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REVIEW

C-type cytochromes in the photosynthetic electron transfer pathways in green sulfur bacteria and heliobacteria

Chihiro Azai · Yusuke Tsukatani · Shigeru Itoh · Hirozo Oh-oka

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Abstract Green sulfur bacteria and heliobacteria are strictly anaerobic phototrophs that have homodimeric type 1 reaction center complexes. Within these complexes, highly reducing substances are produced through an initial charge separation followed by electron transfer reactions driven by light energy absorption. In order to attain efficient energy conversion, it is important for the photooxidized reaction center to be rapidly rereduced. Green sulfur bacteria utilize reduced inorganic sulfur compounds (sulfide, thiosulfate, and/or sulfur) as electron sources for their anoxygenic photosynthetic growth. Membrane-bound and soluble cytochromes *c* play essential roles in the supply of electrons from sulfur oxidation pathways to the P840 reaction center. In the case of gram-positive heliobacteria, the photooxidized P800 reaction center is rereduced by cytochrome *c*-553 (PetJ) whose N-terminal cysteine residue is modified with fatty acid chains anchored to the cytoplasmic membrane.

Keywords Cytochrome · Electron transfer · Green sulfur bacteria · Heliobacteria · Reaction center

Abbreviations

Bchl	Bacteriochlorophyll
Cyt	Cytochrome
ET	Electron transfer
FAP	Filamentous anoxygenic phototroph
P840	Primary electron donor made of a special pair of bacteriochlorophylls *a* in the green sulfur bacterial RC
P800	Primary electron donor made of a special pair of bacteriochlorophylls *g* in the heliobacterial RC
PS I	Photosystem I
PS II	Photosystem II
RC	Reaction center

Introduction

The initial charge separation within the photosynthetic reaction center (RC) complex is driven by light-energy absorption, followed by the production of chemically energized reducing substances. Coupled with this process, the proton gradient across membranes is formed by the function of quinol:cytochrome (cyt) *c* oxidoreductase (cyt *bc* complex) in anoxygenic photosynthetic bacteria or by quinol:cyt c_6 (or plastocyanin) oxidoreductase (cyt *bf* complex) in algae and plants, including cyanobacteria (Gray and Daldal 1995; Cramer et al. 2008). The cyt *bc* (or *bf*) complex translocates protons from the inner side (n side) to the outer side (p side) through quinol oxidation by the Q-cycle mechanism. These dynamic electron transfer reactions are the essence of the photosynthetic energy transduction pathway.

In this review, the old species names of green sulfur bacteria were transcribed to new ones according to Imhoff's definition (Imhoff 2003).

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In oxygenic phototrophs, two different types of RCs, photosystems (PSs) II and I that belong to types 2 and 1 RCs, respectively, are arranged in a straightforward manner through the cyt *bf* complex (Ort and Yocum 1996). Although a lipophilic electron carrier, quinone, functions as a mediator between PS II and the cyt *bf* complex, the electron transfer (ET) reaction from the latter complex to PS I is conducted by soluble metal-containing carriers of cyt c_6 and/or plastocyanin (Hervás et al. 1995; Hope 2000; Dîaz-Quintana et al. 2008). These carriers diffuse over a relatively long distance searching for their reaction partners within the lumen side of thylakoids (see Fig. 1).

On the other hand, in purple nonsulfur bacteria, the cyclic ET system operates between cyt *bc* and type 2 RC complexes; cyt c_2 transfers electrons from the former complex to the latter one, followed by the reduction of the acceptor quinone to quinol in the Q_B pocket, which is then oxidized by the cyt *bc* complex (Okamura and Feher 1995; Meyer and Cusanovich 2003). The physiological role of cyt c_2, as well as its reaction mechanism, has been studied intensively at the molecular level using both biochemical and molecular genetic methods (Meyer and Donohue 1995). It is also well known that membrane-anchored cyt c_y can substitute for the function of cyt c_2 in *Rhodobacter capsulatus* (Daldal et al. 2003).

It is still unknown whether the cyclic ET system operates in green sulfur bacteria and heliobacteria, both of which possess homodimeric type 1 RC complexes (Hauska et al. 2001; Oh-oka 2007). Green sulfur bacteria are strictly anaerobic photoautotrophic bacteria that utilize reduced sulfur compounds (sulfide, thiosulfate, and/or sulfur) as electron sources for their growth (Imhoff 1995). The oxidizing pathways of sulfur compounds have been partly determined from comparative genomic analyses (Frigaard and Bryant 2008). Heliobacteria are also known to be strictly anaerobic; however, they can grow photoheterotrophically as well as chemotrophically in darkness (Madigan and Ormerod 1995). It is noteworthy that secondary electron donors to the RC complexes in both green sulfur bacteria and heliobacteria are membrane-anchored cyts *c*, which directly mediate the ET reactions from cyt *bc* complexes without any soluble carriers (Oh-oka et al. 1998, 2002).

Herein, we review cyts *c* and their function as electron carriers in the unique photosynthetic ET pathways in green sulfur bacteria and heliobacteria and perform a cluster analysis of their amino acid sequences calculated from recent genomic databases. Later, a probably dynamic structural fluctuation of membrane-anchored carriers seems to play key roles in light-energy transduction pathways in anoxygenic photosynthetic bacteria.

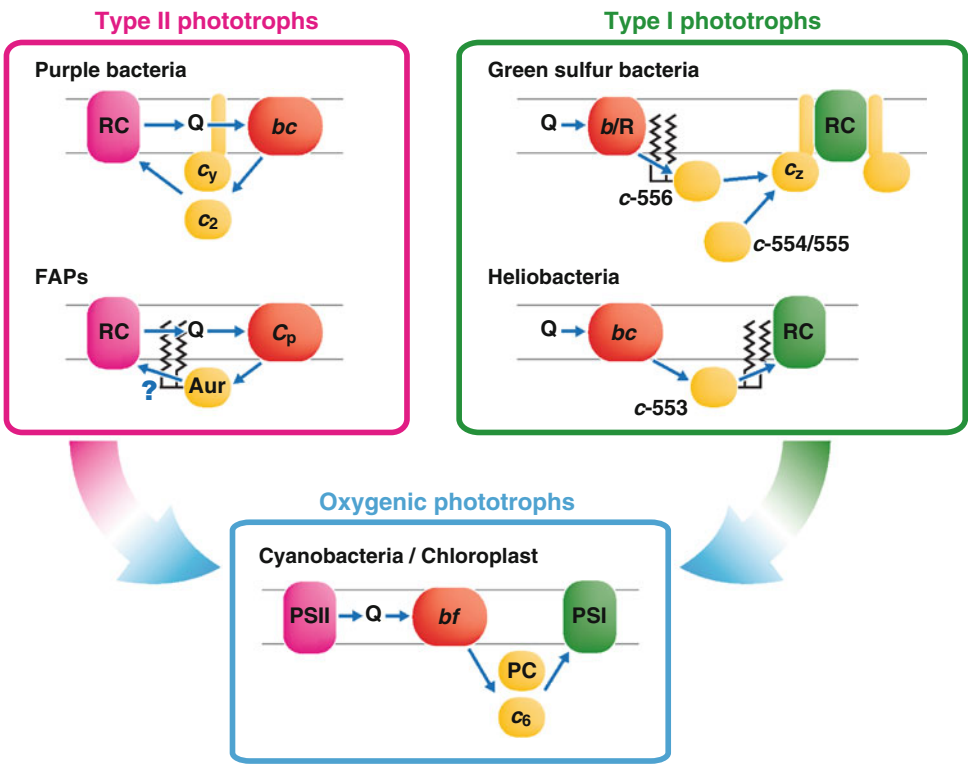


Fig. 1 Diagrammatic representation of photosynthetic electron transfer pathways in anoxygenic and oxygenic phototrophs. *Arrows* indicate the direction of electron flows. RC, PSI, PSII, *bc*, *b*/R, C_p, *bf*, c_2, c_y, c_z, *c*-554/555, *c*-556, *c*-553, c_6, PC, Aur, and Q represent reaction center PS I, PS II, cyt *bc* complex, cyt *b*/Rieske protein, cyt C_p complex, cyt *bf* complex, cyt c_2, cyt c_y, cyt c_z, cyt *c*-554/555, cyt *c*-556, cyt *c*-553, cyt c_6, plastocyanin, auracyanin, and quinone pool, respectively. The function of auracyanin is unknown at present (see text)

Green sulfur bacteria

Cyt *c*-554/555

The presence of low-molecular-weight (approx. 10,000) water-soluble cyt *c* in green sulfur bacteria was first reported in 1961 (Gibson 1961). Later, probably identical cyt *c* was isolated again in high-purity form from *Chlorobaculum* (*Cba.*) *parvum* and characterized in details, mainly by two research groups (Meyer et al. 1968; Yamanaka and Okunuki 1968). The absorption spectrum of the oxidized cyt *c* showed the Soret band at 413 nm, which was shifted to 419 nm upon its reduction, along with the appearance of α and β bands at 555 and 523 nm, respectively; the cyt was thus referred to as cyt *c*-555. It is characteristic that this cyt *c* gives an asymmetric α-absorption peak whose spectral shape resembles that of water-soluble cyt c_6, which functions as an electron donor to PS I RC (Yamanaka and Okunuki 1968; Morand et al. 1994). Soluble cyt *c* in *Cba. tepidum* was also isolated, but revealed an α-absorption peak at 554 nm (Oh-oka et al. 1998; Itoh et al. 2002). Therefore, we use cyt *c*-554/555 when there is no need to distinguish the same cyt *c* molecule based on its α-absorption peak and/or origin. Cyt *c*-554/555 is a basic protein with an isoelectric point (pI) of 10.5; its $E_{m,7}$ is estimated to range from +130 to 150 mV (Meyer et al. 1968; Yamanaka 1972; Selvaraj et al. 1998; Itoh et al. 2002). Its primary structure exhibits relatively high similarity to those of typical class I *c*-type cyts in which the heme-attachment site is located at the N terminus (Ambler 1991) (see Fig. 2).

In 1967, Sybesma and Beugeling suggested the involvement of heme *c* component(s) as electron donor(s) to the P840 RC as judged by absorption changes at the Soret (about 420 nm) and α bands (about 550 nm) in whole cells of *Prosthecochloris* (*Ptc*) sp. after constant illumination (Sybesma and Beugeling 1967). The most probable candidate was therefore assumed to be soluble cyt *c*-555 because its asymmetric α-absorption peak was similar to that of algal cyt c_6 (Yamanaka and Okunuki 1968). Long after, cyt *c*-554 was clearly shown to be oxidized after flash excitation using in vitro reconstitution with membranes from *Cba. tepidum* (Okumura et al. 1994) and then with the purified P840 RC complex from *Cba. tepidum* (Itoh et al. 2002). Furthermore, the direct reaction partner of cyt *c*-554 was revealed to be a secondary electron donor, cyt c_z (PscC), not the primary donor P840 (Itoh et al. 2002) (see Fig. 1). The second-order rate constant of this reaction was estimated to be 1.7×10^7 M^{-1} s^{-1}. If a large amount of cyt *c*-554/555 is assumed to be present in the periplasmic space in vivo at a concentration of a few millimolars, which is similar to the concentration range of cyt c_2 in purple nonsulfur bacteria (Crofts and Wraight 1983; Ortega et al. 1999), one would expect a rapid ET reaction to cyt c_z with a half time ($t_{1/2}$) of several to 10 μs.

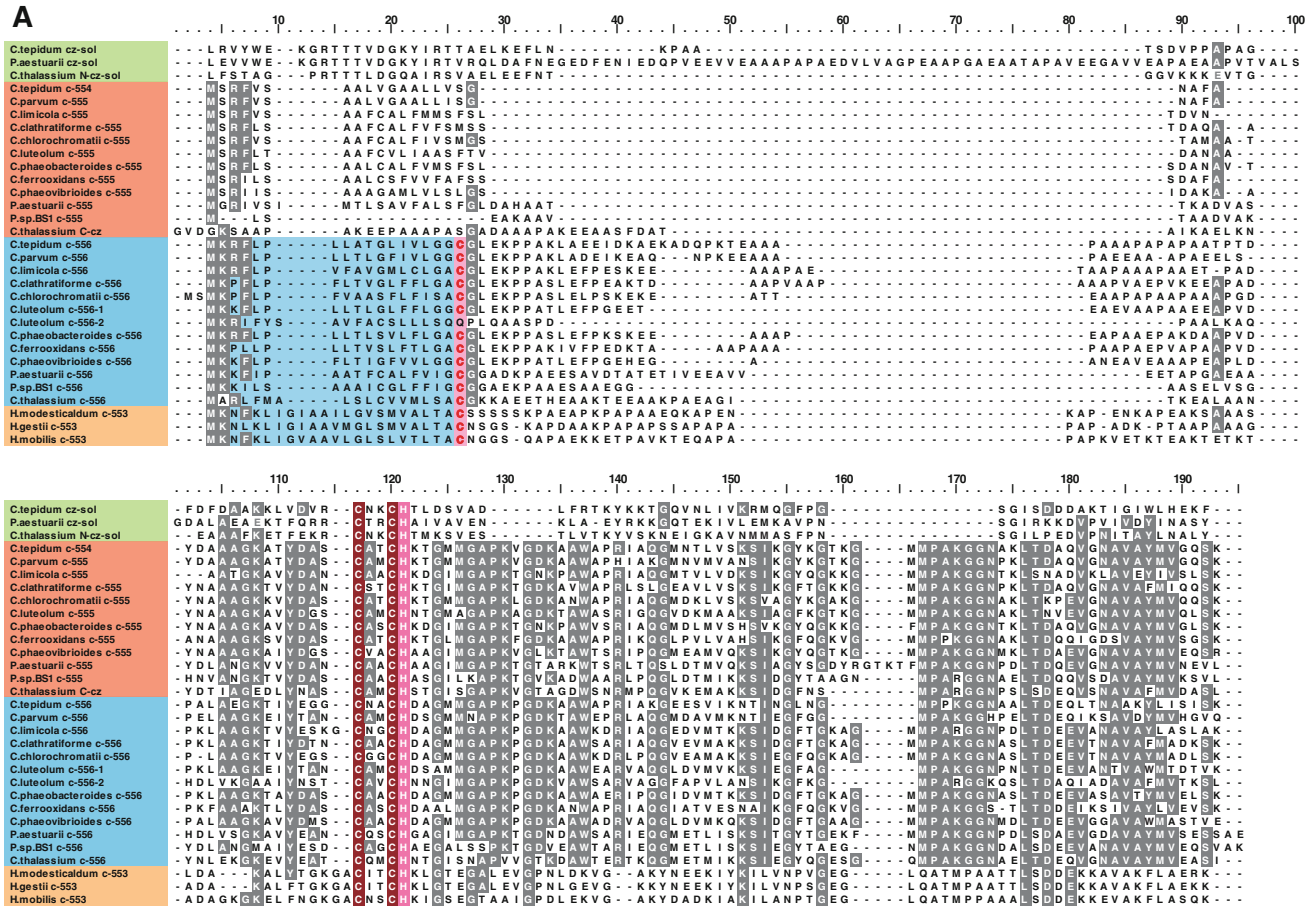
Cyt *c*-554/555 serves as an electron carrier in the oxidation pathways of reduced sulfur compounds. It has been demonstrated that cyt *c*-555 of *Chlorobium limicola* could be an electron acceptor from the thiosulfate-oxidizing enzyme system (the so-called Sox system); a reduction of cyt *c*-551, presumably corresponding to SoxAX, was accelerated by the addition of cyt *c*-555 in the presence of thiosulfate-cyt *c* reductase (Kusai and Yamanaka 1973b; Oh-oka and Blankenship 2004). A recent study confirmed this more clearly by observing the reduction of *Cba. tepidum* cyt *c*-554 in vitro, where the Sox system, including SoxYZ, SoxB, and SoxAX-CT1020, was reconstituted (Ogawa et al. 2008). Cyt *c*-554/555 also appears to be reduced efficiently by a sulfide oxidation system catalyzed by flavocyt *c* sulfide dehydrogenase (SoxEF) (Kusai and Yamanaka 1973a; Frigaard and Bryant 2008). However, cyt *c*-554/555 does not seem to be essential for photosynthetic growth although it functions as an efficient electron acceptor from thiosulfate oxidation (Tsukatani et al. 2006; Azai et al. 2009). It cannot mediate the ET reaction between menaquinol:cyt *c* oxidoreductase (cyt *bc* complex) and the P840 RC (Tsukatani et al. 2008).

Cyt c_z (PscC)

Secondary electron donor to the P840 RC

A monoheme cyt c_z, or PscC (Bryant 1994), one of the subunits in the green sulfur bacterial RC complex, functions as a secondary electron donor to the special pair of bacteriochlorophylls (Bchls) *a*, P840, and can be divided into two domains: one consists of the three membrane-spanning α-helices in the N-terminal half portion, and another comprises the hydrophilic C-terminal half with a single-heme attachment site (Okkels et al. 1992) (Fig. 1). This cyt c_z was designated as a new class of cyt *c*, class V, because its amino acid sequence shows no significant homology with those of already known *c*-type cyts. However, except for the N-terminal hydrophobic region, the C-terminal heme-binding portion could be structurally related to well-known soluble electron carriers like mitochondrial cyt *c* (Meyer and Cusanovich 2003) (see Fig. 2). Therefore, it would be logical to classify cyt c_z into a new subclass belonging to class I in which the heme-binding site is located at the N-terminus (Ambler 1991).

Using membrane preparations, cyt c_z was found to mediate the direct ET reaction from the cyt *bc* complex to the RC complex without any mobile electron carriers (Oh-oka et al. 1998), as mentioned below in detail (Fig. 1). This reaction mode is highly reminiscent of membrane-anchored cyt c_y, which also functions as a mediator



between cyt *bc* and the RC complexes in a purple nonsulfur bacterium, *R. capsulatus* (Jenney et al. 1994; Daldal et al. 2003). The cytochrome *c* subunit in the green sulfur bacterial RC complex was therefore designated as cyt c_z after its functional similarity to cyt c_y (Oh-oka et al. 1998).

A brief history of cyt c_z

The proper identification of cyt c_z has long been controversial: Which cyt *c* molecule—a monoheme- or tetraheme-type one—was responsible for the rereduction of

◀**Fig. 2** **a** Multiple alignment of amino acid sequences of green sulfur and heliobacterial *c*-type cyts (cyts c_z, *c*-554/555, *c*-556, and *c*-553) constructed with a Clustal W program. The typical binding motif for *c*-type heme, C-X-X-C–H, is completely conserved in all cyts *c*, and the relevant cysteine and histidine residues are boxed in *wine-red* and *pink*, respectively. The *blue-shaded* region is a signal-like peptide sequence for lipidation. The conserved cysteine residues just after the signal sequences are written in *red* and shaded in *pink*. **b** Cluster analysis of green sulfur and heliobacterial *c*-type cyts. The unrooted phylogenetic tree was constructed by neighbor-joining method with MEGA4 (Tamura et al. 2007). The bootstrap values were calculated with 1,000 replications and are shown by the sides of the branches. The *asterisks* represent green sulfur bacteria capable of thiosulfate oxidation. The mature proteins start from the 23rd tyrosine and 18th cysteine residues for cyts *c*-554 and *c*-556, respectively, in *Cba. tepidum* and from the 23rd cysteine residue for cyt *c*-553 in *Hbt. modesticaludam*. In the case of cyt c_z, the sequence of its C-terminal hydrophilic and soluble heme-containing domain (referred to as "cz-sol"), which corresponds to the one from the 96th amino acid in *Cba. tepidum*, was used for the analysis. Sequence data of cyt *c*-554/555 and *c*-556 homologs were obtained from the JGI microbial genomic database (http://img.jgi.doe.gov) by blast searches using *Cba. tepidum* *c*-554 (CT0075) and *c*-556 (CT0073) as queries. Although two candidates for cyt *c*-556 in the *Chl. luteolum* genome (*C. luteolum* *c*-556-1 and -2) were hit with high scores, the conserved cysteine residue was found only in the *Chl. luteolum* *c*-556-1. In *Chp. thalassium*, an *orf* gene (Ctha_1874) seems to encode a fused protein of cyt c_z and cyt *c*-555 at the N-terminal and C-terminal halves, respectively. These halves are therefore tentatively referred to as "N-c_z" and "C-c_z," respectively. Other sequence data were obtained from the JGI microbial genomic database and the GenBank database

oxidized $P840^+$? As this issue continues to seem confusing, we herein provide a brief history of how cyt c_z was identified as a secondary electron donor to the P840.

In 1971, Fowler et al. reported that the oxidation of "cyt *c*-553" was accompanied by bleaching of the P840 under continuous illumination in chlorosome-depleted membrane fragments from *Ptc.* sp. (note that "cyt *c*-553" represents an unidentified molecule of *c*-type cyt with an α-absorption peak of 553 nm) (Fowler et al. 1971). The heme *c* content in "cyt *c*-553" was estimated to be about four from the light-minus-dark difference spectrum of its membrane fragments. Prince and Olson (1976), using similar membrane fractions prepared from *Cba. thiosulfatiphilum*, then demonstrated that the rapid rereduction of $P840^+$ was concomitant with "cyt *c*-553" oxidation with a biphasic decay behavior exhibiting half times ($t_{1/2}$) of <5 and 50 μs after a flash excitation. Swarthoff et al. (1981) observed "cyt *c*-553" oxidation showing a single exponential decay phase with a $t_{1/2}$ of 90 μs in detergent-solubilized RC preparations from *Ptc.* sp. However, the activity of "cyt *c*-553" oxidation was unstable and drastically decreased after membrane solubilization, suggesting some damage to the donor side of the RC as a result of the preparation methods used. In membranes from *Cba. parvum*, the flash-induced oxidation of P840 was recovered with a slightly different $t_{1/2}$ of 50–80 μs by a concomitant oxidation of "cyt *c*-553" (Miller et al. 1992). Thus, all of these data indicated that membrane-associated tetraheme-type "cyt *c*-553" appeared to be involved in electron donations to the photooxidized $P840^+$ in green sulfur bacteria, although the time constants and decay phase(s) were variable among preparations, as mentioned above.

In the meantime, a crude fraction containing the P840 RC was obtained from *Cba. thiosulfatiphilum*, which included tetraheme-type cyt *c*-553 with an apparent molecular mass of 35 kDa (Feiler et al. 1992). Although this fraction never showed any photoactivity of cyt *c* oxidation, its α-absorption peak and heme content seemed to meet the criteria of the secondary electron donor, cyt *c*. On the other hand, monoheme-type cyt *c*-551, with an apparent molecular mass of 18 kDa corresponding to cyt c_z, as already explained above, was shown to partly rereduce the flash-oxidized $P840^+$ in the purified RC complex from *Cba. parvum* (Okkels et al. 1992). A crude RC fraction from *Cba.* sp. (ATCC17092) was reported to contain two *c*-551 hemes per P840 (Oh-oka et al. 1993). Therefore, it gave rise to a great deal of discussion with regard to which cyt *c* was truly responsible for the $P840^+$ rereduction. This debate was finally settled by the isolation of the stable RC complex from a mesothermophilic *Cba. tepidum* under anaerobic conditions (Oh-oka et al. 1995). In this research, two copies of membrane-bound monoheme cyt c_z were shown to serve as direct electron donors to the P840. At almost the same time, two other groups obtained similar preparations from *Cba. tepidum* (Kusumoto et al. 1994) and *Cba. thiosulfatiphilum* (Hager-Braun et al. 1995), respectively.

The α-peak wavelength of cyt c_z in our preparation of *Cba. tepidum* RC complex, which was solubilized with sucrose monolaurate, was 552 nm. This was slightly different from the value of 551 nm in other RC preparations from *Cba. thiosulfatiphilum* (Hurt and Hauska 1984; Hager-Braun et al. 1995), *Cba. Parvum* (Okkels et al. 1992), and *Cba.* sp. (ATCC17092) (Oh-oka et al. 1993) or even the value of 553 nm found in membrane preparations from the same species of *Cba. tepidum* (Okumura et al. 1994). The discrepancy may be ascribed to some structural modification of cyt c_z induced by different solubilization procedures and/or it may be a species-dependent difference. In fact, cyt c_z in the *Cba. tepidum* RC complex exhibits an α-absorption peak of 551 nm when solubilized with Triton X-100 (Kusumoto et al. 1994). The RC core complex from *Ptc.* sp. contained no cyt c_z, suggesting that it is rather loosely bound (Francke et al. 1997). It is characteristic that an insertion of 52 amino acid residues can be recognized between the N-terminal and C-terminal domains only in *Ptc.* sp. cyt c_z (see Fig. 2a). Later, the tetraheme-type cyt *c*-553 was isolated from *Cba. thiosulfatiphilum* and characterized in detail with an electron spin

resonance (ESR) spectroscopic method, but its function is still unknown (Albouy et al. 1997).

Reaction characteristics of cyt c_z

The ET rate from cyt c_z to the $P840^+$ was first indicated to be dependent upon temperature: The $t_{1/2}$s were 110 and 340 μs at 297 and 285 K, respectively (Oh-oka et al. 1995). This thermodynamic variability was subsequently verified by examining in detail the effect of viscous medium on the ET reaction (Oh-oka et al. 1997). The rate constant decreased from $5.0 \times 10^3\ s^{-1}$ to $1.0 \times 10\ s^{-1}$ as the glycerol concentration increased from 0 to 60% (V/V) at 296 K. No absorption change ascribable to the oxidation of cyt c_z was observed at low temperature, and the rereduction of photooxidized $P840^+$ proceeded with the time constant $t_{1/e} = 50$ ms due only to the back reaction from $(F_A/F_B)^-$. This dependence on viscosity implies that the C-teminal domain of cyt c_z, which attaches a heme prosthetic group, fluctuates on the surface of the RC by anchoring to membranes through its N-terminal hydrophobic domain while searching for its reaction partners around the P840 (Oh-oka et al. 1998; Tsukatani et al. 2008).

Taking into account the extraordinary high viscosity dependence of the cyt c_z reaction, it would not be unreasonable to find that many different, seemingly conflicting, results were observed regarding the time constants as well as decay phase(s) for the ET reaction from membrane-bound cyt c_z to the $P840^+$. The reactivity of cyt c_z seems to be affected by a wide variety of factors including temperature, viscosity of media, detergents, preparation methods, etc., which might potentially modify the motion of the heme-containing moiety.

An attempt was made to understand the mechanism of the viscosity-dependent reaction of cyt c_z by introducing a solvent-fluctuation-controlled mode to the transition state theory (TST) (Oh-oka et al. 1997). In a high-viscous solvent system, the observed rate constant, k_{obs}, becomes inversely proportional to the fractional power β of the viscosity η (Sumi 1991; Asano et al. 1994).

$$k_{obs} = B\eta^{-\beta} \quad (0 < \beta < 1)$$

However, the exponent β for cyt c_z oxidation was found to be 1.9 at 283 K and 2.4 at 295 K. Although the cyt c_z reaction can be understood within the framework of the Sumi theory, it might be necessary to consider other factor(s) formulated from the particular structural characteristics of cyt c_z since the two copies of cyt c_z appear to function independently and equivalently to each other (Kusumoto et al. 1999).

The C-terminal hydrophilic heme-containing moiety has been overexpressed in *Escherichia coli* and characterized in detail with spectroscopic techniques (Higuchi et al. 2009). The soluble domain contained a *c*-type heme with a low-spin state similar to that found in usual class I cyts *c*. Furthermore, its three-dimensional (3D) structure, which has recently been determined (Hirano et al., a manuscript in preparation), strongly supports the idea that cyt c_z as well as any other membrane-anchored cyt *c* including cyt c_y is a subclass of class I cyts *c*.

Cyt *c*-556

The presence of membrane-bound cyt *c*-556 has been suggested from the chemically reduced-minus-oxidized difference spectrum of purified membranes from *Cba. tepidum* (Oh-oka et al. 1998). In the presence of stigmatellin, an inhibitor of the Q_o site of the cyt *bc* complex, the time-resolved difference absorption spectra indicated the appearance of a clear shoulder at approximately 556 nm. The peak at 552 nm responsible for the cyt c_z oxidation was red-shifted slightly to 556 nm within 3–5 ms after flash excitation. This indicates the sequential oxidations of two distinct hemes *c*; namely, the ET reaction from heme *c*-556 to heme *c*-552 (heme c_z), resulting in the rapid equilibration between them in accordance with the difference in their redox potentials (see Fig. 1). We have recently confirmed the spectral changes at around 552–556 nm, again using membrane preparations from a cyt *c*-554 deletion mutant of *Cba. tepidum* in order to exclude the possibility that residual cyt *c*-554 in the wild-type membranes might be the cause of the shoulder at 556 nm (Tsukatani et al. 2008).

A typical cyt *bc* complex found in *Proteobacteria* consists of three subunits: the Rieske iron-sulfur protein, cyt *b* and cyt c_1, which are encoded by the *fbcF* (*petA*), *fbcB* (*petB*), and *fbcC* (*petC*) genes, respectively, in a single transcriptional unit. In green sulfur bacteria, no *fbcC* gene has been found either downstream or upstream of the unit housing the *fbcF* and *fbcB* genes (Schütz et al. 1994). Therefore, menaquinol:cyt *c* oxidoreductase in *Chlorobia* has been regarded as a cyt *b*/Rieske-type (Hauska et al. 2001). However, our spectroscopic data have strongly suggested that cyt *c*-556 plays a role corresponding to the function of cyt c_1, although its estimated molecular mass of 15–20 kDa, as revealed on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis, is smaller than that of cyt c_1 (Oh-oka et al. 1998).

Comparative genomic analyses have enabled us to specify five candidate genes that probably encode cyt *c*-556 (*CT0073*, *CT0188*, *CT1704*, *CT1734*, and *CT2026*) in *Cba. tepidum*. The gene *CT0073* would be the best candidate, judging from its location upstream of *CT0075* encoding cyt *c*-554, and both products appear to be functionally related to each other. In fact, the adjacent arrangement of *CT0073* and *CT0075* homologs is highly conserved in green sulfur

bacteria whose genome sequences were so far determined (see below). It is noteworthy that the thiol group of the residue cysteine18 in cyt *c*-556 could be modified with fatty acid chains after the cleavage of its signal peptide, as predicted by the ScanProsite analysis of prokaryotic membrane lipoproteins (PROSITE profile: PS51257) on the ExPASy web server (de Castro et al. 2006). Cyt *c*-556 might thus be anchored into membranes and may serve as an electron carrier, substituting for cyt c_1 in the ordinary cyt *bc* complex.

Heliobacteria

Cyt *c*-553 (PetJ)

Heliobacteria are gram-positive bacteria and have no periplasmic space housing soluble electron carriers. The electron donor to the special pair of Bchls *g* (or *g*′), P800, is membrane-bound cyt *c*-553 (PetJ), which has an apparent molecular mass of 17 kDa (Oh-oka et al. 2002) (see Fig. 1). This cyt *c*-553 is supposed to be anchored into membranes through fatty acid chains covalently bound to its amino terminal cysteine residue (Albert et al. 1998). The prediction of its protein motif was also true for the ScanProsite analysis of prokaryotic membrane lipoproteins as mentioned above (data not shown).

The apparent activation energy (E_a) values for the ET reaction from cyt *c*-553 to the P800 were estimated to be 88.2 and 58.9 kJ/mol in whole cells and membranes of *Heliobacterium gestii*, respectively. These values were comparable to that (101 kJ/mol) obtained for cyt c_z oxidation in *Cba. tepidum* (Oh-oka et al. 1997), but much higher, by more than a factor of 10, than that (3.6–8.6 kJ/mol) for the reaction of tetraheme-type cyt *c* in the P860 RC of the purple nonsulfur bacterium, *Blastochloris viridis* (Ortega and Mathis 1993). A slightly lower E_a of 21–27 kJ/mol was also obtained for the ET reaction from soluble cyt c_2 to the P860 RC of *Rhodobacter sphaeroides* (Venturoli et al. 1993). Therefore, the high E_a values for the reaction of cyt *c*-553 in heliobacteria should reflect thermal fluctuations of the heme-binding moiety, as in the case of cyt c_z in green sulfur bacteria. In fact, cyt *c*-553 oxidation was completely inhibited at a temperature below 200 K (Miyamoto et al. 2006).

The ET rate from cyt *c*-553 to P800 in membrane preparations was also drastically increased by the addition of the divalent cation, Mg^{2+} (Oh-oka et al. 2002). This situation is very similar to the reaction between soluble plastocyanin and P700 in PS I (Itoh 1979; Tamura et al. 1983; Hervás et al. 1992; Hope 2000). A reduction in the electrostatic repulsion between negatively charged surfaces of both cyt *c*-553 and the P800 RC would be induced by the addition of the Mg^{+} ion. Alternatively, a decrease in the surface potentials on the membrane might be affected by screening net negative charges as defined by the Gouy–Chapman theory (Itoh 1979). In fact, a slightly positive shift of the apparent redox potential of cyt *c*-553 was observed in the presence of Mg^{2+}, which might have resulted in the acceleration of the ET rate by increasing the amount of photooxidizable heme *c* (Oh-oka et al. 2002). In order to understand these reaction mechanisms on the molecular level, the 3D structures of relevant proteins will be required.

Membrane-anchored cyt *c*-553 in heliobacteria also serves as an electron mediator between cyt *bc* and the P800 RC complexes (Oh-oka et al. 2002) (see Fig. 1). The cyt *bc* complex in heliobacteria was shown to be inhibited by stigmatellin, an inhibitor of Q_0-site, but not antimycin A. The operon encoding this complex consists of four genes, *petC*, *B*, *D*, and *X*, which encode the Rieske [2Fe–2S] protein, cyt b_6, subunit IV, and diheme-type cyt *c*, respectively (Xiong et al. 1998). The complex, therefore, resembles the *bf*-type complex since cyt *b* component is split into cyt b_6 and subunit IV compared to the typical one in *Proteobacteria*.

However, the whole complex has not ever been isolated for biochemical characterization except for the recent report of a crude fraction rich in cyt *b* and Rieske protein (Ducluzeau et al. 2008). The gene encoding cyt *c*-553 (*petJ*) is located upstream of the *petCBDX* operon, implying that cyt *c*-553 is functionally related to cyt *bc* complex.

Evolutionary aspects of green sulfur and heliobacterial cyts *c*

Recent genomic analyses of 12 species of green sulfur bacteria have revealed that an orthologous gene encoding a low-molecular-weight, soluble monoheme cyt *c*-554/555 is present in all of them (Fig. 2a). Although their deduced amino acid sequences exhibit a relatively wide range of identities (41–91%) to each other, the identities in species containing the Sox gene cluster exhibit a higher identity proportion (approximately 64–91%). An orthologous gene encoding cyt *c*-556 is also present as probable membrane-bound *c*-type cyt in all species, and their amino acid sequences exhibit 31–75% identities to each other. Except for *Chloroherpeton thalassium* and *Chl. chlorochromatii*, the gene for cyt *c*-556 is located immediately upstream of the gene for cyt *c*-555; however, in the *Cba. tepidum* genome only, an ORF (*CT0074*) is located between two genes for cyts *c*-554 and *c*-556, although the annotation of *CT0074* is doubtful. These genomic organizations strongly suggest that the ET pathways involving both cyts *c*-554/555 and

c-556 are evolutionarily conserved in green sulfur bacteria regardless of their capability for thiosulfate oxidation.

The cluster analysis of green sulfur bacterial cyts c_z, *c*-554/555, and *c*-556, as well as heliobacterial cyt *c*-553, has clarified three major groups, as shown in Fig. 2b. Cyts *c*-554/555 and *c*-556 are closely related to each other but distantly related to cyt c_z, suggesting that the former two share a paralogous relationship with each other as electron donors to cyt c_z. Cyts *c*-554/555 in four thiosulfate-oxidizing species (*Cba. tepidum*, *Cba. parvum*, *Chl. phaeovibriodes*, and *Chl. clathratiforme*) do not form a monophyletic cluster, implying that they might have evolved independently in accordance with physiological demands of individual species. Furthermore, cyt *c*-555 of *Chl. ferroxidans* shows a high sequence identity (approximately 69%) compared to those of thiosulfate-oxidizing species. *Chl. ferroxidans* does not utilize any reduced sulfur compounds as electron sources but oxidizes ferrous ion to ferric ion probably by using cyt *c*-555 as an electron carrier. In general, cyts would be evolutionarily "adaptive" or "flexible" to physiological variations and have different functions depending on species (Meyer and Cusanovich 2003).

Implication of membrane-anchored electron carriers in anoxygenic phototroph

The ET reaction from cyt *bc* complex to the RC, which could be mediated by a membrane-anchored electron carrier, was first discovered in *R. capsulatus* (Jenney and Daldal 1993) (see Fig. 1). The time constant of this reaction, operated by cyt c_y, was estimated to be sufficiently fast enough (in the microsecond time range) to compete with cyt c_2. However, only ca. 30% of the photooxidized RC could be rereduced by direct contact with cyt c_y (Myllykallio et al. 1998). In contrast, the tightly coupled or stoichiometric reactions could be observed in the case of cyt c_z (PsaC) of green sulfur bacteria and cyt *c*-553 (PetJ) of heliobacteria, indicating most RC fractions are directly associated with cyts *c*.

Little is known about photosynthetic ET pathway in filamentous anoxygenic phototrophs (FAPs; formerly called green nonsulfur bacteria), which are not closely related to green sulfur bacteria with the exception of having a common photosynthetic apparatus—the large chlorosome antenna complex. Recent studies have suggested that membrane-bound auracyanin, a blue-copper protein with an E_m of 200–250 mV, might transfer electrons from membranous oxidoreductase (presumably a multi-subunit complex homologous to molybdopterin oxidoreductase and containing *c*-type cyts; cyt C_p) to the type 2 RC (Yanyushin et al. 2005; Tsukatani et al. 2009).

Why do photosynthetic bacteria utilize membrane-anchored electron carriers? These carriers would help to increase the efficiency of the ET reactions between adjacent membranous complexes by shuttling across the limited spaces. This reaction mode might therefore be the most primitive one in the evolutionary stage of the photosynthetic energy transduction pathway. However, the restriction to diffusible regions in searching for ET partners along the membrane surface would be unfavorable for the development of complicated acclimation mechanisms in response to environmental alterations. Instead, water-soluble electron carriers (cyt c_6 or plastocyanin) would have evolved in cyanobacteria, algae, and higher plants to allow them to acclimate more easily, e.g., to reorganize light-harvesting proteins in thylakoid membranes (Takahashi et al. 2006) and to change the ratio of PS I/II at the transcription and/or translation level as well (Murakami and Fujita 1991; Kulkarni and Golden 1994).

Concluding remarks

In the past decade, we have revealed the photosynthetic ET pathway around the green sulfur bacterial type 1 RC, focusing especially on the function of electron donors cyts c_z (PscC) and *c*-554/555. Cyt c_z is a monoheme type and not a tetraheme type, contrary to the previous anticipation, and two copies of cyt c_z function equivalently to donate electrons to the P800. Cyt *c*-554/555 does not serve as a shuttle-like electron carrier between cyt *bc* and the RC complexes. On the other hand, the ET pathway in heliobacteria still remains unknown except for the role of the membrane-anchored cyt *c*-553 (PetJ) in mediating a direct ET reaction from the cyt *bc* complex to the RC. The most difficult factor in the research on these ET pathways is the fragile nature of ET components (iron-sulfur centers and Bchls) since they can easily be damaged upon exposure to oxygen. However, clarification of the molecular architecture in both primitive photosynthetic anaerobes is undoubtedly important to our understanding of the primordial process that built up the versatile photosynthetic ET pathways that confer tremendous bioenergetic benefits to terrestrial creatures.

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