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Isolation and Characterization of a Novel Membrane-bound Cytochrome c_{553} from the Strictly Anaerobic Phototroph, *Heliobacillus mobilis*

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Heliobacillus mobilis **is a strictly anaerobic Gram-positive bacterium which contains a primitive Photosystem I-type reaction center. The membrane-bound cytochrome c_{553} from the heliobacterium suggested to be the immediate electron donor to the photooxidized pigment (P 798+) has been isolated and characterized. The heme protein was visualized as a major component with an apparent molecular size of 17 kDa in TMBZ-staining analysis of the membrane preparation and showed characteristic α (552.5 nm), β (522 nm), and Soret absorption (416 nm) peaks of a typical reduced *c*-type cytochrome in the partially purified sample. The internal 43 amino acid sequence of the electron donor was obtained by chemical agent and protease treatments followed by N-terminal sequencing of the resulting fragments. The internal sequence carries lots of lysine residues and a Cys-X-X-Cys-His sequence motif which are the characteristics of typical *c*-type cytochromes. The analysis of the sequence by FAST or FASTA program, however, did not show any significant similarity to other known heme proteins.**

Key words: Isolation, characterization, novel membrane-bound *c*-type cytochrome, secondary electron donor, heliobacteria

It was shown that the reaction centers of photosynthetic bacteria can be grouped into two classes based on the natures of early electron acceptors. One class, found in purple bacteria and the green gliding bacterium *Chloroflexus aurantiacus*, is homologous to Photosystem II (PSII) in plants and cyanobacteria (1, 3, 6). The other class consists of the reaction centers found in heliobacteria and green sulfur bacteria. The newly discovered heliobacteria, together with green sulfur bacteria, have implied clues for understanding the evolution of the photosynthetic apparatus as primitive Photosystem I (PSI) models (2).

The secondary electron donor systems have also recently been recognized to be quite different in both types of photosynthetic bacteria. The ancestral PSII-type bacteria (the green gliding and purple bacteria) contain either a soluble *c*-type cytochrome or a reaction center associated with four heme *c* protein as the immediate electron donor to the photooxidized pigment in the reaction center (4, 16). A novel-type monoheme cytochrome c_{551} as the secondary electron donor has been identified in the preparation of the reaction center from *Chlorobium vibrioforme* (14) and a similar protein has been found in other green sulfur bacteria (13, 15). The report on the unique membrane-bound cytochrome has triggered a strong interest in the structure of the secondary electron donor in the other primitive PSI-type family, the Gram-positive heliobacteria which is distant from the Gram-negative green sulfur bacteria in 16S ribosomal RNA analysis (23).

A membrane-bound cytochrome c_{553} with a similar midpoint potential (+170 mV) to that of the green sulfur bacterial monoheme cytochrome has been suggested to be the immediate electron donor to the heliobacterial reaction center (17). A polypeptide with an apparent mass of 16 kDa, which is comparable to the 18 kDa (the calculated molecular size based on the amino acid sequence is 22.8 kDa) monoheme electron donor of *C. vibrioforme*, has been found as a major membrane-bound heme protein in the membrane preparation from *Helobacillus mobilis* (20). Therefore, the 16 kDa membrane-bound cytochrome has been suggested to be the electron donor to the reaction center in

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heliobacteria (14).

The recently described strictly anaerobic heliobacteria are the unique photosynthetic representatives of the Gram-positive bacteria. The previous studies on the heliobacteria have shown a strong character of the genus *Clostridium* (9, 22, 23). The 16S ribosomal RNA classification has indicated that the ancestral phenotype for Gram-positive bacteria was fermentative, which is similar to the strict anaerobes such as the clostridia with the deepest branches in the phylum. It has been, therefore, considered that the heliobacteria may imply a key to the evolutionary scenario of the phylum. Besides the special interest in the ancestral phenotypes of the heliobacteria in terms of Photosystem and the Gram-positive phylum, it seems that the evolutionary history of bacteria is becoming more elusive as structural information on cytochromes *c* from various organisms becomes more available.

In this report, the isolation and characterization of the membrane-bound cytochrome c_{553} from the heliobacterium are first described. Peptide mapping was carried out to obtain a partial sequence for future study on the complete primary structure of the protein using molecular biological techniques. The protein is indicated to be a novel *c*-type cytochrome based on the internal 43 amino acid sequence determined by peptide mapping.

Materials and Methods

Materials

H. mobilis was obtained from Dr. Blankenship at Arizona State University. Ammonium sulfate, sodium dodecyl sulfate (SDS), dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), cyanogen bromide, 3-bromo-3-methyl-2-(2-nitrophenyl mercapto)-3H-indole (BNPS-skatole), and phenyl-sepharose were purchased from Sigma Chemicals Co. DEAE-Biogel A and 3, 3', 5, 5'-tetramethylbenzidine (TMBZ) were obtained from Bio-Rad and Aldrich Chemical Co., respectively. All other chemicals were of the highest purity commercially available.

Cell growth

Cells of *H. mobilis* were anaerobically grown, harvested by centrifugation at 10,000× g, and stored at -20°C under nitrogen until used as previously described (7).

Membrane preparation

About 50 g of wet-packed cell paste was suspended in a total volume of 200 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM sodium ascorbate, 5 mM $MgCl_2$, 1 mM EDTA, and 5% glycerol. The cell suspension was cooled to 0°C on a salt ice bath and sonicated three times for 1 min each at a power setting of 8 on a Branson Model 350 sonifier. Just before the first and second sonication, 1 ml of the protease inhibitor PMSF (100 mM stock solution in isopropanol) was added to the cell solution. The cell debris and unbroken cells were removed by centrifugation at 10,000× g. The pelleted membrane proteins were obtained by ultracentrifugation at 200,000× g for two h in a Beckman 45 Ti rotor.

Isolation of cytochrome c_{553}

All isolation steps were carried out at 4°C or on an ice bath. The procedures are outlined in Fig. 1. The cytochrome c_{553} was followed by taking ascorbate reduced minus ferricyanide oxidized difference spectra.

The membrane fragments were resuspended adjusted to a final optical density of about 20 at 798 nm in 50 mM Tris-HCl pH 8.0 buffer containing 1% Deriphat detergent. 5 mM $MgCl_2$, 1 mM EDTA, 1 mM PMSF for the inhibition of proteases, and 5% glycerol for the stabilization of integral membrane proteins were added into the solubilization buffer. The solution was incubated at 4°C for 1 h with gentle shaking. The solubilized fraction was obtained by ultracentrifugation at 200,000× g for 2 h. The detergent extract was fractionated by ammonium sulfate. The remaining solution after 70% ammonium sulfate salting out which contained most of the cytochrome c_{553} (confirmed by SDS-PAGE followed by a specific stain for heme with TMBZ) was applied directly onto phenyl-sepharose hydrophobic interaction column (2.5× 20) previously equilibrated with 70% ammonium sulfate saturated buffer containing 0.05% Deriphat detergent. The column was washed with about 2 volumes of the equilibration buffer, and then the proteins were eluted by stepwise manner in the concentration of ammonium sulfate. The heme containing fraction was eluted with 50% saturated solution of ammonium sulfate. The heme fraction was dialyzed against about 10 volumes of 50 mM Tris-HCl pH 8.0 buffer containing 0.05% Triton X-100 for one day with two or three buffer changes. The dialyzed material was loaded on DEAE-Biogel A column (2.5× 30) equilibrated with the salt free 50 mM Tris-HCl buffer containing 0.05% Triton X-100. The proteins were developed by a gradient elution of 0 to 20 mM NaCl without washing the column. The reddish fraction was separated from the first eluting green fraction during the anionic exchange column chromatography. The reddish proteins showed the characteristic light absorptions of a typical *c*-type cy-

tochrome. Further purification of the cytochrome was attempted by gel filtrations with Sephadex G-100 or Sephacryl S-300 and DEAE Sephacel anion exchange, but the separations were not successful. During the attempts, the proteolytic degradation of the heme protein was observed. It seems that protease-like enzyme in the fraction becomes active or easy to contact with the membrane-bound cytochrome after the partial purification. Therefore, preparative SDS-PAGE was performed to get the pure protein. The heme protein was extracted by passive elution with 0.1% SDS containing $(NH_4)HCO_3$ solution from the SDS-PAGE band showing reddish color due to heme *c* groups covalently attached to the polypeptide. The isolated protein was stored frozen at -20°C until used.

Chemical and proteolytic cleavage of the cytochrome

One hundred molar excess of cyanogen bromide over the protein was added to the reaction mixture containing 70% formic acid. The reaction was carried out at room temperature in the dark for over night. For tryptophan-specific cleavage reaction, 100 µl of BNPS-skatole stock solution (5 mg/ml 50% acetic acid) was added to 100 µl of the protein (1 mg) solution containing 50% acetic acid. The reaction tube was covered with aluminum foil and incubated at room temperature for 24 h.

For Staphylococcus V_8 protease treatment, 200 µl (about 1 mg of the protein) of the ammonium bicarbonate solution containing the cytochrome was washed 4 or 5 times with 50 mM Tris-HCl pH 8.0 buffer containing 0.1% SDS over a YM-10 membrane (Amicon, Inc.) and the final volume was adjusted to 100 µl. Stock solutions of DTT and EDTA were added to the protein solution in final concentrations of 5 and 1 mM, respectively. The sample was heated at 60°C for 1 h. 10 µl (2 µg) of V_8 protease solution (freshly prepared) was mixed with the protein solution and the reaction mixture was incubated at 37°C for 24 h. An additional 2 µg of the enzyme was added to the reaction solution after about 12 h. Other proteolytic digestion of the isolated cytochrome c_{553} was carried out essentially as previously described (12).

The peptides resulted from the chemical and proteolytic cleavage of the cytochrome were size-fractionized on SDS-PAGE. The peptides on the gel were either electroblotted onto a PVDF membrane as previously described (11) or extracted with $(NH_4)HCO_3$ solution containing 0.1% SDS for sequencing.

A Porton 2090E protein sequencer was used for the automated Edman degradation sequencing of the peptide samples.

Other methods

SDS-PAGE was carried out according to Schägger and von Jagow's system (18) with samples incubated at room temperature for 30 min. The polypeptides were stained with Coomassie brilliant blue and the heme proteins were visualized by TMBZ as previously described (19). The apparent molecular weights of proteins were measured by comparison to high or low molecular weight markers from Sigma.

Light absorption spectra were taken on a Shimadzu UV-160 or a Varian Cary 219 spectrophotometer. Chemically induced difference spectra were recorded on the same instruments. The cytochrome was reduced and oxidized by adding a few solids of ascorbate and ferricyanide, respectively.

Results

Isolation and partial characterization

The isolation of the membrane-bound cytochrome c_{553} from *H. mobilis* is summarized in Fig. 1. During the isolation, SDS-PAGE analysis followed by heme staining with TMBZ is shown in Fig. 2. Most of the membrane-bound proteins were successfully solubilized with the anionic detergent Deriphat. Two heme proteins with apparent molecular sizes of 31 and 19 kDa were detected in both membrane preparations and after the detergent extraction. The 31 kDa heme protein is expected to be a member of cytochrome bc complex (8). The present data (Fig. 2) show a 19 kDa heme protein as a major membrane-bound cytochrome instead of the 16 kDa

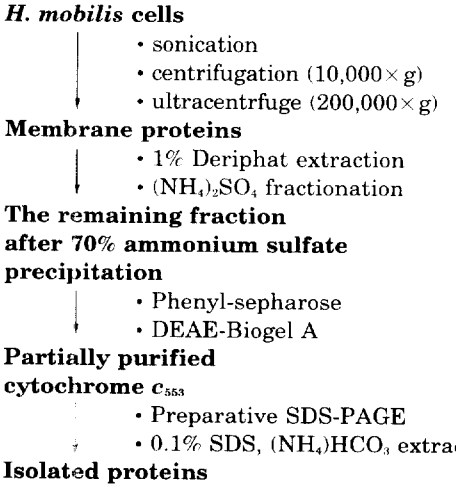

Fig. 1. The isolation scheme of cytochrome c_{553} from *H. mobilis*.

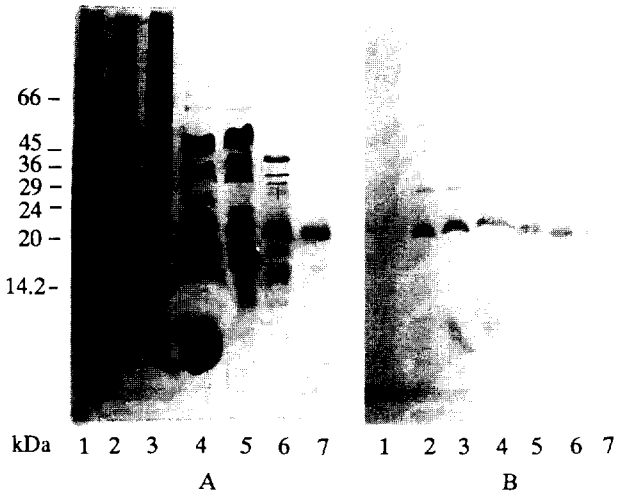

Fig. 2. SDS-PAGE analysis followed by the heme specific staining (TMBZ) of the cytochrome c_{553} during isolation. A, Coomassie blue staining; B, TMBZ staining. Lane 1, molecular mass standards; lane 2, membrane preparation; lane 3, Deriphat detergent extract; lane 4, the remaining solution after 70% ammonium sulfate precipitation; lane 5, the fraction containing cytochrome c_{553} after phenyl-sepharose column; lane 6, the partially purified cytochrome from DEAE-Biogel A; lane 7, the cytochrome c_{553} isolated by preparative SDS-PAGE from the partially purified sample.

heme protein reported previously (20). However, when the isolated cytochrome was run with low molecular markers from Sigma on SDS-PAGE, the apparent size of the heme protein seemed to be 16 or 17 kDa based on the markers (see Fig. 5). The cytochrome is, therefore, designated as a 17 kDa heme protein in this communication. The slower movement in the remaining fraction after 70% ammonium sulfate precipitation is due to the high

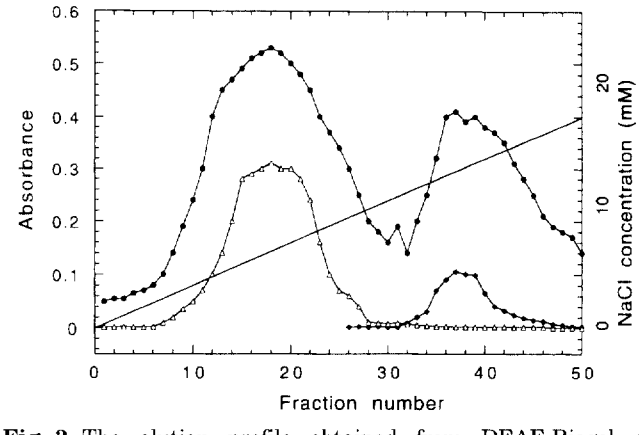

Fig. 3. The elution profile obtained from DEAE-Biogel A column chromatography. The protein was separated by a NaCl gradient elution (0 to 20 mM). ●, and ▲, and ◆ depict the absorbances at 280 nm (protein), 670 nm (bacteriochlorophyll *g* protein), and 550 nm (heme protein), respectively.

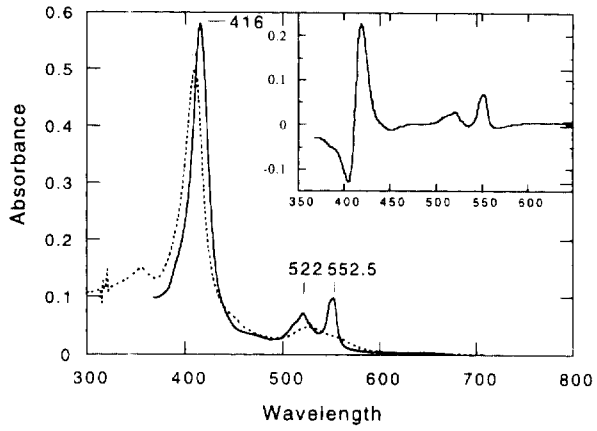

Fig. 4. Light absorption spectra of the partially purified cytochrome c_{553} from *H. mobilis*: Solid line, ascorbate reduced; dashed line, ferricyanide oxidized; inset, ascorbate reduced minus ferricyanide oxidized difference spectrum.

salt concentration in the sample. The major cytochrome was apparently enriched by successive protein techniques of ammonium sulfate fractionation, hydrophobic and anionic exchange column chromatography.

The chromatogram of DEAE-Biogel A column is shown in Fig. 3. The reddish fraction obtained from the anionic exchange column shows characteristic α (552.5 nm), β (522 nm), and Soret absorption (416 nm) peaks of a typical reduced *c*-type

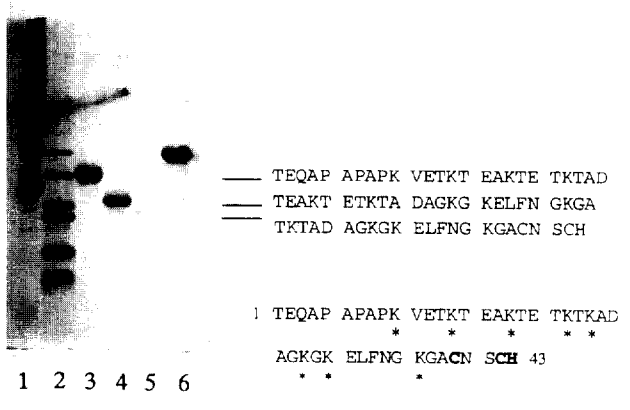

Fig. 5. SDS-PAGE and sequence analyses of *H. mobilis* cytochrome *c* fragments obtained by V_8 protease treatment and from the degraded cytochromes of the partially purified sample: lane 1, high molecular markers (66, 45, 36, 29, 24, 14.2 kDa); lane 2, low molecular markers (16.9, 14.4, 10.6, 8.16, 6.21, 3.46 kDa); lane 3 and 4, F1 and F2 fragments, respectively (see Table I); lane 5, a major fragment obtained by V_8 protease treatment; lane 6, the isolated *H. mobilis* cytochrome. The 43 amino acid sequence was obtained by overlapping the fragment sequences: Lys residues were marked by asterisks under the residue. The heme binding sequence (CXXCH) was shown in bold.

cytochrome (Fig. 4) and contains the 17 kDa TMBZ-staining protein as a major component (lane 6 in Fig. 2). The cytochrome appeared to be chopped up quickly by contaminant(s) after the partial purification (data not shown). It seems that a PMSF-independent or weakly dependent enzyme is able to be more active or to gain access to the cytochrome more easily after the partial purification than before.

The partially purified sample shows another significant protein with an apparent molecular weight of 45 kDa on the SDS-PAGE. The reaction center complex isolated from *H. mobilis* has exhibited a single protein band of 45 kDa on SDS-PAGE (21). The 45 kDa protein in the partially purified sample might be the apoprotein of the reaction center generated during the isolation so that the tight association between the cytochrome and the reaction center protein in the sample may reflect their functional relationship *in vivo*.

The final preparation by the passive elution of the protein from the gel with the ammonium bicarbonate solution showed a single band in both Coomassie blue and TMBZ staining (lane 7 in Fig. 2). Unfortunately, the heme protein appeared to be N-terminally blocked as judged from the failure of direct N-terminal sequencing.

Cleavage of the isolated cytochrome by chemical agents and proteases

Cleavage of the cytochrome was carried out by chemical reagents and site-specific proteases to get partial sequences of the protein. The results are summarized in Table I. Most of the chemical or proteolytic fragments separated by SDS-PAGE did not undergo Edman degradation, which indicates that either only the C-terminal region was exposed to the chemicals and enzymes in most cases under the present conditions or the N-termini of the fragments were artificially blocked during SDS-PAGE. Clostripain which is able to recognize Arg residue did not react with the cytochrome. Some of SDS-PAGE analyses of the fragmentations and those N-terminal sequences are presented in Fig. 5. The F1 and F2 fragments were the proteolytic degraded products isolated from the partially purified sample. These fragments were detected by heme staining of the partially purified sample and extracted from the gel with the ammonium bicarbonate solution.

The appropriate overlap of the fragment sequences produces an internal protein sequence of 43 amino acids. The internal sequence indicates that the protein is quite distant from other known heme proteins but is indeed a typical *c*-type cytochrome. The internal sequence containing 43 amino acids were analyzed by TFASTA or FASTA programs of the Genetics Computer Group's (GCG) sequence analysis software package and did not show any significant similarity to other known heme proteins, even to the chlorobial cytochrome which is the immediate electron donor to the reaction center (14). It seems, however, that the apparent structures of the two proteins are similar by being composed of hydrophobic N-terminal and functional C-terminal domains. The 43 amino acids are highly hydrophilic and characterized by a lot of Lys residues, which have been known to form a ring of positive charges in typical *c*-type cytochromes to interact with the redox partner (5, 10). The sequence Cys-Asn-Ser-Cys-His at the C-terminal part of the partial sequence corresponds to the characteristic motif CXXCH for binding of a heme group.

Discussion

The cytochrome c_{553} appears to be a membrane anchoring polypeptide since it is not released from membrane fragments by salt washing, which is similar to the situation of the secondary electron donor

Table 1. The chemical and proteolytic fragments of the isolated *H. mobilis* cytochrome c_{553} and their N-terminal sequences

Reagents/Proteases	Specific cleavage sites	Fragments/Sequences
Degraded products[a]		F1(14)[b]: TEQAP APAPK VETKT EAKTE TKTAD
		F2(11): TEAKT ETKTA DAGKG KELFN GKGA
CNBr	Met-X	C1(15), C2(14)
BNPS-skatole	Trp-X	B1(14.5), B2(13)
Clostripain	Arg-X	No reaction
Trypsin	(Arg, Lys)-X	T1(15), T2(14.5), T3(13), T4(6), T5(5)
Chymotrypsin	(Trp, Phe Tyr, Met Leu, Ala)-X	Ch1(15), Ch2(14), Ch3(13.5)
V_8 protease	(Glu, Asp)-X	V1(9): TKTAD AGKGK ELFNG KGACN SCH

[a] The degraded heme proteins were found in the partially purified fraction (obtained from DEAE-Biogel A). Fragments were isolated by cutting the reddish bands on preparative SDS-PAGE.
[b] The numbers in parentheses indicate apparent molecular sizes (kDa) based on SDS-PAGE.

in the green sulfur bacteria. The isolated membrane-bound cytochrome in current preparation exhibits an apparent molecular size of 17 kDa, comparable to that detected in the previous membrane preparation (20).

The sequence overlap of the proteolytic fragments (F1 and F2) indicates that the contaminant responsible for the proteolytic degradation of the cytochrome in the partially purified sample is a membrane-bound Trypsin-like Lys specific protease. One more fragment (about 10 kDa, data not shown) was obtained from the partially purified sample, and its N-terminal sequence confirmed the Lys specific cleavage. So the next N-terminal residue to the internal 43 amino acid sequence would be Lys.

A Met (located at the position 182 of the C-terminal part in the 206-aa-long polypeptide) has been suggested to be one axial ligand of the heme group in the secondary electron donor of *C. vibrioforme* (14). The N-terminally blocked 15 kDa fragment obtained by Met cleavage suggests that *H. mobilis* cytochrome may conserve Met at a similar position. The spectral data of the partially purified sample, together with the partial internal sequence containing the CXXCH motif and the putatively conserved Met, indicates strongly that the isolated protein in present work is a typical *c*-type cytochrome where the heme *c* group is bound to the polypeptide by the covalent linkage of the two Cys and coordinated by two axial ligands of His and Met. The computer search with FASTA program implies that the cytochrome *c* would be a novel-type, though. The gene encoding the membrane-bound cytochrome is being currently searched to deduce its complete primary structure which provides important information on the functional and evolutionary aspects of the protein.

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References

1. **Blankenship, R.E.** 1985. Electron transport in green photosynthetic bacteria. *Photosynth. Res.* **6**, 317-333.
2. **Blankenship, R.E.** 1992. Origin and early evolution of photosynthesis. *Photosynth. Res.* **33**, 91-111.
3. **Bruce, B.D., R.C. Fuller, and R.E. Blankenship.** 1982. Primary photochemistry in the facultatively aerobic green photosynthetic bacterium *Chloroflexus aurantiacus*. *Proc. Natl. Acad. Sci. USA* **79**, 6532-6536.
4. **Deisenhofer, J. and H. Michel.** 1989. The photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis*. *EMBO J.* **8**, 2149-2170.
5. **Jenney, F.E., R.C. Prince, and F. Daldal.** 1994. Roles of the soluble cytochrome c_2 and membrane associated cytochrome c_y of *Rhodobacter capsulatus* in photosynthetic electron transfer. *Biochemistry* **33**, 2496-2502.
6. **Kirmair, C. and D. Holten.** 1987. Primary photochemistry of reaction centers from the photosynthetic purple bacteria. *Photosynth. Res.* **13**, 225-260.
7. **Lee, W.Y., D.C. Brune, R. LoBrutto, and R.E. Blankenship.** 1995. Isolation, characterization and primary structure of rubredoxin from the photosynthetic bacterium, *Heliobacillus mobilis*. *Arch. Biochem. Biophys.* **318**, 80-88.
8. **Liebl, U., A.W. Rutherford, and W. Nitschke.** 1990. Evidence for a unique Rieske iron-sulfur centre in *Heliobacterium chlorum*. *FEBS Lett.* **261**, 427-430.
9. **Madigan, M.T.** 1992. The Prokaryotes, 2nd ed. Vol. II, pp. 1982-1992. *In* Balows A., H.G. Trüper, M. Dworkin, K.H. Schleifer, and W. Harder (eds.), Springer-Verlag, Berlin.
10. **Bosshard, H.R. and M. Zürrer.** 1980. The conformation of cytochrome *c* in solution. *J. Biol. Chem.* **255**, 6694-6699.
11. **Matsudaira, P.** 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.* **262**, 10035-10038.
12. **McManus, J.** 1990. Ph. D. thesis. pp. 52-56. Arizona State University.
13. **Oh-oka, H., S. Kamei, H. Matsubara, M. Iwaki, and S. Itoh.** 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.
14. **Okkels, J.C., B. Kjær,. ÖHansson, I. Svendsen, B.L. Møller, and H.V. Scheller.** 1992. A membrane-bound monoheme cytochrome *c*-551 of a novel type is the immediate electron donor to P840 of the *Chlorobium vibrioforme* photosynthetic reaction center complex. *J. Biol. Chem.* **267**, 21139-21145.
15. **Okumura, N., K. Shimada, and K. Matsuura.** 1994. Photo-oxidation of membrane-bound and soluble cytochrome *c* in the green sulfur bacterium *Chlorobium tepidum*. *Photosynth. Res.* **41**, 125-134.
16. **Prince, R.C.** 1990. The Bacteria, Vol. XII, pp. 111-149. *in* T.E. Krulwich (ed.), Academic Press, Sand Diego.
17. **Prince, R.C., H. Gest, and R.E. Blankenship.** 1985. Thermodynamic properties of the photochemical reaction center of *Heliobacterium chlorum*. *Biochim. Biophys. Acta* **810**, 377-384.
18. **Schägger, H. and G. von Jagow.** 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **166**, 368-379.
19. **Thomas, P.E., D. Ryan, and W. Levin.** 1976. An improved staining procedure for the detection of the peroxidase activity of cytochrome p-450 on sodium dodecyl sul-

fate polyacrylamide gels. *Anal. Biochem.* **75**, 168-176.
20. **Trost, J.T. and R.E. Blankenship.** 1990. Current research in Photosynthesis. Vol. II, p. 703-706. *In* Baltscheffsky, M. (ed.), Kluwer Academic Publishers, Dordrecht, The Netherlands.
21. **Trost, J.T. and R.E. Blankenship.** 1989. Isolation of a photoactive photosynthetic reaction center-core antenna complex from *Heliobacillus mobilis*. *Biochemistry* **28**, 9898-9904.
22. **Woese, C.R.** 1987. Bacterial evolution. *Microbiol Rev.* **51**, 221-271.
23. **Woese, C.R., B.A. Debrunner-Vossbrinck, H. Oyaizu, E. Stackebrandt, and W. Ludwig.** 1985. Gram-positive bacteria: Possible photosynthetic ancestry. *Science* **229**, 762-765.