A gene coding for the photosynthetic reaction center-bound cytochrome subunit, pufC, of Blastochloris viridis, which belongs to the α-purple bacteria, was introduced into Rubrivivax gelatinosus, which belongs to the β-purple bacteria. The cytochrome subunit of B. viridis was synthesized in the R. gelatinosus cells, in which the native pufC gene was knocked out, and formed a chimeric reaction center (RC) complex together with other subunits of R. gelatinosus. The transformant was able to grow photosynthetically. Rapid photo-oxidization of the hemes in the cytochrome subunit was observed in the membrane of the transformant. The soluble electron carrier, cytochrome _c_2, isolated from _B. viridis_ was a good electron donor to the chimeric RC. The redox midpoint potentials and the redox difference spectra of four hemes in the cytochrome subunit of the chimeric RC were almost identical with those in the _B. viridis_ RC. The cytochrome subunit of _B. viridis_ seems to retain its structure and function in the _R. gelatinosus_ cell. The chimeric RC and its mutagenesis system should be useful for further studies about the cytochrome subunit of _B. viridis_.

The L and M subunits of RC are encoded by the _pufL_ and _pufM_ genes, respectively, which form an operon called _puf_ together with the _pufB_ and _pufA_ genes that encode the β and α subunits of the light-harvesting 1 complex. In species containing the RC-bound cytochrome subunit, the _pufC_ gene coding for this subunit is located immediately downstream of the _pufM_ gene. Amino acid sequences derived from the nucleotide sequences of the _pufC_ genes of purple bacteria contain four _CXXCH_ sequence motifs to bind _c_-type hemes (3), with an exception in the _Rhodovulum_ species (4). The hemes have been numbered as heme-1 to heme-4 according to the order of the appearance of the respective binding motifs in the amino acid sequence (5).

The RC complex purified from a purple nonsulfur bacterium belonging to the α-subclass, _Blastochloris viridis_ (formally called _Rhodopseudomonas viridis_) (6, 7), has been crystalized (8–10). Its crystal structure showed that the four _c_-type hemes are arranged in a roughly linear manner, almost perpendicularly to the membrane. The spectrophotometric and thermodynamic properties of the four hemes and their arrangement in the structure have been extensively studied (11–16). The four hemes have high-low-high-low midpoint potentials from the special pair to the periplasmic space. They are distinguishable in terms of the peak positions in their _α_-absorption bands.

A gene manipulation system for _B. viridis_ was established previously (17), and some studies had been done using site-directed mutagenesis manipulated into the LM core complex and into the cytochrome subunit of _B. viridis_ (18). _B. viridis_ cannot be inoculated aerobically and grows with a doubling time of 24 h under microaerobic respiratory conditions (17). The very low growth rate makes it difficult to obtain a sufficient number of the mutant strains of _B. viridis_. The expression of the _B. viridis_ cytochrome subunit gene in _Escherichia coli_ cells was also challenged. However, it resulted in the accumulation of the inclusion body without heme incorporation (19).

The cytochrome subunit of _Rubrivivax gelatinosus_, which belongs to the β-subclass of purple bacteria, has also been studied and shown to be similar to that of _B. viridis_ in terms of the redox potentials of hemes (20). _R. gelatinosus_ grows well under respiratory conditions (about 2 h of the doubling time) as well as under photosynthetic conditions (about 2.5 h of the doubling time); therefore, it is easy to introduce mutations into the photosynthetic apparatus (21). The photosynthetic growth rate of the _R. gelatinosus_ mutant lacking the cytochrome subunit was about a half of that of the wild type, showing that the cytochrome subunit is not essential but is advantageous for photosynthesis (21). Based on the mutational replacements of charged amino acid residues distributed on the surface of the subunit, Osyczka _et al._ (22–24) have shown that the low potential heme-1 located at the most distant position from the special pair is a direct electron acceptor from the soluble electron carrier.
carrier, which suggests that all four hemes are involved in the electron transfer to the special pair.

Several water-soluble electron carrier proteins are present in the periplasmic space of *R. gelatinosus*. Three of these proteins (high potential iron-sulfur protein (HiPIP), high potential cytochrome *c*$_{55}$, and low potential cytochrome *c*$_{6}$), have been shown to work as the electron donors to the RC-bound cytochrome *c*$_{55}$ in this study. The physiological electron donor to the *R. gelatinosus* RC is the cytochrome *c*$_{55}$. The kinetic strength dependence studies on the reaction of the cytochrome *c*$_{55}$ with the *B. viridis* RC have suggested that the heme-1 of the cytochrome subunit is the primary electron acceptor from the cytochrome *c*$_{55}$ (28). However, direct evidence for the precise interaction mechanisms between the soluble electron donor and heme-1 in *B. viridis* is not yet available.

In this study, the gene for the cytochrome subunit of *R. gelatinosus* was replaced by that of *B. viridis* using a common restriction site at a conserved DNA region in both species. The mutated cells of *B. viridis* produced a chimeric RC complex containing the cytochrome subunit derived from *B. viridis* and the other subunits from the host. The chimeric RC was isolated and characterized.

**EXPERIMENTAL PROCEDURES**

**Bacteria and Growth Media—** *R. gelatinosus* strain IL144RL2, which is a spontaneous mutant of the wild-type strain IL144 and shows greatly depressed production of the light-harvesting 2 complex, was used as a host strain for gene manipulations in this study. The *R. gelatinosus* strain ΔC constructed in a previous study was also used in this study, in which the pufC gene was knocked out by insertion of a kanamycin resistance gene (22). *R. gelatinosus* strains were grown under aerobic-dark or anaerobic-light conditions at 30 °C with a PYS medium (21). *B. viridis* cells were grown under anaerobic-light conditions at 30 °C in a malate–base salt medium containing 0.5% sodium malate, 0.1% yeast extract (Difco), 0.1% ammonium sulfate, 1% basal salt solution (29), 0.1% vitamin solution (29), and 20 mM sodium phosphate (pH 7.0). *E. coli* strain JM109 was used as a host for plasmids and grown aerobically with a Luria-Bertani medium or an SOB medium at 37 °C (30). When needed, ampicillin, kanamycin, or chloramphenicol was added to the *E. coli* and *R. gelatinosus* cultures at a final concentration of 50 μg/ml.

**Plasmid Construction and Transformation—** The plasmid pGELPUFC containing the 4.6-kb whole puf operon of *R. gelatinosus* was created by connecting two DNA fragments, pHG37 and pG7T, previously cloned from the genomic DNA library of *R. gelatinosus* strain IL144 (31). The 3.7-kb region flanked by *Sal I* sites was the puf operon of *R. gelatinosus* replaced by an ampicillin resistance gene. This plasmid was named pGELPUF (Fig. 1A) and used for construction of the *R. gelatinosus* mutant lacking the puf operon. A 2.5-kb DNA fragment flanked by *Bam H1* restriction sites containing the 3′-region of *pufM* and the entire *pufC* of *B. viridis* was cloned from the genomic library of *B. viridis* DNA using a pUC119 plasmid as a cloning vector. This plasmid was named pHGPF (Fig. 1B). The nucleotide sequence of *pufC* in the pHGPF plasmid was confirmed to be identical to that previously reported for the *B. viridis* *pufC* (5). Both the genes for the M subunits of *R. gelatinosus* and *B. viridis* have a common *Sal I* restriction site at the conserved 3′-regions. The region downstream of *Sal I* site in the inserted DNA of the plasmid pGELPUF was removed and replaced by the DNA fragment containing the region downstream of the corresponding *Sal I* site of the pGELPF plasmid, in which the entire *pufC* of *B. viridis* was contained (Fig. 1B). The DNA fragment containing this “chimeric” *pufC* operon was cloned into a pHSG298 plasmid. The p2GFPV-KF plasmid was then isolated. The plasmid was transformed into *E. coli* by electroporation (21).

**Preparation of Membranes and Soluble Electron Donors—** Cells in the late logarithmic growth phase were harvested by centrifugation at 7,200 × g for 20 min, washed, and suspended in 10 mM MOPS-NaOH (pH 7.0). The cells were disrupted by passage through a French press at 1100–1200 kg/m$^2$ in the presence of a few grains of DNase I. The disrupted cell suspension was centrifuged at 28,000 × g for 15 min to remove the debris and ultracentrifuged at 340,000 × g for 20 min. The sediment was used as the membrane preparation. The cytochrome *c*$_{55}$ of *B. viridis* and HiPIP of *R. gelatinosus* were isolated from the remaining supernatant according to the methods previously reported (26). The protein content in the membrane was determined with a Protein Assay Kit (Bio-Rad), using bovine serum albumin (fraction V) as a standard protein. Reduced-minus-oxidized difference spectra were measured with a UV-3000 spectrophotometer (Shimadzu). Two millimolar (final concentration) of potassium ferricyanide or a few grains of sodium dithionate were added for the oxidation or the reduction of the membrane preparations.

**SDS-PAGE—** SDS-PAGE was carried out according to Laemmli (32). The proteins with a concentration of 5 mg/ml were denatured in 1% SDS by boiling for 1 min. Ten micrograms of protein was applied to each lane of the gel. After electrophoresis, the gel was treated with Coomasie Brilliant Blue using a Quick-CBB Kit (Wako, Japan) for staining the proteins or treated with TMBZ for detection of the hemes according to the method of Thomas et al. (33).

**Isolation of the RC Complex of *R. gelatinosus—** The membranes prepared were suspended in a buffer containing 10 mM MOPS-NaOH (pH 7.0) to give an absorbance of 40 at 875 nm. The suspension was mixed with an equal volume of precooled 1.6% (v/v) β-d-ethylicthioglucoiside and incubated on ice for 40 min with stirring. Subsequently, the suspension was applied on the top of a sucrose density gradient (from top to bottom, 10–40%, v/v) containing 10 mM MOPS-NaOH (pH 7.0) and 0.05% Triton X-100 and ultracentrifuged at 146,000 × g for 20 h. A brownish red band that formed at a sucrose density of about 25% was recovered as an RC fraction and concentrated with Centrifo CP25 membrane capsules (Amicon). The RC fraction was diluted with a buffer containing 100 mM KCl, 20 mM MOPS-KOH (pH 7.0), and 0.05% Triton X-100.

**Kinetic Measurements—** Xenon flash-induced absorption changes were measured with a single beam spectrophotometer designed and assembled in our laboratory (34). The membranes were suspended in a 20 mM MOPS-KOH buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, and 10 μM DAD. The concentration of the ascorbate was adjusted to give an absorbance of 1 at 860 nm for the *R. gelatinosus* strain ΔC, at 875 nm for other *R. gelatinosus* strains, and at 1012 nm for *B. viridis*. One micromolar valinomycin was added to the membrane preparation of the *R. gelatinosus* strain ΔC to reduce the effect of the band shift of carotenoids responding to the membrane potential. The kinetics of an electron transfer from the soluble electron donor to the RC was measured in a buffer containing 5 mM MOPS-NaOH (pH 7.0). The *R. gelatinosus* HiPIP and the *B. viridis* cytochrome *c*$_{55}$ were added at the final concentrations of 1 μM. One millimolar sodium ascorbate, 10 μM DAD, and 0.03% Triton X-100 were also added.

**Redox Titration—** Redox titration of hemes in the RC-bound cytochrome *c* was carried out as described previously (35) using a double beam spectrophotometer UV-3000 (Shimadzu). The redox potential was monitored with an Ag/AgCl electrode (TOA) connected to an HM-40S voltmeter (TOA). The concentration of the sample was adjusted to give an absorbance of 2 at 875 nm for *R. gelatinosus* and at 1012 nm for *B. viridis*. The buffer for the measurements contained 100 mM KCl, 20 mM MOPS-KOH (pH 7.0), and 0.05% Triton X-100. The redox mediators used are as follows: 100 μM potassium ferricyanide, 10 μM Fe-EDTA, 10 μM phenazine methosulfate, 10 μM vitamin K$_1$, 10 μM 2-hydroxy-1,4-naphthoquinone, and 3 μM pyocyanin. The oxygen was eliminated by a continuous stream of nitrogen gas. At the beginning of the measurements, the redox potential was reduced by addition of a solution of sodium ascorbate or sodium dithione. Subsequently, the redox potential was raised stepwise by additions of a solution of potassium ferricyanide.

**RESULTS**

**Expression of *B. viridis* pufC Gene in Cells of *R. gelatinosus—** The plasmid pAPUF2, which contains a kanamycin resistance gene and the *R. gelatinosus* puf operon disrupted by the insertion of an ampicillin resistance gene (Fig. 1A), was introduced into the cells of the *R. gelatinosus* strain IL144RL2. A strain showing resistance to ampicillin but sensitivity to kanamycin was picked up after successive cultivation under the pressure of ampicillin and named DP2. An absorption spectrum of the membrane prepared from the strain DP2 cells showed no accumulation of the RC-light-harvesting 1 complex (Fig. 2).
Southern hybridization, PCR, and DNA sequencing experiments for the genomic DNA isolated from the strain DP2 showed that the 3.7-kb NotI-NotI DNA region containing \(puf^{BALMC}\) was replaced by the ampicillin resistance gene (data not shown), probably due to a double cross-over recombination between the genomic DNA and the \(p^B/H9004\) p\(\text{PUF2}\) plasmid. The strain DP2 could not grow photosynthetically.

The plasmid p2GPVC-KF, which contains the majority of the \(puf\) genes of \(R.\ gelatinosus\) and the entire \(pufC\) of \(B.\ viridis\) (Fig. 1), was introduced into the \(R.\ gelatinosus\) strain DP2 cells. A new strain showing resistance to kanamycin was obtained and named VC-F. The VC-F cells were able to grow under anaerobic light conditions, although the growth rate was about 3 times slower than that of the strain IL144RL2. An absorption spectrum of the strain VC-F membrane was nearly identical with that of the strain IL144RL2 membrane (Fig. 2), suggesting that the strain VC-F recovered the ability to synthesize the RC-light-harvesting 1 complex without significant alterations in the environments of the photosynthetic pigments. The presence of \(puf\) genes in the strain VC-F cells was confirmed by Southern hybridization experiments. The p2GPVC-KF plasmid was detected in the cell lysate of the strain VC-F and seemed to be reproducible in the VC-F cells.

The synthesis of the RC-bound cytochrome subunit derived from the \(B.\ viridis\) \(pufC\) gene in the cells of the \(R.\ gelatinosus\) strain VC-F was examined by SDS-PAGE of the membranes, as shown in Fig. 3. The band patterns stained with Coomassie Brilliant Blue were almost identical among the membrane samples of the three strains of \(R.\ gelatinosus\). Minor differences between the patterns lower than 14 kDa for the strain \(p^B/H9251\) and for two other strains, IL144RL2 and VC-F, may reflect the difference in the contents of the light-harvesting 2 complexes. In the gel stained for hemes, a peptide with a molecular mass of 43 kDa was detected in the membrane of the strain IL144RL2, which was assigned to the cytochrome subunit (36). No heme-stained peptides were observed in the membrane of the strain \(p^B/H9252\), which lacks the cytochrome subunit (22). In the membrane of the strain VC-F, a heme-stained peptide was detected at the 39-kDa molecular mass, which coincided with that of the RC-bound cytochrome subunit of \(B.\ viridis\). This result suggested that the RC-bound cytochrome subunit derived from \(B.\ viridis\) was expressed in the \(R.\ gelatinosus\) strain VC-F.

**Redox and Spectroscopic Characteristics of Hemes in the Cytochrome Subunit**—Fig. 4 shows the reduced-minus-oxidized difference spectrum in the \(\alpha\)-absorption band region of the

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**Fig. 1.** Schematic representation of gene manipulation performed in this study. The rectangles represent the photosynthesis genes of the \(puf\) operon coding for the \(L, M,\) and cytochrome subunits of the RC (\(L, M,\) and \(C,\) respectively) and the \(\alpha\) and \(\beta\) subunits of the light-harvesting 1 complex (\(A\) and \(B,\) respectively). Genes for bacteriochlorophyll and carotenoid biosynthesis (\(bchZ\) and \(crtA,\) respectively) and unknown open reading frames are also presented. Shaded rectangles show genes derived from \(B.\ viridis.\)

**Fig. 2.** Absorption spectra of the membranes of \(R.\ gelatinosus\) strains IL144RL2 (dotted line), VC-F (solid line), and DP2 (broken line). Membranes were prepared from cells grown under anaerobic-light conditions (IL144RL2 and VC-F) and under aerobic-dark conditions (IL144RL2 and DP2). Each of the membrane samples contained 200 \(\mu\)g of protein/ml.
cytochromes c in the membrane of the strain VC-F, together with those of the strains IL144RL2 and ΔC of *R. gelatinosus* and *B. viridis*. The difference spectrum of the strain IL144RL2 membrane, which has two low potential hemes that peaked at 551 nm and two high potential hemes that peaked at 555 nm in the cytochrome subunit, showed an absorption maximum at 552 nm, which was consistent with the results obtained from the wild type of *R. gelatinosus* shown in a previous study (36). In the strain ΔC, the absorption bands derived from cytochromes were very small, as expected (21). The broad and small bands detected in the strain ΔC membrane were probably due to the cytochrome bc₁ complex. The membrane of the strain VC-F, on the other hand, showed a different spectrum with an absorption maximum at 553 nm with a shoulder around 559 nm, which was similar to that obtained for the membrane of *B. viridis*.

Hemes contained in the cytochrome subunit of the *R. gelatinosus* strain VC-F were further characterized by redox titration (Fig. 5). Four redox components were detected when the peak heights of the α-absorption bands of the cytochromes c in the membrane preparation were plotted against the ambient potentials. When the data were fitted with Nernst equations, the redox midpoint potentials (\(E_m\)) values of these components were estimated to be \(-60, +32, +320,\) and \(+419\) mV in the membrane and \(-60, +32, +310,\) and \(+398\) mV in the solubilized RC complex (Fig. 5). These values were consistent with those reported for the four hemes in the *B. viridis* cytochrome subunit (Table I). Fig. 6 shows the reduced-minus-oxidized difference spectra of the four components in the strain VC-F membrane. Four c-type hemes with different absorption maxima in the α-band regions were resolved. The heme with the highest \(E_m\) value showed a narrow α-band peaked at 559 nm with a shoulder at near 552 nm. The heme with the second highest \(E_m\) had a somewhat broad α-band peaked at 556 nm. The two low potential hemes showed narrow α-bands with peaks at 552 and 553 nm. These spectral and redox characteristics in the strain VC-F were almost identical with those reported for *B. viridis* (11). This indicated that the environments surrounding the hemes in the cytochrome subunit in the strain VC-F were almost the same as those in the original RC complex of *B. viridis*.

The redox and spectroscopic properties of the four hemes in the membrane preparations of the *R. gelatinosus* strain IL144RL2 are summarized in Table I. The values of the redox midpoint potentials in *R. gelatinosus* IL144RL2 were \(+64\) mV for the two low potential hemes peaking at 551 nm and \(+281\) and \(+348\) mV for the two high potential hemes peaking at 555 nm. These \(E_m\) values were consistent with those for the *R. gelatinosus* strain IL144 (36) and for the *R. gelatinosus* strain 52 (20). Thus, the redox midpoint potentials of the four...
was adjusted to give an absorbance of 2 at 875 nm.

Poised at various redox potentials.

Chromes /CH9251 induced absorption changes in the

IL144RL2.

Hemes determined in the membrane of the

R. gelatinosus

in the membranes prepared from the cells of

R. gelatinosus

and

B. viridis

peak position in α-band (nm)

Heme

1

2

3

4

Reference/source

Cytochrome subunit of R. gelatinosus

Strain IL144RL2

Pakushima et al.

Nitschke et al.

Cytochrome subunit of B. viridis

Strain VC-F

B. viridis

Wild type

Dracheva et al.

Nitschke and Rutherford

Frizsch et al.

Shinker et al.

Alegria and Dutton

Fritz et al.

551

555

555

551

This work

+64

+281

+348

+64

Ref. 36

+90

+330

+330

+90

Ref. 20

+70

+300

+320

+130

Ref. 20

553

556

559

552

This work

−60

+310

+398

+32

This work

−73

+310

+384

+35

This work

−60

+310

+380

+20

Ref. 11

−80

+320

+400

+20

Ref. 12

−60

+300

+370

+10

Ref. 13

−50

+312

+360

+20

Ref. 14

−51

+295

+370

+50

Ref. 15

−49

+321

+383

+33

Ref. 16

a Numbering of hemes was based on the appearance in the amino acid sequence (5).

b Wavelength of absorption maximum in the redox difference spectrum measured in this study.

FIG. 6. Reduced-minus-oxidized difference spectra of cytochromes c in the membrane of the R. gelatinosus strain VC-F poised at various redox potentials. The concentration of the sample was adjusted to give an absorbance of 2 at 875 nm.

Flash-induced Redox Change of Hemes—Fig. 7 shows flash-induced absorption changes in the α-band region of the cytochromes c in the membranes prepared from the cells of R. gelatinosus strains and B. viridis. For these measurements, the two high potential hemes in the cytochrome subunit were reduced prior to the flash activation by the addition of 1 mM sodium ascorbate. In the membrane of the strain VC-F, rapid oxidation of the cytochrome was observed, as in the membranes of R. gelatinosus IL144RL2 and B. viridis. The flash-induced spectra derived from this rapid oxidation showed a peak at 556 nm in the membranes of the strain VC-F and B. viridis, indicating that the second high potential heme, c556 (heme-2), was oxidized by the flash. In the flash-induced absorption change of the cytochromes in the membrane of the strains IL144RL2, the major oxidized component showed a peak at 553 nm. This suggested that the low potential hemes peaked at 551 nm in the IL144RL2 cytochrome subunit were partially reduced prior to the flash activation possibly due to their relatively high redox potentials. No remarkable peaks were observed in the flash-induced spectrum in the membrane preparation of the strain ΔC due to the lack of the cytochrome subunit.

Fig. 8 shows the kinetics of the electron transfer from the soluble electron carrier proteins to the cytochrome subunit in the prepared membrane. When the R. gelatinosus HiPIP was added, re-reduction of the cytochrome was observed with a second order rate constant of 1.82 × 10−7 in the IL144RL2 membrane but not in the membranes of the strain VC-F and B. viridis. This suggested that HiPIP, which is the physiologically main electron donor to the oxidized cytochrome subunit in the R. gelatinosus wild-type cells, was not a good electron donor to the RC in the strain VC-F cells. When the B. viridis cytochrome c2 was added to the membrane of the strain VC-F, re-reduction of the RC-bound cytochrome and concomitant oxidation of the cytochrome c2 were observed with a second order rate constant of 7.75 × 10−6, which was comparable with the value obtained from the measurement for the B. viridis membrane, 5.05 × 10−6. The membrane of the strain IL144RL2 did not show an apparent electron transfer reaction with the B. viridis cytochrome c2.

DISCUSSION

In this study, the RC-bound cytochrome subunit of B. viridis was shown to be synthesized in the distantly related species of purple bacteria, R. gelatinosus. Measurements of spectra and flash-induced redox changes of the cytochromes c showed that the B. viridis cytochrome subunit was not only synthesized in R. gelatinosus but also functioned as the rapid electron donor to the photooxidized special pair. This means that the B. viridis cytochrome subunit can form a functional “chimeric” RC complex together with the R. gelatinosus LM core polypeptides.
This was somewhat surprising, since the amino acid sequence identities of the L, M, and cytochrome subunits between *B. viridis* and *R. gelatinosus* are only 66, 61, and 45%, respectively. It has been reported in the fine structure of the *B. viridis* RC that 30 amino acids in the cytochrome subunit are bound to the LM core via hydrogen bonds and salt bridges (10). These amino acids are indicated with the primary structure shown in Fig. 9. In the cytochrome subunit of *R. gelatinosus*, only 80 of these 30 amino acids are conserved, based on the comparison of the amino acid sequences. Some of the amino acid residues located near the binding residues are absent in the *R. gelatinosus* cytochrome subunit (Fig. 9). These amino acid sequences around these residues also seemed to be well conserved. These findings suggest that the surfaces on the LM core that bind to the cytochrome subunit are similar in the two species and that the *R. gelatinosus* LM core can interact rather tightly with the *B. viridis* cytochrome subunit. In addition, the C-terminal residues of the M subunit of *R. gelatinosus* were well conserved in the L and M subunits of *R. gelatinosus* (Fig. 9). The amino acid sequences around these residues also seemed to be well conserved.

**Fig. 7.** Flash-induced redox changes and spectra of the cytochrome subunit in the membrane. Redox changes of hemes in the membranes of the *R. gelatinosus* strains IL144RL2 (a), ΔC (b), and VC-F (c), and *B. viridis* (d) were measured at 540 nm (upper traces) and 551-minus-540 nm (lower traces). Spectra were obtained by plotting the extent of the absorption changes taken at 1.6 ms after the flash (e). The concentration of the membrane was adjusted to give an absorbance of 1 at 875 nm (for *R. gelatinosus*) or at 1012 nm (for *B. viridis*) in 100 mM KCl and 20 mM MOPS-KOH (pH 7.0) containing 1 mM sodium ascorbate and 10 μM DAD.

**Fig. 8.** Kinetics of flash-induced electron transfer from soluble electron donors to the cytochrome subunit. The membranes from *R. gelatinosus* IL144RL2 (traces a, d, and g), *R. gelatinosus* VC-F (traces b, e, and h), and *B. viridis* (traces c, f, and i) were prepared to give an absorbance of 1 at 875 nm for *R. gelatinosus* and at 1012 nm for *B. viridis* in 5 mM MOPS-NaOH (pH 7.0) containing 1 mM sodium ascorbate, 10 μM DAD, and 0.03% Triton X-100. Traces a–c were recorded in the presence of 1 μM HiPIP purified from *R. gelatinosus*. Other traces (d–i) were the result of the measurements for the samples containing 1 μM cytochrome c₂ purified from *B. viridis*. The redox change of the hemes in the cytochrome subunit was measured at 556-minus-540 nm (traces a–f). Oxidation of the cytochrome c₂ was measured at 549 minus 540 nm (traces g–i).
Fig. 9. Comparison of the amino acid sequences of L, M, and cytochrome subunits between R. gelatinosus and B. viridis. The regions responsible for binding the cytochrome subunit to the LM core shown in the fine structure of the B. viridis RC (10) were presented. Identical residues between the two species are shown in reverse contrast. Residues directly interacting between the cytochrome subunit and the LM core subunits via hydrogen bonds and salt bridges, shown in B. viridis (10), are marked by asterisks. An arrow shows the amino acid position corresponding to the Sall restriction site on the gene (pufM), whose site was used for connection of the puf operons of R. gelatinosus and B. viridis, as shown in Fig. 1. This means that the amino acid sequence in the C-terminal side of the arrow is identical between the M subunits of B. viridis and the R. gelatinosus strain VC-F.

The rate of electron transfer from soluble electron carriers to the cytochrome subunit of the strain VC-F were derived from B. viridis. It has been suggested that the C terminus of the M subunit is strongly associated with the cytochrome subunit in B. viridis as well as in other species (37). Therefore, the cytochrome subunit of B. viridis in the strain VC-F were closely associated with the “chimeric” M subunit.

It is known that the N-terminal 20 amino acid residues in the precursor form of the cytochrome subunit of B. viridis function as a signal peptide and are removed possibly after secretion for the periplasmic space (5). The N-terminal first amino acid of the mature form of the subunit is a cysteine residue, which is modified by two fatty acid molecules (38). The sequence LVAGC at positions 17–21 in the precursor protein of B. viridis may be responsible for this modification and for the cleavage by signal peptidase II (38). The cytochrome subunit of R. gelatinosus also has a similar motif, LLAGC, at positions 19–23 (31). The post-translational modification shown in B. viridis presumably occurs in the cells of R. gelatinosus. Evidence for this comes from the results of SDS-PAGE, which showed that the mobility of a band corresponding to the B. viridis cytochrome subunit synthesized in the R. gelatinosus strain VC-F was identical with that in the B. viridis membrane, as shown in Fig. 3.

The redox midpoint potentials and the spectroscopic characteristics of four hemes in the cytochrome subunit of the strain VC-F were almost the same as those in the original B. viridis complex. This result suggests that the association with the R. gelatinosus LM core did not significantly affect the electrochemical properties of the hemes, except possibly for heme c559. Thus, the cytochrome subunit derived from B. viridis gene probably holds its original structure and functions in the chimerical RC complex in the R. gelatinosus strains VC-F. The results of flash-induced spectrophotometric measurements in the membrane preparation of the R. gelatinosus strain VC-F indicated that the cytochrome subunit derived from B. viridis functioned as an efficient electron donor to the photooxidized special pair in the strain VC-F.

The rate of electron transfer from soluble electron carriers to the hemes in the cytochrome subunit in the strain VC-F was largely different from that in the wild-type strains of R. gelatinosus. This can be explained by the fact that the major electron donor to the cytochrome subunit is cytochrome c5 in B. viridis but HiPIP in R. gelatinosus. A previous study showed that the R. gelatinosus HiPIP is a very poor electron donor to the B. viridis RC (39). Consistently, an addition of the R. gelatinosus HiPIP to the strain VC-F membrane had no detectable effects on the reduction of the cytochrome, as shown in Fig. 8. This may be one of the reasons that the growth rate of the strain VC-F is considerably lower than those of the wild-type strains under photosynthetic conditions. On the other hand, the B. viridis cytochrome c5 worked as a good electron donor to the chimeric RC in the strain VC-F membrane, as observed for the native RC of B. viridis. The native structure of the B. viridis cytochrome subunit seems to be well maintained in the R. gelatinosus strain VC-F.

Previous studies have shown that the cluster of acidic residues immediately surrounding the heme-1 of the RC-bound cytochrome subunit forms an electrostatically favorable binding site to cytochromes in R. gelatinosus (22–24). A comparison of the three-dimensional structure of the cytochrome subunit solved in B. viridis and that predicted for R. gelatinosus suggested that these species are different in the distribution of the acidic residues around the heme-1. As part of a future study, it will be interesting to introduce site-specific mutations to the chimeric RC to clarify the interaction between the cytochrome subunit and the cytochrome c5 of B. viridis.

Further physiological and kinetic studies and additional site-directed mutagenesis on the cytochrome subunit of the strain VC-F should be useful for the clarification of the precise mechanisms of the electron transfer and of the physiological roles of the cytochrome subunit. A great number of studies have been conducted on the cytochrome subunit of B. viridis, resulting in a significant body of available structural and kinetic information. This information and the system established for R. gelatinosus in this study have made it easier than before to obtain further information about the relationships between the structure and function in the electron transfer protein.

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