A New Cytochrome Subunit Bound to the Photosynthetic Reaction Center in the Purple Bacterium, *Rhodovulum sulfidophilum*

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The nucleotide sequence of the *puf* operon, which contains the genes encoding the B870 light-harvesting protein and the reaction center complex of the purple photosynthetic bacterium, *Rhodovulum sulfidophilum*, was determined. The operon, which consisted of six genes, *pufQ*, *pufB*, *pufA*, *pufL*, *pufM*, and *pufC*, is a new variety in photosynthetic bacteria in the sense that *pufQ* and *pufC* coexist. The amino acid sequence of the cytochrome subunit of the reaction center deduced from the *pufC* sequence revealed that this cytochrome contains only three possible heme-binding motifs; the heme-1-binding motif of the corresponding tetraheme cytochrome subunits was not present. This is the first exception of the "tetraheme" cytochrome family in purple bacteria and green filamentous bacteria. The *pufC* sequence also revealed that the sixth axial ligands to heme-1 and heme-2 iron were not present in the cytochrome either. This cytochrome was actually detected in membrane preparation as a 43-kDa protein and shown to associate functionally with the photosynthetic reaction center as the immediate electron donor to the photo-oxidized special pair of bacteriochlorophyll. This new cytochrome should be useful for studies on the role of each heme in the cytochrome subunit of the bacterial reaction center and the evolution of proteins in photosynthetic electron transfer systems.

The photosynthetic pigment-protein system of purple bacteria consists of a reaction center (RC) complex and two light-harvesting complexes, LH1 and LH2. The light energy captured by LH1 and LH2 is transferred to the RC, where the primary photochemical reaction takes place. Two types of RC are known in purple bacteria. One has a tightly bound subunit of a c-type cytochrome at the periplasmic side that donates electrons to the photo-oxidized RC core complex. The other does not have the cytochrome subunit and accepts electrons directly from water-soluble electron carriers such as cytochrome *c*<sub>2</sub> (1–4). A three-dimensional structure of the RC of *Blastochloris* (formerly called *Rhodospseudomonas*) *viridis* showed that the cytochrome subunit has four c-type hemes aligned along the long axis of this subunit (5). These four hemes are distinguishable in terms of the peak wavelengths of the α-bands and the redox midpoint potentials in *B. viridis*. It has been shown that the hemes are arranged sequentially with high-low-high-low midpotentials from the special pair of bacteriochlorophylls in the LM core, the core part of the reaction center complex composed of L and M subunits and cofactors (6–8). This alternate arrangement of hemes seems to be conserved through the cytochrome subunits of various purple bacteria, although its significance in the function has not been clarified (4).

Amino acid sequences of the cytochrome subunits of various purple bacteria have been reported, the sequence identities among the subunits being over 40% (9). All of the sequences consistently conserve four heme-binding motifs (Cys-Xaa-Xaa-Cys-His) and methionine and histidine residues as the sixth axial ligands for the heme iron. The four heme-binding motifs were also conserved in a green filamentous bacterium, *Chloroflexus aurantiacus*, which is phylogenetically distant from purple bacteria (10). Thus, the cytochrome subunit has often been called a "tetraheme cytochrome."

When hemes are numbered according to the order in the amino acid sequence from the N terminus, the four hemes of the cytochrome subunit are arranged in the structure of the *B. viridis* RC in the order of heme-3, heme-4, heme-2, and heme-1 from the special pair in the membrane (11). Recently, we showed direct evidence through mutagenesis on the cytochrome subunit of *Rubrivivax gelatinosus* that electron transfer to the cytochrome subunit from soluble cytochromes occurred via electrostatic interactions between negatively charged amino acids surrounding heme-1 and positively charged amino acids on the soluble cytochromes (12), which is consistent with a suggestion by Knaff et al. (13). These charged residues on the cytochrome subunit are well conserved among many purple bacteria so far examined, suggesting that the most distant heme-1 works as a direct electron acceptor from the soluble electron carriers (9). This indicates that all four hemes are involved in the electron transfer from the soluble carrier to the special pair.

The RC complexes of purple bacteria are known to consist, at least, of L, M, and H subunits. Light-harvesting (LH) complexes are composed of two membrane spanning polypeptides, α and β subunits, which bind bacteriochlorophyll and carotenoids. In purple photosynthetic bacteria, β and α polypeptides of the LH1 and the L and M polypeptides of RC are encoded by *pufB*, *pufA*, *pufL*, and *pufM* genes, respectively, which form an operon called "puf operon." The H polypeptide of RC is encoded by the *pufH* gene that is out of the *puf* operon (14–16). In species with the bound cytochrome subunit, the *pufC* gene coding for the RC-bound c-type cytochrome is located immediately downstream of *pufM* in the operon. Some species have other genes in their *puf* operons. *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* have the *pufQ* gene upstream of *pufB* and the *pufX* gene downstream of *pufM* (17–20). In *R. gelati-
genes have been reported only in two \textit{Rhodobacter} species do not contain this subunit (1, 17–19). \textit{R. denitrificans} contains the cytochrome subunit (2, 3, 25), although \textit{Roseobacter denitrificans} contains the \textit{bchZ} gene (17–20). Why species in classes \textit{Rhodovulum} and the \textit{3} subclass show such varied structures of RCs and putative \textit{puf} operons has not been determined yet.

In the present study, we determined the nucleotide sequence of the \textit{puf} operon of a \textit{purple nonsulfur bacterium, \textit{Rhodovulum sulfidophilum}}. Results indicate that this bacterium has a unique RC-bound cytochrome subunit that has only three heme-binding motifs, one of which, in addition, lacks the amino acid residue functioning as the sixth ligand for the heme iron.

**EXPERIMENTAL PROCEDURES**

**Media and Growth Conditions**—Cells of \textit{Rhodobacter sulfidophilum} and \textit{R. sphaeroides} were grown photosynthetically at 30 °C in screw-capped bottles filled with a \textit{PYS medium}, as described by Nagashima et al. (26).

For \textit{R. sulfidophilum}, the \textit{PYS medium} was supplemented with 0.35 M sodium chloride. Cells of \textit{Rhodovulum sulfidophilum} were grown aerobically in the \textit{PYS medium}, supplemented with 0.35 M sodium chloride, 1 mM EDTA, and 100 mM sodium acetate, pH 7.8, supplemented with 100 mM sodium chloride, 1 mM EDTA, and 1 mM phenylmethylsulfon fluoride. Cells were disrupted with sonication to screen the whole \textit{puf} operon of \textit{R. sulfidophilum} (Fig. 1, probe A). Nine positive clones were selected from the cosmid library. Inserted DNA fragments in one of the nine cosmids vectors were digested with EcoRI and screened by Southern blot hybridization using the same probe as described in the cosmid screening. An approximately 10-kb DNA fragment giving a positive signal was identified and cloned into the plasmid pUC118, being named pUFS101. This plasmid was used as the template for DNA sequencing, as described below. DNA manipulation, colony hybridization, Southern blot hybridization, and plasmid isolation were carried out according to a manual of molecular cloning.

**DNA Sequencing**—Sequencing of pUFS101 (see "Screening and Cloning of the \textit{puf} Genes") was performed using a Dye Terminator Cycle Sequencing kit and a 310A DNA Sequencer or a 377A DNA Sequencer (Applied Biosystems). Oligonucleotides designed to generate overlapping DNA sequences to complete the DNA sequence analysis (primer walking) were ordered from Life Technologies, Inc. The DNA sequences were analyzed using the DNASIS program (Hitachi).

**Extraction of RNA and Northern Hybridization**—The total RNA of \textit{R. sulfidophilum} was extracted with a RNeasy kit (QIAGEN). Electrophoresis of the total RNA of \textit{R. sulfidophilum} was performed in 1.2% agarose gels containing formaldehyde (40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA, and pH 7.0). After electrophoresis, the RNA was transferred to the positively charged nylon membranes (Boehringer Mannheim). The probe used for hybridization was the polymerase chain reaction product used for colony hybridization to screen the whole \textit{puf} operon of \textit{R. sulfidophilum} (Fig. 1, probe A) or the 1.2-kb DNA fragment corresponding to the \textit{puf} operon of \textit{R. sulfidophilum} (Fig. 1, probe B). The DNA fragment was labeled with digoxigenin-dUTP as instructed by the manufacturer (Boehringer Mannheim). RNA Molecular Weight Marker I (Boehringer Mannheim) was used as a molecular weight standard. Hybridization was carried out according to a manual of molecular cloning.

**Preparation of Membrane Samples**—Cells of \textit{R. sphaeroides} were harvested by centrifugation and washed once with distilled water. Cells of \textit{R. sulfidophilum} and \textit{R. denitrificans} were harvested by centrifugation and washed once with 100 mM sodium chloride. Washed cells were then centrifuged and suspended in a 25 mM sodium phosphate buffer, pH 7.8, supplemented with 100 mM sodium chloride, 1 mM EDTA, and 1 mM phenylmethylsulfon fluoride. Cells were disrupted with sonication and treated with DNaseI. Membrane fragments were collected by a method of differential centrifugation as a sedimented fraction between 7000 × g for 20 min and 280,000 × g for 20 min. To obtain membrane preparations free of soluble electron carrier proteins, the membrane preparations were suspended in a 25 mM sodium phosphate buffer, pH 7.8, supplemented with 100 mM sodium chloride and 0.01% Triton X-100 and centrifuged at 280,000 × g for 20 min and then resuspended in the same buffer.

**Detection of Heme-containing Proteins in Membrane Preparations**—SDS-PAGE was carried out according to Laemmli (30). Heme staining was performed by the method of Thomas et al. (31).
Flash-induced absorbance change spectrophotometry—The absorbance changes due to the photo-oxidation of cytochromes induced by xenon flash illumination in the membrane preparations free of soluble electron carrier proteins were recorded with a single beam spectrophotometer, as described previously (32).

RESULTS

Structure of the puf Operon of R. sulfidophilum—A 10-kb DNA fragment showing a positive hybridizing signal to a polymerase chain reaction product containing R. sulfidophilum pufB, pufA, and pufL genes (Fig. 1, probe A) was cloned into pUC118 and named pUFS101. The 5.4-kb region in the inserted DNA fragment was sequenced and analyzed, as shown in Fig. 2. The nucleotide sequence had six ORFs, each of which had a consensus Shine-Dalgarno sequence, GGAG (one GAGG), preceding the start codon, ATG. Comparisons with the puf genes of other photosynthetic bacteria revealed that five of the

FIG. 2. The nucleotide sequences of puf genes and the deduced amino acid sequences of the products of R. sulfidophilum. The putative ribosome-binding sites are underlined. The stop codons are indicated by asterisks. Head-to-head arrows indicate presumed hairpin structures giving minimum free energies in the respective regions.

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six ORFs were \( \text{pufB, pufA, pufL, pufM, and pufC} \), which encode the \( \beta \) and \( \alpha \) subunits of the LH1 light-harvesting complex, and the L, M, and cytochrome subunits of the RC complex, respectively. The amino acid sequence of the remaining ORF upstream of \( \text{pufB} \) showed significant sequence identities to those of \( \text{pufQ} \) gene products of \( \text{R. capsulatus} \) and \( \text{R. sphaeroides} \), as shown in Fig. 3. The ORF was identified as \( \text{pufQ} \), because it encodes a protein with 73 amino acids showing 37 and 38% identities to the \( \text{pufQ} \) gene products of \( \text{R. capsulatus} \) and \( \text{R. sphaeroides} \), respectively. The role of this gene product has not been fully clarified yet but has been suggested to be involved in the assembly of pigment-protein complexes and bacteriochlorophyll biosynthesis (33, 34).

The upstream region of the \( \text{pufQ} \) gene of \( \text{R. sulfidophilum} \) contained a nucleotide sequence showing significant sequence identity to \( \text{bchZ} \), which encodes an enzyme for bacteriochlorophyll biosynthesis found in many purple bacteria (21, 35–38). An incompletely sequenced ORF found downstream of \( \text{pufC} \) in \( \text{R. sulfidophilum} \) showed a high identity to the 5' region of ORF 641 encoding the \( \beta \)-chain of pyruvate dehydrogenase, which is located downstream of the \( \text{puf operon} \) in \( \text{R. capsulatus} \) and \( \text{R. denitrificans} \) (DDBJ, EMBL, and GenBank accession numbers Z11165 and X83392, respectively). Two putative hairpin structures were found between \( \text{pufC} \) and this ORF (Fig. 2). One of these structures had a 10-base pair stem part with a calculated free enthalpy of \(-28.1\) kcal/mol followed by poly(T) residues. These findings indicate that the \( \text{puf operon of R. sulfidophilum} \) is terminated after the \( \text{pufC} \) gene, and no other ORFs were found in \( \text{R. sulfidophilum} \) \( \text{puf operon} \), leading to the conclusion that the \( \text{puf operon in this species is constructed with an order of pufQ, pufB, pufA, pufL, pufM, and pufC} \), the combination of which has not been reported previously in other purple bacteria.

Two additional putative hairpin loop structures were found between \( \text{pufQ} \) and \( \text{pufB} \) and \( \text{pufA} \) and \( \text{pufL} \) (Fig. 2). The location of these two hairpin-loop structures are the same as those in the \( \text{puf operon of R. capsulatus} \) (Fig. 1) (17). The hairpin loop between \( \text{pufA} \) and \( \text{pufL} \) has been suggested to work as an mRNA decay terminator for the 5'-3' exonuclease activity, providing the necessary mRNA stability for the proper functioning of the \( \text{puf operon} \) (39–41).

Lack of a Heme-1-binding Motif in the Cytochrome Subunit—A \( \text{pufC} \) gene coding for the cytochrome subunit of RC was found in \( \text{R. sulfidophilum} \) \( \text{puf operon} \) (Figs. 1 and 2). The deduced amino acid sequence of \( \text{PufC} \) in \( \text{R. sulfidophilum} \) consisted of 356 amino acids with the calculated molecular weight of 39,145. The protein had the highest similarity to its homologue of \( \text{R. denitrificans} \) (40% identity). An amino acid sequence alignment of the cytochrome subunits of \( \text{R. sulfidophilum} \) and various purple bacteria is shown in Fig. 4. Surprisingly, one of the four conserved heme-binding motifs (Cys-Xaa-Xaa-Cys-His), corresponding to heme-1 in the tetraheme subunit of other species, was not detected in \( \text{R. sulfidophilum} \), whereas three other possible heme-binding sites were conserved. Only in this bacterium, methionine residues functioning as the axial ligands to the first heme and the second heme iron (positions 118 and 157, respectively) (5) were not conserved either.

Gene Coding for LH1 and L and M Subunits of RC in \( \text{R. sulfidophilum} \)—The putative \( \alpha \) and \( \beta \) subunits of LH1 were composed of 54 and 48 amino acid residues, respectively, and showed the highest identities, exceeding 70%, with \( \text{R. capsulatus} \). This subunits contained almost all amino acid residues commonly conserved in the corresponding polypeptides of other purple bacteria, including the histidine residues (32nd and 39th of the \( \alpha \) and \( \beta \) subunits, respectively) presumed to bind bacteriochlorophylls (42). The alignment of the C-terminal amino acid sequences of M subunits from various purple bacteria is shown in Fig. 5. The additional 17–20 amino acids at the C terminus of the M subunit have been reported only in bacteria having RC-bound cytochrome subunits and are thought to contribute to the binding between the cytochrome subunit and the LM core (4). \( \text{R. sulfidophilum} \) had the additional C-terminal sequence of the M subunit as well, although it was a little shorter than the others.

Analysis of Transcripts—Because the gene combination of the \( \text{puf operon of R. sulfidophilum} \) was revealed to be different from those of other purple bacteria (Fig. 1) and the gene coding for the RC-bound cytochrome was unique, as described above, we performed Northern hybridization experiments to identify the transcripts of this \( \text{puf operon} \). Results are shown in Fig. 6. The total RNA was extracted from photosynthetically grown cells. Two probes were used for Northern hybridization (Fig. 1, probes A and B). One of the two probes corresponding to \( \text{pufQBA} \) and the part of \( \text{pufL} \) (probe A, Fig. 6, lane 1) was hybridized strongly with a 0.6-kb band and weakly with an approximately 4.5-kb band. Another probe corresponding to \( \text{pufC} \) (probe B, Fig. 6, lane 2) was only hybridized with the approximately 4.5-kb band. In the \text{Rhodobacter} species, the transcript corresponding to \( \text{pufQ} \) was detected with the specific probes to the \( \text{pufQ} \) gene, and its band was almost the same in size as the \( \text{pufBA} \) transcript (34). The 0.6-kb band in Fig. 6, therefore, was likely to contain both the \( \text{pufQ} \) and the \( \text{pufBA} \) transcripts. The 4.5-kb transcript probably includes the whole \( \text{puf operon, pufQ, pufB, pufA, pufL, pufM, and pufC} \). The 0.6-kb transcripts were more abundant than the 4.5-kb transcript. This difference may be due to abundance in \( \text{pufBA} \) transcripts, a factor thought to adjust the ratio of LH1 peptides to RC proteins (39, 40).

Detection of the RC-bound Cytochrome in Membrane Preparations—Membrane proteins from \( \text{R. sulfidophilum} \) and from phylogenetically related species, \( \text{R. denitrificans} \) and \( \text{R. sphaeroides} \), were subjected to SDS-PAGE, and proteins containing c-type cytochromes were specifically stained (Fig. 7). The band at 43.1 kDa in \( \text{R. sulfidophilum} \) corresponds to the RC-bound cytochrome (lane 1). A similar band at 48.3 kDa was observed in \( \text{R. denitrificans} \) (lane 2) but not in \( \text{R. sphaeroides} \) (lane 3), consistent with the presence of the RC-bound cytochrome in the former two species and its absence in the last species (4, 24). Bands seen at 31.6, 35.7, and 34.5 kDa in lanes 1, 2, and 3, respectively, correspond to cytochrome \( \text{c1} \) in the cytochrome \( \text{bC} \) complex.

Photo-oxidation of the RC-bound Cytochrome—The flash-induced absorbance changes in the \( \alpha \)-band region of c-type cytochromes were observed in membrane preparations from \( \text{R. sulfidophilum} \) and the related species (Fig. 8). The absence of soluble cytochromes in the preparation was ensured by treatment with a salt and a detergent (see “Experimental Procedures”). Fast photo-oxidation of the RC-bound cytochrome in \( \text{R. sulfidophilum} \) and \( \text{R. denitrificans} \) was observed as absorbance
decreased at 554–540 nm, which is a characteristic feature of the RC-bound cytochrome subunit (Fig. 8, traces a and b). The transient spectra of cytochrome photo-oxidation are clearly seen in Fig. 8B for R. sulfidophilum (circles) and R. denitriicans (triangles). On the other hand, no photo-oxidation of cytochromes was seen in the kinetics and spectrum of R. sphaeroides (Fig. 8, A, trace c, and B, squares).

**DISCUSSION**

In this study, we found new characteristics in the nucleotide sequence of the puf operon of R. sulfidophilum and confirmed the presence and function of the product of a unique cytochrome gene. The R. sulfidophilum puf operon contained, from upstream, pufQ, pufB, pufA, pufL, pufM, and pufC genes, the combination of which has not been reported in other purple bacteria investigated so far in the sense that both pufQ and pufC are present in the operon. The amino acid sequence alignment of the RC-bound cytochrome subunits of R. sulfidophilum and various purple bacteria revealed that the heme-1-binding site (Fig. 4, position 131–135) is not conserved in R. sulfidophilum, although three other possible heme-binding sites were observed. Methionine residues at positions 118 and 157, which are thought to be the axial ligands to heme-1 and heme-2 irons, respectively, were not conserved either (Fig. 4). No alternatives for the heme-1-binding site and the two ligands were found in the sequence. Therefore, only two heme-binding sites bear similarity to those of the tetraheme cytochrome subunits in other purple bacteria in addition to the unusual heme-2-binding site.

No. 5. Alignment of the C-terminal amino acid sequences of M subunit of reaction center complex. The upper five organisms have RC-bound cytochrome subunits. The lower three organisms do not have the cytochrome.

**Fig. 6.** Northern hybridization analysis of total RNA from R. sulfidophilum with probes specific for the puf DNA region. Total RNA was extracted from R. sulfidophilum grown photosynthetically. The digoxigenin-dUTP-labeled probe A (lane 1) and probe B (lane 2) shown in Fig. 1 were used. The lengths of the standard RNAs are indicated on the left.
from the RC-bound cytochrome subunit.

The SDS-PAGE analysis in combination with the heme-staining method (Fig. 7) indicates that pufC in R. sulfidophilum is indeed translated in vivo and the product is integrated into the membrane. Furthermore, the RC-bound cytochrome in the membrane of R. sulfidophilum is photoactive, as shown by the flash-induced absorbance changes (Fig. 8). The cytochrome subunit is presumed to accept electrons from water-soluble cytochromes and to transfer them to the photooxidized RC core complex.

It has been shown that electron transfer reactions from soluble electron donors to the cytochrome subunit are controlled by charge interactions (12, 13). The study of site-directed mutagenesis in R. gelatinosus has shown that negatively charged amino acids (Glu) surrounding the heme-1 (positions 82, 113, and 129 in Fig. 4), which are well conserved among purple bacteria, have a stimulative effect on the rate of electron transfer, suggesting that the heme-1 of the RC-bound cytochrome subunit is a direct electron acceptor from soluble electron donors in purple bacteria (9, 12). The absence of a heme-1-binding domain in R. sulfidophilum suggests that the site of interaction with soluble cytochromes is different from that in usual purple bacteria. This idea is supported by the charge distribution on the surface of the cytochrome subunit, because the above-mentioned three glutamate residues that are suggested to be important for the interaction are not conserved in R. sulfidophilum (Fig. 4). These observations suggest that the electron transfer between the cytochrome subunit and soluble electron donors does not occur on the surface around the heme-1 but may occur around the other hemes of the cytochrome subunit in R. sulfidophilum. An unidentified interaction site on the cytochrome subunit will be revealed by the method of site-directed mutagenesis, as has been done in R. gelatinosus (12).

The physiological significance of the cytochrome subunit in RC is still unclear, because some species of purple bacteria lack this subunit. Until now, the following properties of the subunit have been shown: 1) the four hemes are arranged sequentially with high-low-high-low midpoint potentials; 2) the subunit can reduce the photo-oxidized special pair of bacteriochlorophylls faster than the soluble cytochromes; and 3) the heme-1 of the cytochrome is a site involved in the electron flow from soluble electron carriers, indicating that all four hemes of the subunit are likely to be involved in electron transfer toward the photo-oxidized special pair of bacteriochlorophylls (12, 13). The existence of a cytochrome subunit containing only three hemes, including one unusual heme in R. sulfidophilum, suggests that all four hemes and the arrangement of high-low-high-low midpoint potentials are not essential requirements for the functions of the subunit.

We have previously reported that a R. gelatinosus mutant lacking the cytochrome subunit is able to grow photosynthetically (43). Possibly, the main role of the cytochrome subunit is to reduce the photo-oxidized special pair of bacteriochlorophyll fast enough to avoid the electron backflow (“back reaction”) from the ubiquinone to the oxidized special pair. Rhodobacter species do not have the pufC gene coding for the subunit, having a pufX gene at that position instead (Fig. 1). Because the PufX has been suggested to be involved in efficient electron transfer from the RC to the bc1 complex (19, 20), it may also reduce the back reaction. Thus, PufX may be a functional alternative of the cytochrome subunit in photosynthetic electron transport in the Rhodobacter species. However, some purple photosynthetic bacteria, at least Rhodospirillum rubrum, have neither pufC nor pufX genes in the puf operon. This bacterium may have other systems to reduce the possibilities of back reaction.

It should be noted that the pufQ gene was found in R. sulfidophilum puf operon that had been detected only in the Rhodobacter species (Fig. 1). This gene product was suggested to be an integral membrane protein involved in the assembly of pigment-protein complexes and bacteriochlorophyll biosynthetic process.
s (33, 34). The hydropathy profile of the puFQ gene product of \textit{R. sulfidophilum} showed high similarities to those of \textit{R. capsulatus} and \textit{R. sphaeroides} (data not shown). Characterization of puFQ gene in \textit{R. sulfidophilum} would be useful for further understanding of its role.

Finally, the study presented here clearly demonstrated that \textit{R. sulfidophilum} utilizes a unique RC-bound cytochrome subunit that contains only three heme-binding sites. Our preliminary experiments of the membrane redox titration showed the unique characteristic of the subunit in that the redox potentials of these three hemes were $-380$, $-20$, and $+360$ mV, the middle one showing an unusual absorbance spectrum. Further biochemical and biophysical studies of this cytochrome subunit will help us to understand not only the physiological significance of the RC-bound cytochrome subunit but also the evolution of RC complexes and electron transfer systems in photosynthetic bacteria.

REFERENCES

1. Dutton, P. L., and Prince, R. C. (1978) in \textit{The Photosynthetic Bacteria: Reaction-center-driven Cytochrome Interactions in Electron and Proton Translocation and Energy Coupling} (Clayton, R. K., and Sistrom, W. R., eds) pp. 525–570. Plenum Press, New York

2. Matsuura, K., and Shimada, K. (1990) in \textit{The Photosynthetic Bacteria: Reaction Center Associated Cytochromes} (Blankenship, R. E., Madigan, M. T., and Bauer C. E., eds) pp. 775–805, Kluwer Academic Publishing, Dordrecht, The Netherlands

3. Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985) \textit{Nature} \textbf{318}, 618–624

4. Dracheva, S. M., Drachev, L. A., Konstantinov, A. A., Semenov, A. Y., Skulachev, V. P., Arutjunjan, A. M., Shuvalev, V. A., and Zaberezhnaya, S. M. (1988) \textit{Eur. J. Biochem.} \textbf{171}, 253–264

5. Vermeglio, A., Richaud, P., and Breton, J. (1989) \textit{FEBS Lett.} \textbf{245}, 259–263

6. Alegría, G., and Dutton, P. L. (1991) \textit{Biochim. Biophys. Acta} \textbf{1057}, 239–257

7. Nagashima, K. V. P., Sogabe, S., Miki, K., Yoshida, M., Shimada, K., and Matsura, K. (1995) \textit{Photosynth. Res.} \textbf{55}, 349–355

8. Dracheva, S., Williams, J. A., Van Driessche, G., Van Beemen, J. J., and Blankenship, R. E. (1991) \textit{Biochemistry} \textbf{30}, 11451–11458

9. Weyer, K. A., Lottspeich, F., Gruenenberg, H., Lang, F., Oesterhelt, D., and Michel, H. (1987) \textit{EMBO J.} \textbf{6}, 2197–2202

10. Oszyczka, A., Nagashima, K. V. P., Sogabe, S., Miki, K., Yoshida, M., Shimada, K., and Matsura, K. (1998) \textit{Biochemistry} \textbf{37}, 11732–11744

11. Knafl, D. B., Willie, A., Long, J. E., Kriasucianas, A., Durham, B., and Millett, F. (1991) \textit{Biochemistry} \textbf{30}, 1303–1319

12. Vagyv, D. C., Bylina, E. J., Alberti, M., Begusch, H., and Hearst, J. E. (1984) \textit{Cell} \textbf{37}, 949–957

13. Michel, H., Weyer, K. A., Gruenenberg, H., Dunger, I., Oesterhelt, D., and Lottspeich, F. (1986) \textit{EMBO J.} \textbf{5}, 1149–1158

14. Bauer, C. E. (1995) in \textit{Anoxygenic Photosynthetic Bacteria: Regulation of Photosynthesis Gene Expression} (Blankenship, R. E., Madigan, M. T., and Bauer C. E., eds) pp. 1221–1234, Kluwer Academic Publishing, Dordrecht, The Netherlands

15. Bauer, C. E., Young, D. A., and Marrs, B. L. (1988) \textit{J. Biol. Chem.} \textbf{263}, 4820–4827

16. Davis, J., Donohue, T. G., and Kaplan, S. (1988) \textit{J. Bacteriol.} \textbf{170}, 320–329

17. Farchaus, J. W., Gruenberg, H., and Oesterhelt, D. (1990) \textit{J. Bacteriol.} \textbf{172}, 767–785

18. Dilburn, T. G., Haith, C. E., Prince, R. C., and Beatty, J. T. (1992) \textit{Biochim. Biophys. Acta} \textbf{1100}, 160–170

19. Nagashima, K. V. P., Matsuura, K., Ohyama, S., and Shimada, K. (1994) \textit{J. Biol. Chem.} \textbf{269}, 2477–2484

20. Nagashima, K. V. P., Matsuura, K., Wakaio, N., Hiraishi, A., and Shimada, K. (1997) \textit{Plant Cell Physiol.} \textbf{38}, 1249–1258

21. Woese, C. R. (1987) \textit{Microbiol. Rev.} \textbf{51}, 221–271

22. Hirota, A., and Ueda, Y. (1984) \textit{Int. J. Syst. Bacteriol.} \textbf{44}, 15–23

23. Kortlake, C., Breese, K., Gao, N., Labahn, A., and Dews, G. (1997) \textit{J. Bacteriol.} \textbf{179}, 5247–5258

24. Nagashima, K. V. P., Hiraishi, A., Shimada, K., and Matsuura, K. (1997) \textit{J. Mol. Biol.} \textbf{45}, 131–136

25. Shini, Y. (1986) \textit{Plant Cell Physiol.} \textbf{27}, 567–572

26. Masuda, S., Matsunoto, Y., Nagashima, K. V. P., Shimada, K., Inoue, K., Bauer, C. E., and Matsuura, K. (1998) in \textit{Proceedings of Xth International Congress on Photosynthesis}, Kluwer Academic Publishing, Dordrecht, The Netherlands, in press

27. Mänttäri, T., Fritsch, F. P., and Sambrook, J. (1989) \textit{Molecular Cloning: A Laboratory Manual}, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

28. Laemmli, U. K. (1970) \textit{Nature} \textbf{227}, 680–685

29. Thomas, P. E., Ryan, D., and Levin, W. (1976) \textit{Anal. Biochem.} \textbf{75}, 168–176

30. Matsuura, K., Fukushima, A., Shimada, K., and Satoh, T. (1988) \textit{FEBS Lett.} \textbf{237}, 21–25

31. Bauer, C. E., and Marrs, B. L. (1988) \textit{Proc. Natl. Acad. Sci. U.S.A.} \textbf{85}, 9774–9778

32. Nagashima, K. V. P., Matsuura, K., and Shimada, K. (1994) \textit{J. Bacteriol.} \textbf{173}, 2946–2961

33. Taylor, D. P., Cohen, S. N., Clark, W. G., and Marrs, B. L. (1985) \textit{J. Bacteriol.} \textbf{154}, 580–590

34. Wiessner, C., Dunger, I., and Michel, H. (1990) \textit{J. Bacteriol.} \textbf{172}, 2877–2887

35. Lieder, R., Hornberger, U., and Dews, G. (1991) \textit{Mol. Microbiol.} \textbf{5}, 1459–1468

36. Nagashima, K. V. P., Matsuura, K., and Shimada, K. (1996) \textit{Photosynth. Res.} \textbf{50}, 61–70

37. Zhu, Y. S., Wiley, P. J., Donohue, T. J., and Kaplan, S. (1994) \textit{J. Bacteriol.} \textbf{176}, 10366–10374

38. Klug, G., Adams, C. W., Belaske, J., Doerge, B., and Cohen, S. N. (1987) \textit{EMBO J.} \textbf{6}, 3515–3520

39. Heck, C., Rothfus, R., Jager, A., Rauthut, R., and Klug, G. (1996) \textit{Mol. Microbiol.} \textbf{20}, 1165–1178

40. Zuber, H., and Cogdell, R. J. (1995) in \textit{Anoxygenic Photosynthetic Bacteria: Structure and Organization of Purple Bacterial Antenna Complexes} (Blankenship, R. E., Madigan, M. T., and Bauer C. E., eds) pp. 315–348, Kluwer Academic Publishing, Dordrecht, The Netherlands

41. Nagashima, K. V. P., Shimada, K., and Matsuura, K. (1996) \textit{FEBS Lett.} \textbf{385}, 209–213