core protein (right) of *Hbt. modesticaldum*. An ordinate scale of 100 corresponds to an absorbance change of 0.01. The upward changes represent the absorption decrease. (B) Temperature dependence of the decay rate (left) and relative amplitude (right) of each exponential decay component in the membranes: (○) 19−30 ms component, (■) 3−5 ms component, and (▲) 3 ms component. (C) Same as in panel B except for the RC core complex: (○) 11−19 ms component and (■) 3−5 ms component. The kinetic analyses were carried out by curve fittings.

disappeared below 200 K (Figure 4B, right panel). The suppression could be interpreted as the decrease in the level of collision between cytochrome *c* and $P800^+$ on cooling, as seen in the reaction of cytochrome c_z and $P840^+$ in green sulfur bacteria (*27*). An additional faster 3−5 ms component appeared on further cooling and became dominant below 150 K, showing an apparent crossover temperature at 175 K.

The isolated RC core complex, which is depleted of F_A/F_B centers, exhibited similar kinetic behavior (Figure 4A, right panel, and Figure 4C). The 11−19 ms decay phase that was dominant at high temperatures seemed to represent the charge recombination reaction between $P840^+$ and F_X^-. This phase was replaced by the faster 3−5 ms phase at low temperatures. The crossover from the fast to the slow phase occurred at 160 K. This temperature was almost comparable to the apparent crossover temperature of 175 K in the membranes and would reflect a common transition mechanism in both preparations. Similar results have been reported in *Hba. mobilis* membranes by Chiou et al. (*37*). They attributed the fast decay component (2−4 ms) to charge recombination between $P800^+$ and quinone-type acceptor A_1 on the basis of the absorbance change at 390−470 nm. However, the ESR measurement in this study indicated that the $P800^+F_X^-$ state was transiently formed even at 5 K (see Figures 2C and 3). Since we measured the decay time constant ($t_{1/2}$ = 10 ms) of the F_X^- signal only with a slow time constant because of the low signal-to-noise ratio, it is possible that charge recombination between $P800^+$ and F_X^- occurred with a somewhat shorter time constant ($t_{1/2} \sim 3-5$ ms) at 5 K, as detected optically at 160−77 K.

Assessment of Reactions in the Faster Time Range at 77 K: Spectral Changes of Acceptor A_0. The heliobacterial RC contains two molecules of 8^1-OH-Chl *a* that function as primary electron acceptor A_0 (*20*−*22*). We measured the absorption changes in the 670 nm region to detect the reaction of A_0 molecules and their electrochromic shift in response to the formation of $P800^+$ in a nanosecond to millisecond time range after laser flash excitation in the core complex at 77 K with a 10 ns instrument time constant.

A small and very rapid absorption decrease was detected immediately after the flash excitation at 10% of the total change, as shown in Figures 5A and 6A. This phase with a decay time of <10 ns seemed to represent the decay of the singlet excited state of 8^1-OH-Chl *a*. The shift-type spectral changes produced by the excitation continued for a longer time and decayed with a time constant of 2 ms (see Figure 6A). This time constant agreed well with that of $P800^+$ decay monitored in the 800 nm region (data not shown). The shiftlike absorption change thus represents the electrochromic shift of 8^1-OH-Chl *a* accompanied by the formation of $P800^+$.

After the exposure of the core complex to air for 30 min, a larger degree of bleaching was detected at 666 nm just

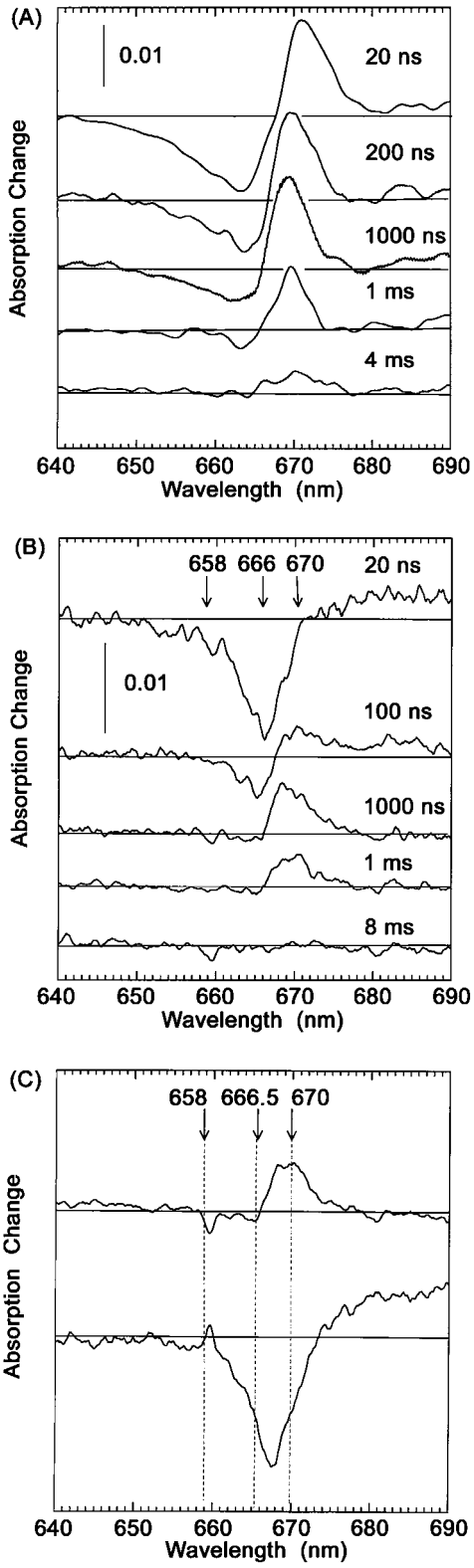

FIGURE 5: Time-resolved difference spectra of the intact core complex (A) and the air-damaged complex (B) of *Hbt. modesticaldum* in a 670 nm region at 77 K. (C) The difference spectrum of A_0 (bottom) was calculated as the difference between the shift-type spectrum of Chl-670 at 10 μs (top) and the spectrum at 20 ns in the air-exposed complex.

after laser excitation (Figure 5B). The difference spectra measured within 1 μs were somewhat different from those measured in the untreated core complex, and those in the 10 μs to millisecond time range revealed the electrochromic

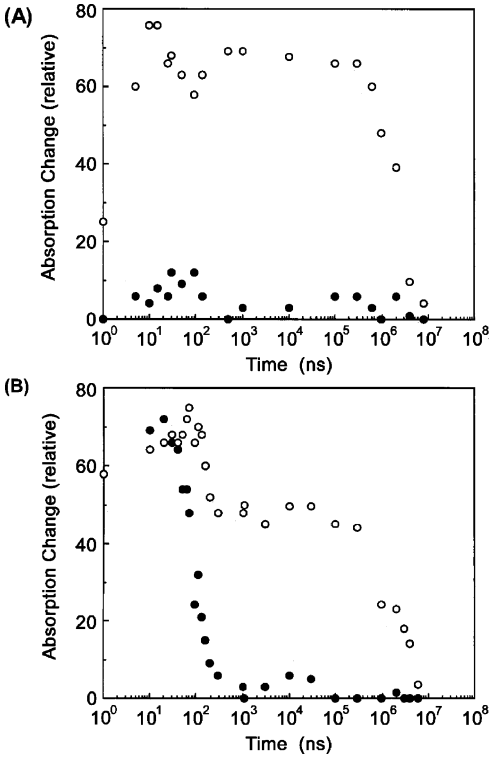

FIGURE 6: Time courses of the amounts of $P800^+$ and A_0^- after flash excitation in the intact core complex (A) and the partially air-degraded complex (B) of *Hbt. modesticaldum* at 77 K. The level of A_0^- (●) was calculated as the difference between the absorption changes at 666 and 658 nm and that of $P800^+$ (○) as the difference between those at 670 and 666 nm (see the text for details).

shift of 8^1-OH-Chl *a*. The measurements separately conducted in the 800 nm region gave two decay phases with time constants of 100 μs and 2 ms (data not shown). The difference spectrum of the 100 μs phase exhibited bleaching at 800 nm with a small increase at 820 nm but no increase at 780 nm, giving the feature of the $P800^T$ state (*43*). On the other hand, the 2 ms component was assigned to the decay of $P800^+$.

The absorption change in the 670 nm region within 1 μs of laser excitation in the air-treated core complex, then, should include the absorption change of A_0^- mixed with the electrochromic shift of 8^1-OH-Chl *a* associated with $P800^+$. We calculated the A_0^- spectrum by subtracting the spectrum at 10 μs from the spectrum at 20 ns in the air-exposed core complex, as shown in Figure 5C. The difference spectrum of $(P800^+)A_0^-/(P800)A_0$ thus calculated gave a peak at 668 nm with a bandwidth of 6.5 nm. $P800^+$ itself did not contribute significantly to this spectral range. Using this $(P800^+)A_0^-/(P800)A_0$ difference spectrum, we estimated the extent of the $P800^+A_0^-$ state as the absorption difference between 666 and 658 nm, since these two wavelengths were the isosbestic points of the shift spectrum. We also estimated the extent of $P800^+$ as the absorption difference between 670 and 666 nm; the contributions of A_0^-/A_0 to these wavelengths were almost negligible. $P800^+$ was recovered in two phases with $t_{1/2}$ values of 100 ns and 2 ms (Figure 6B). The 2 ms decay time of the slower phase completely agreed with that detected in the untreated complex. On the other hand, the 100 ns decay component could be ascribed to the charge recombination between $P800^+$ and A_0^- to form $P800^T$. These results indicated that electron acceptors other

than A_0 were destroyed in 40% of the RC core complex by exposure to air.

DISCUSSION

Iron−Sulfur Centers F_A and F_B. In this study, F_A and F_B were fully reduced in the dark by an excess of dithionite at pH 8.0 in the isolated membranes of *Hbt. modesticaldum* (Figure 2C); in an earlier study, Nitschke et al. (*3*) had failed to chemically reduce them in the membranes of *Hbt. chlorum*. Our results suggested the E_m values of F_A and F_B to be around −400 to −450 mV, respectively, in agreement with the estimations by the titration of the $P800^+$ decay kinetics in *Hbt. chlorum*, which gave values of −440 mV (*43*) and/or −510 mV (*44*).

The amount of F_B^- photoreduced after the 5 min illumination at 14 K was ∼25% of the total amount of F_A^-/F_B^- accumulated after the 20 min illumination at 230 K. This amount was larger than that reported by Nitschke et al. (*3*), who estimated only 8% accumulation after a 10 min illumination at 4 K in *Hbt. chlorum* membranes. Although the reason for the discrepancy remains unknown, it could be attributed to the strict anaerobic experimental procedure in this study.

The ESR spectrum in Figure 2B suggests the magnetic interactions between the spins on F_A^- and F_B^- because the g_z signal of F_B^- at 2.069 was shifted to 2.052 under the fully reduced condition. The effect is similar to that observed in the RC complexes of PS I (*45*) and green sulfur bacteria (*46*) and implies the relatively short distance between spins on F_A and F_B, i.e., their locations within the same protein. The proteins that hold centers F_A and F_B are PsaC with an apparent molecular mass of 9 kDa in PS I (*45*, *47*) and PscB (24 kDa) in green sulfur bacteria, respectively (*5*). The amino acid sequences of PsaC and PscB are slightly similar (16%), except for the conserved binding motifs for the two [4Fe-4S] clusters (*5*). The iron−sulfur protein with centers F_A and F_B in heliobacteria has not been identified yet. The ESR signals of F_A and F_B centers were also detected in the crude RC fraction obtained by treatment with a detergent milder than that used in this study, indicating the F_A/F_B protein is associated with the RC core protein (*48*).

The preferential reduction of F_B by the 14 K illumination suggested that the redox potential of F_B is more positive than that of F_A, as indicated by Nitschke et al. (*3*), and similar to that in green sulfur bacteria (*46*, *49*). The situations in heliobacteria and green sulfur bacteria, however, were different from that in PS I, in which the E_m value of F_B (−590 mV) is more negative than that of F_A (−540 mV) (*42*, *45*).

Properties of Iron−Sulfur Center F_X. The amino acid sequence of PshA has a binding motif to bridge a [4Fe-4S] cluster at the center of the PshA−PshA homodimer, as in the PsaA−PsaB PS I heterodimeric and PscA−PscA homodimeric RCs of green sulfur bacteria (*4*). However, an ESR signal comparable to F_X^- in PS I or the green sulfur bacterial RC has not been found in heliobacteria for a long time. This is the first study in which the ESR signal of the F_X^--type cluster has been measured. The signal could be detected only during illumination or just transiently after laser excitation at 5 K at a high microwave power in the presence of pre-reduced F_A^-/F_B^-.

In the membranes and RC complex of green sulfur bacteria, the ESR spectra of photoaccumulated F_X^- have been shown with low signal-to-noise ratios (*49*−*51*). The spectrum of F_X^- with a g_z of 2.17, a g_y of 1.92, and a g_x of 1.77 has been identified by its characteristic low-field peak and high-field trough (*51*), as observed in PS I (*42*, *45*). On the other hand, the F_X^- spectrum in heliobacteria, giving a g_z of 2.040, a g_y of 1.911, and a g_x of 1.896, was somewhat different from that in PS I and closer to the resonance field of the F_A^-/F_B^- signals (see Figure 2). Therefore, it is natural that it has been difficult to detect the F_X^- signal in heliobacterial RC.

Table 1: Comparison of the *g* Values of the ESR Signals of Iron−Sulfur Center F_X in Type 1 Homodimeric RCs of *Hbt. modesticaldum* and *Chl. tepidum* and the Heterodimeric Spinach PS I RC

	Hbt. modesticaldum RC[a]	*Chl. tepidum* RC[b]	spinach PS I[b]
g_z	2.04	2.17	2.08
g_y	1.911	1.92	1.88
g_x	1.896	1.77	1.78

[a] From this study. [b] From refs *51* and *52*.

The F_X^- spectrum in the RC core complex was, however, undetectable, although the RC preparation was fully functional judging from the kinetics measurements of $P800^+$ by optical (Figures 4 and 6) and transient ESR spectroscopy (R. Miyamoto, unpublished results), and the kinetics were essentially similar to those in the membranes. Changes in the spin relaxing mode or modifications of the structure of the F_X cluster might have obscured the F_X^- signal in the isolated RC core complex. Further studies are required to know the function of F_X in the core complex.

The isotropic spectrum of the F_X^- signal in heliobacterial RC makes a clear contrast to the anisotropic ones in PS I and the green sulfur bacterial RC, as listed in Table 1. The [4Fe-4S] cluster of F_X in the heliobacterial RC, thus, seems to have a cubic structure with a symmetry higher than those in PS I and/or the green sulfur bacterial RC. However, the amino acid sequences around the F_X-binding loop are highly similar (Figure 7A); thus, a small change in the structure around the binding site might affect the symmetry of the spin density on the F_X-type cluster.

Electron Transfer in the RC of Heliobacteria at a Cryogenic Temperature. The re-reduction kinetics of $P800^+$ after flash excitation exhibited 19−30 and 12−19 ms phases in the membranes and isolated RC core complex, respectively, from room temperature to 200 K. These different time constants seemed to represent the charge recombination of $P800^+$ with $(F_A/F_B)^-$ in the membranes and that with F_X^- in the core complex, respectively. Both re-reduction rates of $P800^+$ slowed slightly on cooling until 200 K and were replaced by the faster 3−5 ms phases on further cooling, exhibiting crossover temperatures of 175 and 160 K in the membranes and the core complex, respectively. In both preparations, the faster phases became dominant below 150 K and exhibited almost constant decay rates of 3−5 ms until 77 K (see Figure 4), in agreement with the reports by Smit et al. (*53*) and Chiou et al. (*37*).

Nitschke et al., in their pioneering work (*3*), demonstrated two decay phases of $P800^+$ with 850 μs and 2.5 ms time constants after flash excitation at 10 K in *Hbt. chlorum* membranes and assumed the faster one to the reduction by a quinone acceptor A_1. The time constant of the slower phase agreed with that measured in this study but not with the faster

(A)

```
Hmd    406 RMVFFSKVSPWTTKVGMKAKRDGEFPCLGPAYGGTCSISLVDQ
Hg     406 RMVFFSKVSPWTTKVGMKAKRDGEFPCLGPAYGGTCSISLVDQ
Hmb    407 RMVFFSKVSPWTTKVGMKAKRDGEFPCLGPAYGGTCSISLVDQ
Cl     501 RIAFFAHTSPLWDDLGLKKN-SYSFPCLGPVYGGTCGVSIQDQ
Ct     502 RIAFFAHTSPLWDDLGLKKN-SYSFPCLGPVYGGTCGVSIQDQ
Se     555 KGVLFARSS---RLIPDKANLGFRFPCDGPGRGGTCQVSGWDH
                : .:*:: *     :  * : .  *** **  **** :*  *:
```

(B)

```
Hmd    533 LLGAHFIWAFTFSMLFQYRGSRDEGAMVLKWAHE    578 N-R--ALSLKEGKAIG
Hg     533 LLGAHFIWAFTFSMLFQYRGSRDEGSMVLKWAHE    578 N-R--ALSLKEGKAIG
Hmb    534 LLGAHFIWAFTFSMLFQYRGSRDEGAMVLKWAHE    579 N-R--ALSLKEGKAIG
Cl     616 LMIGHLVWFISFAVWFEDRGSRLEGADIQTRTIR    661 NFRFPVLTISDSKLAG
Ct     617 LMIGHLVWFISFAVWFEDRGSRLEGADIQTRTIR    662 NFRFPVLTISDSKLAG
Se     676 FLGAHFIWAFSLMFLFSGRGYWQELIESIVWAHN    719 P-R--ALSIIQGRAVG
                :: .*::* ::: . *. **   *       : .          *  :*:: :.:  *
```

(C)

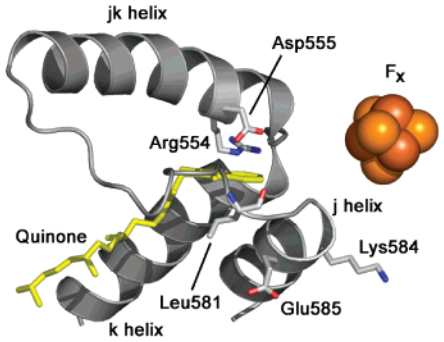

FIGURE 7: (A and B) Comparison of the amino acid sequences around the F_X- and quinone-binding sites in *S. elongatus* PS I and those in RCs of heliobacteria and green sulfur bacteria, respectively. The numbers indicate the residue positions in the corresponding proteins. The names of species are abbreviated as follows: *Hmd*, *Hbt. modesticaldum*; *Hg*, *Hbt. gestii*; *Hmb*, *Hbc. mobilis*; *Cl*, *Chl. limicola*; *Ct*, *Chl. tepidum*; and *Se*, *S. elongatus*. (C) Structural homology modeling of the heliobacterial RC in a portion of the putative quinone-binding site (see the text for details). The software used for the modeling was Swiss-PdbViewer (http://kr.expasy.org/spdbv/), and the figure was prepared using PyMOL (http://pymol.sourceforge.net/).

one. The discrepancy might come from differences in either the bacterial species, the reduction states, or the contributions of the $P800^T$ signal.

The features of the temperature dependence above are different from that in PS I (*45*), in which the reaction time between $P700^+$ and $(F_A/F_B)^-$ is 30 ms at room temperature, slows on cooling, and stops at cryogenic temperatures. The reaction time between $P700^+$ and F_X^- increases from 250 μs at room temperature to 100 ms at 200 K and becomes significantly slower upon further cooling. Moreover, most of $P700^+$ is reduced by A_1^- rapidly with a $t_{1/2}$ of 100 μs below 200 K due to the suppression of the electron transfer from A_1^- to F_X.

We assessed the reaction between $P800^+$ and F_X^- with a time constant shorter than 10 ms even at 5 K. This makes a clear contrast to the very slow oxidation of F_X reported in PS I (*54*, *55*). The switchover of the $P800^+$ re-reduction rate from the slow to the fast one may indicate either the changes in the protein dynamics, as suggested by Chiou et al. (*37*), or the involvement of menaquinone through an unidentified mechanism.

Reaction of Primary Electron Acceptor A_0. Primary electron acceptor A_0 in the heliobacterial RC is 8^1-OH-Chl *a* esterified with farnesol (8^1-OH-Chl *a*), which gives an absorption peak at 670 nm (*21*); its full stereochemistry has been recently determined (*23*). A_0^- is formed within 25−30 ps of flash excitation and is reoxidized with a time constant of 500−800 ps (*20*, *22*, *56*). In green sulfur bacteria, A_0 is also chlorophyll *a* esterified with Δ-2,6-phytadienol (Chl a_{670}) (*57*), which is reoxidized with a time constant of 550−700 ps (*58*, *59*). The slow reoxidation times of A_0^- were also confirmed by pulse ESR analysis (*38*). These reoxidation times of A_0^- are significantly slower than the time for reoxidation of A_0^- by the A_1 phylloquinone in PS I (21−25 ps) (*60*, *61*). In both heliobacterial and green sulfur bacterial RCs, F_X has been assumed to be directly reduced by A_0^-, since no positive evidence has been obtained for the function of quinone (A_1) (*17*, *28*, *29*, *31*).

In this study, we partially modified the electron transfer pathway in the purified heliobacterial RC by exposing it to air. Approximately 40% of the RC was damaged and exhibited fast charge recombination between $P800^+$ and A_0^- with a $t_{1/2}$ of 100 ns (see Figure 6B) to form $P800^T$, which decayed with a $t_{1/2}$ of 100 μs at 77 K. This situation was somewhat different from that of PS I, in which F_X could be selectively eliminated by urea treatment (*62*) or by heat treatment in the presence of ethylene glycol (*63*) while retaining the activity of the quinone acceptor A_1.

When the A_0^-/A_0 difference spectrum is compared with the shift-type spectral change of A_0 that accompanied the

formation of $P800^+$, it is clear that the bleaching peak (668 nm) of A_0^- is slightly different from the peak (670 nm) of the shift, giving the isosbestic point at 665.5 nm (see Figure 5C), suggesting the heterogeneity of A_0 components. One way to interpret this observation is that two A_0 molecules are located in symmetrical positions and that one loses its ground-state absorption band upon being reduced while the other gives the electrochromic shift in response to $P800^+$ formation. In the homodimeric RC of heliobacteria, it is not known whether the 8^1-OH-Chl *a* molecules on both sides function as A_0 by chance or whether the function is limited to a molecule on one side because of a slight asymmetry caused by some unknown factors.

Homology Modeling of the Structure of the Heliobacterial RC. Figure 7B compares the amino acid sequences around the binding regions of the P700 and A_1 phylloquinone in PS I with the corresponding sites in the RCs of heliobacteria and green sulfur bacteria. The sequences in this region are highly similar (~50%) compared to the level of similarity calculated for the whole sequences (10−15%).

We constructed a three-dimensional homology model for the *Hbt. modesticaldum* RC on the basis of the atomic coordinates of the PS I structure of *S. elongatus* and their amino acid sequence similarity. The model suggested that most of the electron transfer cofactors reside at sites similar to those in PS I. The structure and physicochemical property of the binding site of F_X were expected to be almost conserved, judging from the highly conserved amino acid sequences (not shown). Figure 7C shows the probable quinone (A_1)-binding pocket in the RC of *Hbt. modesticaldum*. In PS I, the phylloquinone on the PsaA side is known to be fixed to the hydrophobic site through a hydrogen bond between the carbonyl oxygen of quinone and the NH group of a leucine residue (LeuA722) and the $\pi-\pi$ interaction between the aromatic rings of quinone and a tryptophan residue (TrpA697). LeuA722 in PS I is conserved as Leu581 in the *Hbt. modesticaldum* RC, and TrpA697 is replaced by the positively charged Arg554. The model also indicates the presence of positively (Lys584) and negatively charged residues (Asp555 and Glu585) in place of hydrophobic residues around the quinone pocket in PS I and suggests that the pocket is more hydrophilic than that in PS I, as predicted for the green sulfur bacterial RC (*64*). The model in Figure 7C suggests either the absence of hydrophobic quinone or its loose binding at this site. If menaquinone exists in this site, its redox potential should be different from that of phylloquinone in PS I. It has even been suggested that the reduced quinones in bacterial type 1 RCs exchange with the oxidized ones in a quinone pool in the membrane (*18*, *65*).

An edge-to-edge distance of 14 Å can be estimated between A_0 and F_X in the model of the heliobacterial RC. The empirical calculation proposed by Moser et al. (*66*), which is based on the electron transfer theory of Marcus (*67*), predicted the direct electron transfer time between A_0 and F_X over this distance to be longer than 200 ns even at the maximum matching of the energy gap. The fast reaction time of 500−800 ps, then, suggests the mediation by some other component such as menaquinone or an aromatic amino acid residue such as tyrosine that functions on the donor side of PS II (*68*), although we found no such residues in the predicted model.

We identified an ESR signal of F_X in the heliobacterial RC that exhibited a very fast spin relaxation rate, a rapid fully reversible turnover rate, and a rather symmetrical spectrum at 5 K. Although these features were somewhat different from those of F_X in PS I (*41*), the structural and functional relationship between these RCs was clearly shown to be similar in that they both contained F_X. The unresolved issue in this heliobacterial RC is the mechanism for electron transfer from A_0 to F_X. The charge recombination rate of F_X^- did not slow significantly even at a cryogenic temperature, in contrast to that in PS I. The reaction, thus, may also involve the activity of menaquinone, especially at low temperatures. Although the properties of quinone-binding sites in heliobacterial and green sulfur bacterial RCs could be predicted by homology modeling, the precise locations of menaquinone as well as its function are still unknown. The elucidation of the electron transfer mechanism in bacterial type 1 RCs will be essential in exploring the evolution mechanism of photosynthetic RCs from homodimeric to heterodimeric and from anoxygenic to oxygenic.

ACKNOWLEDGMENT

We thank Mr. Shohei Miyago (Osaka University) for his technical assistance in the determination of the DNA sequence. We also thank Prof. Keiichi Fukuyama and Dr. Kei Wada for their participation in stimulating discussions.

REFERENCES

1. Madigan, M. T. (1992) The Family Heliobacteria, in *The Prokaryotes* (Balows, A., Trüper, H. G., Dworkin, M., and Schleifer, K.-H., Eds.) 2nd ed., pp 1981−1992, Springer, Berlin.
2. Amesz, J. (1995) The Antenna-Reaction Center Complex of Heliobacteria, in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 687−697, Kluwer Academic Publishers, Dordrecht, The Netherlands.
3. Nitschke, W., Sétif, P., Liebl, U., Feiler, U., and Rutherford, A. W. (1990) Reaction center photochemistry of *Heliobacterium chlorum*, *Biochemistry 29*, 11079−11088.
4. Liebl, U., Mockensturm-Wilson, M., Trost, J. T., Brune, D. C., Blankenship, R. E., and Vermaas, W. (1993) Single core polypeptide in the reaction center of the photosynthetic bacterium *Heliobacillus mobilis*: Structural implication and relations to other photosystems, *Proc. Natl. Acad. Sci. U.S.A. 90*, 7124−7128.
5. Büttner, M., Xie, D.-L., Nelson, H., Pinther, W., Hauska, G., and Nelson, N. (1992) Photosynthetic reaction center genes in green sulfur bacteria and in photosystem 1 are related, *Proc. Natl. Acad. Sci. U.S.A. 89*, 8135−8139.
6. Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., and Krauss, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution, *Nature 411*, 909−917.
7. Schubert, W.-D., Klukas, O., Saenger, W., Witt, H. T., Fromme, P., and Krauss, N. (1998) A common ancestor for oxygenic and anoxygenic photosynthetic systems: A comparison based on the structural model of photosystem I, *J. Mol. Biol. 280*, 297−314.
8. Heathcote, P., Fyfe, P. K., and Jones, M. R. (2002) Reaction centres: The structure and evolution of biological solar power, *Trends Biochem. Sci. 27*, 79−87.
9. Okamura, M. Y., and Feher, G. (1995) Proton-coupled electron transfer reactions of Q_B in reaction centers from photosynthetic bacteria, in *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 577−594, Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. Guergova-Kuras, M., Boudreaux, B., Joliot, A., Joliot, P., and Redding, K. (2001) Evidence for two active branches for electron transfer in photosystem I, *Proc. Natl. Acad. Sci. U.S.A. 98*, 4437−4442.
11. Ramesh, V. M., Gibasiewicz, K., Lin, S., Bingham, S. E., and Webber, A. N. (2004) Bidirectional electron transfer in photo-

system I: Accumulation of A_0^- in A-side or B-side mutants of the axial ligand to chlorophyll A_0, *Biochemistry 43*, 1369−1375.
12. Cohen, R. O., Shen, G., Golbeck, J. H., Xu, W., Chitnis, P. R., Valieva, A. I., van der Est, A., Pushkar, Y., and Stehlik, D. (2004) Evidence for asymmetric electron transfer in cyanobacterial photosystem I: Analysis of a methionine-to-leucine mutation of the ligand to the primary electron acceptor A_0, *Biochemistry 43*, 4741−4754.
13. Fairclough, W. V., Forsyth, A., Evans, M. C., Rigby, S. E., Purton, S., and Heathcote, P. (2003) Bidirectional electron transfer in photosystem I: Electron transfer on the PsaA side is not essential for phototrophic growth in *Chlamydomonas*, *Biochim. Biophys. Acta 1606*, 43−55.
14. Santabarbara, S., Kuprov, I., Fairclough, W. V., Purton, S., Hore, P. J., Heathcote, P., and Evans, M. C. (2005) Bidirectional electron transfer in photosystem I: Determination of two distances between $P700^+$ and A_1^- in spin-correlated radical pairs, *Biochemistry 44*, 2119−2128.
15. Rigby, S. E. J., Evans, M. C. W., and Heathcote, P. (2001) Electron nuclear double resonance (ENDOR) spectroscopy of radicals in photosystem I and related type 1 photosynthetic reaction centres, *Biochim. Biophys. Acta 1507*, 247−259.
16. Noguchi, T., Fukami, Y., Oh-oka, H., and Inoue, Y. (1997) Fourier transform infrared study on the primary donor P798 of *Heliobacterium modesticaldum*: Cysteine S-H coupled to P798 and molecular interactions of carbonyl groups, *Biochemistry 36*, 12329−12336.
17. Iwaki, M., Itoh, S., Kamei, S., Matsubara, H., and Oh-oka, H. (1999) Time-resolved spectroscopy of chlorophyll-*a* like electron acceptor in the reaction center complex of the green sulfur bacterium *Chlorobium tepidum*, *Plant Cell Physiol. 40*, 1021−1028.
18. Hauska, G., Schoedl, T., Remigy, H., and Tsiotis, G. (2001) The reaction center of green sulfur bacteria, *Biochim. Biophys. Acta 1507*, 260−277.
19. Kobayashi, M., van de Meent, E. J., Erkelens, C., Amesz, J., Ikegami, I., and Watanabe, T. (1991) Bacteriochlorophyll *g* epimer as a possible reaction center component of heliobacteria, *Biochim. Biophys. Acta 1057*, 89−96.
20. Nuijs, A. M., van Dorssen, R. J., Duysens, L. N. M., and Amesz, J. (1985) Excited states and primary photochemical reactions in the photosynthetic bacterium *Heliobacterium chlorum*, *Proc. Natl. Acad. Sci. U.S.A. 82*, 6865−6868.
21. van de Meent, E. J., Kobayashi, M., Erkelens, C., van Veelen, P. A., Amesz, J., and Watanabe, T. (1991) Identification of 8^1-hydroxychlorophyll *a* as a functional reaction center pigment in heliobacteria, *Biochim. Biophys. Acta 1058*, 356−362.
22. Lin, S., Chiou, H. C., Kleinherenbrink, F. A. M., and Blankenship, R. E. (1994) Time-resolved spectroscopy of energy and electron transfer processes in the photosynthetic bacterium *Heliobacillus mobilis*, *Biophys. J. 66*, 437−445.
23. Mizoguchi, T., Oh-oka, H., and Tamiaki, H. (2005) Determination of stereochemistry of bacteriochlorophyll g_F and 8^1-hydroxychlorophyll a_F from *Heliobacterium modesticaldum*, *Photochem. Photobiol. 81*, 666−673.
24. Albert, I., Rutherford, A. W., Grav, H., Kellermann, J., and Michel, H. (1998) The 18 kDa cytochrome c_{553} from *Heliobacterium gestii*: Gene sequence and characterization of the mature protein, *Biochemistry 37*, 9001−9008.
25. Xiong, J., Inoue, K., and Bauer, C. E. (1998) Tracking molecular evolution of photosynthesis by characterizatin of a major photosynthetic gene cluster from *Heliobacillus mobilis*, *Proc. Natl. Acad. Sci. U.S.A. 95*, 14851−14856.
26. Oh-oka, H., Iwaki, M., and Itoh, S. (2002) Electron donation from membrane-bound cytochrome *c* to the photosynthetic reaction center in whole cells and isolated membranes of *Heliobacterium gestii*, *Photosynth. Res. 71*, 137−147.
27. Oh-oka, H., Iwaki, M., and Itoh, S. (1998) Membrane-bound cytochrome c_z couples quinol oxidoreductase to the P840 reaction center complex in isolated membranes of the grenn sulfur bacterium *Chlorobium tepidum*, *Biochemistry 37*, 12293−12300.
28. Lin, S., Chiou, H.-C., and Blankenship, R. E. (1995) Secondary electron transfer processes in membranes of *Heliobacillus mobilis*, *Biochemistry 34*, 12761−12767.
29. Kleinherenbrink, F. A. M., Ikegami, I., Hiraishi, A., Otte, S. C. M., and Amesz, J. (1993) Electron transfer in menaquinone-depleted membranes of *Heliobacterium chlorum*, *Biochim. Biophys. Acta 1142*, 69−73.
30. Brettel, K., Leibl, W., and Liebl, U. (1998) Electron transfer in the heliobacterial reaction center: Evidence against a quinone-type electron acceptor functioning analogous to A_1 in photosystem I, *Biochim. Biophys. Acta 1363*, 175−181.
31. Kusumoto, N., Sétif, P., Brettel, K., Seo, D., and Sakurai, H. (1999) Electron transfer kinetics in purified reaction centers from the green sulfur bacterium *Chlorobium tepidum* studied by multiple-flash excitation, *Biochemistry 38*, 12124−12137.
32. Takaichi, S., and Oh-oka, H. (1999) Pigment composition in the reaction center complex from the thermophilic green sulfur bacterium, *Chlorobium tepidum*: Carotenoid glucoside esters, menaquinone and chlorophylls, *Plant Cell Physiol. 40*, 691−694.
33. Frankenberg, N., Hager-Braun, C., Feiler, U., Fuhrmann, M., Rogl, H., Schneebauer, N., Nelson, N., and Hauska, G. (1996) P840-reaction centers from *Chlorobium tepidum*: Quinone analysis and functional reconstitution into lipid vesicles, *Photochem. Photobiol. 64*, 14−19.
34. Permentier, H., Schmidt, K. A., Kobayashi, M., Akiyama, M., Hager-Braun, C., Neerken, S., Miller, M., and Amesz, J. (2000) Composition and optical properties of reaction centre core complexes from the green sulfur bacteria *Prosthecochloris aestuarii* and *Chlorobium tepidum*, *Photosynth. Res. 64*, 27−39.
35. Muhiuddin, I. P., Rigby, S. E. J., Evans, M. C. W., Amesz, J., and Heathcote, P. (1999) ENDOR and special TRIPLE resonance spectroscopy of photoaccumulated semiquinone electron acceptors in the reaction centers of green sulfur bacteria and heliobacteria, *Biochemistry 38*, 7159−7167.
36. Kjær, B., Frigaard, N.-U., Yang, F., Zybailov, B., Miller, M., Golbeck, J. H., and Scheller, H. V. (1998) Menaquinone-7 in the reaction center complex of the green sulfur bacterium *Chlorobium vibrioforme* functions as the electron acceptor A_1, *Biochemistry 37*, 3237−3242.
37. Chiou, H.-C., and Blankenship, R. E. (1996) Temperature dependence of charge recombination in *Heliobacillus mobilis*, *Photochem. Photobiol. 64*, 32−37.
38. van der Est, A., Hager-Braun, C., Leibl, W., Hauska, G., and Stehlik, D. (1998) Transient electron paramagnetic resonance spectroscopy on green-sulfur bacteria and heliobacteria at two microwave frequencies, *Biochim. Biophys. Acta 1409*, 87−98.
39. Oh-oka, H., Kamei, S., Matsubara, H., Iwaki, M., and Itoh, S. (1995) Two molecules of cytochrome *c* function as the electron donors to P840 in the reaction center complex isolated from a green sulfur bacterium, *Chlorobium tepidum*, *FEBS Lett. 365*, 30−34.
40. Liebl, U., Rutherford, A. W., and Nitschke, W. (1990) Evidence for a unique Rieske iron-sulphur centre in *Heliobacterium chlorum*, *FEBS Lett. 261*, 427−430.
41. Evans, M. C., Sihra, C. K., and Cammack, R. (1976) The properties of the primary electron acceptor in the Photosystem I reaction centre of spinach chloroplasts and its interaction with P700 and the bound ferredoxin in various oxidation−reduction states, *Biochem. J. 158*, 71−77.
42. Malkin, R. (1996) Photosystem I electron transfer reactions: Components and kinetics, in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 313−332, Kluwer Academic Publishers, Dordrecht, The Netherlands.
43. Smit, H. W. J., Amesz, J., and van der Hoeven, M. F. R. (1987) Electron transport and triplet formation in membranes of the photosynthetic bacterium *Heliobacterium chlorum*, *Biochim. Biophys. Acta 893*, 232−240.
44. Prince, R. C., Gest, H., and Blankenship, R. E. (1985) Thermodynamic properties of the photochemical reaction center of *Heliobacterium chlorum*, *Biochim. Biophys. Acta 810*, 377−384.
45. Golbeck, J. H., and Bryant, D. A. (1991) *Photosystem I*, Vol. 16, Academic Press, Inc., San Diego.
46. Nitschke, W., Feiler, U., and Rutherford, A. W. (1990) Photosynthetic reaction center of green sulfur bacteria studied by EPR, *Biochemistry 29*, 3834−3842.
47. Oh-oka, H., Takahashi, Y., Kuriyama, K., Saeki, K., and Matsubara, H. (1988) The protein rersponsible for center A/B in spinach photosystem I: Isolation with iron−sulfur cluster(s) and complete sequence analysis, *J. Biochem. 103*, 962−968.
48. Oh-oka, H., Iwaki, M., Miyamoto, R., Mino, H., and Itoh, S. (2005) in *Photosynthesis: Fundamental Aspects to Global Perspectives* (van der Est, A., and Bruce, D., Eds.) pp 50−52, Allen Press, Lawrence, KS.
49. Oh-oka, H., Kakutani, S., Matsubara, H., Malkin, R., and Itoh, S. (1993) Isolation of the photoactive reaction center complex that contains three types of Fe-S centers and a cytochrome *c* subunit

from the green sulfur bacterium *Chlolobium limicola* f. *thiosulfatophilum*, strain Larsen, *Plant Cell Physiol. 34*, 93−101.
50. Hager-Braun, C., Jarosch, U., Hauska, G., Nitschke, W., and Riedel, A. (1997) EPR studies of the terminal electron acceptors of the green sulfur bacterial reaction centre. Revisited, *Photosynth. Res. 51*, 127−136.
51. Vassiliev, I. R., Ronan, M., Hauska, G., and Golbeck, J. (2000) The bound electron acceptors in green sullfur bacteria: Resolution of the *g*-tensor for the F_X iron−sulfur cluster in *Chlorobium tepidum*, *Biophys. J. 78*, 3160−3169.
52. Warden, J. T., and Golbeck, J. H. (1986) Photosystem I charge separation in the absence of centers A and B. II. ESR spectral characterization of center 'X' and correlation with optical signal 'A_2', *Biochim. Biophys. Acta 849*, 25−31.
53. Smit, H. W. T., van Dorssen, R. J., and Amesz, J. (1989) Charge separation and trapping efficiency in membranes of *Heliobacterium chlorum* at low temperature, *Biochim. Biophys. Acta 973*, 212−219.
54. Evans, M. C., and Heathcote, P. (1980) Effects of glycerol on the redox properties of the electron acceptor complex in spinach photosystem I particles, *Biochim. Biophys. Acta 590*, 89−96.
55. Parrett, K. G., Mehari, T., Warren, P. G., and Golbeck, J. H. (1989) Purification and properties of the intact P-700 and F_x-containing Photosystem I core protein, *Biochim. Biophys. Acta 973*, 324−332.
56. van Kan, P. J. M., Aartsma, T. J., and Amesz, J. (1989) Primary photosynthetic processes in *Heliobacterium chlorum* at 15 K, *Photosynth. Res. 22*, 61−68.
57. Kobayashi, M., Oh-oka, H., Akutsu, S., Akiyama, M., Tominaga, K., Kise, H., Nishida, F., Watanabe, T., Amesz, J., Koizumi, M., Ishida, N., and Kano, H. (2000) The primary electron acceptor of green sulfur bacteria, bacteriochlorophyll 663, is chlorophyll a esterified with Δ2,6-phytadienol, *Photosynth. Res. 63*, 269−280.
58. Nuijs, A. M., Vasmel, H., Joppe, H. L. P., Duysens, L. N. M., and Amesz, J. (1985) Excited states and primary charge separation in the pigment system of the green photosynthetic bacterium *Prosthecochloris aestuarii* as studied by picosecond absorbance difference spectroscopy, *Biochim. Biophys. Acta 807*, 24−34.
59. Shuvalov, V. A., Amesz, J., and Duysens, L. N. M. (1986) Picosecond spectroscopy of isolated membranes of the photosynthetic green sulfur bacterium *Prosthecochloris aestuarii* upon selective excitation of the primary electron donor, *Biochim. Biophys. Acta 851*, 1−5.
60. Kumazaki, S., Iwaki, M., Ikegami, I., Kandori, H., Yoshihara, K., and Itoh, S. (1994) Rates of primary electron transfer reactions in the photosystem I reaction center reconstituted with different quinones as the secondary acceptor, *J. Phys. Chem. 98*, 11220−11225.
61. Hastings, G., Kleinherenbrink, F. A. M., Lin, S., McHugh, T. J., and Blankenship, R. E. (1994) Observation of the reduction and reoxidation of the primary electron acceptor in photosystem I, *Biochemistry 33*, 3193−3200.
62. Parrett, K. G., Mehari, T., and Golbeck, J. H. (1990) Resolution and reconstitution of the cyanobacterial photosystem I complex, *Biochim. Biophys. Acta 1015*, 341−352.
63. Hoshina, S., Sakurai, R., Kunishima, N., Wada, K., and Itoh, S. (1990) Selective destruction of iron−sulfur centers by heat/ethylene glycol treatment and isolation of photosystem I core complex, *Biochim. Biophys. Acta 1015*, 61−68.
64. Heathcote, P., Jones, M. R., and Fyfe, P. K. (2003) Type I photosynthetic reaction centres: Structure and function, *Philos. Trans. R. Soc. London, Ser. B 358*, 231−243.
65. Baymann, F., Brugna, M., Muhlenhoff, U., and Nitschke, W. (2001) Daddy, where did (PS)I come from? *Biochim. Biophys. Acta 1507*, 291−310.
66. Moser, C. C., and Dutton, P. L. (1992) Engineering protein structure for electron transfer function in photosynthetic reaction centers, *Biochim. Biophys. Acta 1101*, 171−176.
67. Marcus, R. A., and Sutin, N. (1985) Electron transfers in chemistry and biology, *Biochim. Biophys. Acta 811*, 265−322.
68. Diner, B. A., and Babcock, G. T. (1996) Structure, dynamics, and energy conversion efficiency in photosystem II, in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 213−247, Kluwer Academic Publishers, Dordrecht, The Netherlands.

BI0519710