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Invited Article

Comparative and Evolutionary Aspects of the Photosynthetic Electron Transfer System of Purple Bacteria

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The cyclic electron transfer system in purple bacteria is composed of the photosynthetic reaction center, the cytochrome bc_1 complex, cytochrome c_2, and ubiquinone. These components share many characteristics with those of photosynthesis and respiration in other organisms. Studies of the cyclic electron transfer system have provided useful insights about the evolution and general mechanisms of membranous energy-conserving systems. The photosynthetic system in purple bacteria may represent a prototype of highly efficient, energy-conserving electron transfer systems in the organisms.

Key words: Photosynthesis — Electron transfer — Purple bacteria — Reaction center — Cytochrome *c* — Cytochrome *bc* complex

There are many similarities in the electron transfer systems of photosynthesis and respiration in various organisms, in spite of large variations in energy sources and terminal electron donors and acceptors in these organisms. Comparative studies have contributed in revealing the structure and function of these components, especially at the molecular and submolecular levels, and have began to explain the evolution of energy-conserving electron transfer systems.

The purple photosynthetic bacteria constitute one of five groups of photosynthetic eubacteria (Woese 1987). Some species of purple bacteria have been used extensively in studies of photosynthesis, as a model of more complicated oxygenic photosynthesis in cyanobacteria and chloroplasts. The phylogenetic group of purple bacteria is also thought to include the prokaryotic ancestor of mitochondria. Thus, the purple photosynthetic bacteria are key organisms for understanding the membranous energy-conserving systems of photosynthesis and respiration.

The present paper summarizes current understandings about the photosynthetic electron transfer system in purple bacteria with special attention to the author's studies, and compares the structure and function of its components within the purple bacteria, and in other bacteria, chloroplasts, and mitochondria. The evolutionary implications of these findings are also discussed.

Photosynthetic Electron Transfer System in Purple Bacteria

The evolutionary position of purple bacteria

The group of purple photosynthetic bacteria is one of five groups of photosynthetic eubacteria (Fig. 1), and includes both sulfur and non-sulfur purple bacteria. This group conducts anoxygenic photosynthesis, and uses sulfur compounds, organic compounds, or hydrogen molecules as terminal electron donors. Each organism in this group contains one type of photosynthetic reaction center (RC) complex in the photosynthetic membrane, in contrast to cyanobacteria and chloroplasts, which conduct oxygenic photosynthesis and have two kinds of RCs. The light-harvesting pigment systems of purple bacteria are also simpler than those in oxygenic organisms. The simplicity of the photosynthetic systems of purple bacteria make this group useful for studying the general mechanisms of photosynthesis.

Based on the 16S rRNA sequence analysis of various organisms, Woese *et al.* (Woese and Fox 1977, Woese 1987, Woese *et al.* 1990) classified all organisms into three kingdoms : archaebacteria (Archaea), eubacteria (Bacteria), and eukaryotes (Eucarya). Eubacteria were classified into at least ten divisions, five of which include

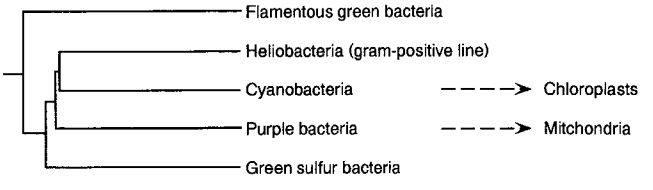


Fig. 1. Phylogenetic relationships of five groups of photosynthetic bacteria. The phylogenetic tree is based on Woese (1978).

* Recipient of the Botanical Society Award of Young Scientists, 1992, Abbreviations : BChl, bacteriochlorophyll ; $(BChl)_2$, the special pair of bacteriochlorophyll in the photosynthetic reaction center complex ; RC, photosynthetic reaction center.

photosynthetic bacteria. The group of purple bacteria discussed in the present paper comprises one of these divisions, and is further divided into five subclasses: alpha, beta, gamma, delta, and epsilon. The phylogenetic group of purple bacteria also includes many non-photosynthetic bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, so it has been the most widely investigated bacterial division. The non-photosynthetic members in the division appear to have lost their capacity for photosynthesis through evolution; the common ancestor of the division is thought to be a photosynthetic bacterium. Moreover, since the basic photosynthetic mechanisms are shared by all photosynthetic eubacteria, including oxygenic cyanobacteria, the ancestor of most eubacteria is suggested to be a photosynthetic bacterium that had chlorophyll-type pigments and membranous, photosynthetic electron transfer systems.

Cyclic electron transfer system

The photosynthetic electron transfer system of purple bacteria is cyclic (Fig. 2), and is composed of four components: two are membrane-spanning large complexes of proteins and prosthetic groups, i.e., the RC complex and the cytochrome bc_1 complex; the other two are the mobile electron carriers, ubiquinone and cytochrome c_2. The energy of light absorbed by bacteriochlorophylls and carotenoids in the light-harvesting systems are transferred to the special pair of bacteriochlorophylls in the RC complex and release an electron from the special pair. Through several intermediates in the photosynthetic complex, the electron is transferred to the quinone molecules and delivered to the cytochrome bc_1 complex. After a complicated electron transfer in the complex, the electron returns to the RC via soluble cytochrome c_2. Protons are transferred across the membrane by energy-conserving reactions coupled to the electron transfer through the bc_1 complex.

All photosynthetic organisms, except a group of Halobacteria in archaebacteria, have corresponding components to the four components of the cyclic electron system in purple bacteria. In the following sections, three proteinous components are discussed.

Photosynthetic Reaction Center Complexes

Two types of RC in purple bacteria

The RC complexes of purple bacteria can be classified into two groups: those with and those without a bound cytochrome subunit containing four c-type hemes (Dutton and Prince 1978, Matsuura and Shimada 1990). Three-dimensional structures of the RCs of *Rp. viridis* (Deisenhofer *et al.* 1985) and *Rb. sphaeroides* (Allen *et al.* 1987) have been revealed by X-ray analysis of their crystals. In the RC of *Rp. viridis*, the cytochrome subunit is bound to the periplasmic side of the RC complex, and that of *Rb. sphaeroides* does not have the subunit (Fig. 2). Except for the presence of the bound cytochrome c, the two types of RC complexes are similar to each other with respect to pigments, redox centers, and polypeptide compositions. The role of the RC in the cyclic electron system is also identical irrespective of the type of RC (Fig. 2).

In the RC of purple bacteria, photosynthetic energy transduction is initiated by photo-excitation of the special pair of bacteriochlorophyll ($(BChl)_2$). An electron from the excited $(BChl)_2$ is donated to a quinone acceptor (QA) through another BChl and a bacteriopheophytin so that a stable charge separation state, $(BChl)_2^+ : Q_A^-$, is generated. In the case of an RC with a bound cytochrome c subunit, the oxidized special pair is rapidly re-reduced by one of the hemes in the subunit. The particular heme that acts as a stable electron donor varies depending on the ambient redox potential. The electron from any of the hemes, except the one adjacent to $(BChl)_2$, is considered to pass through other hemes on the way to the $(BChl)_2^+$. In species without tightly bound cytochrome *c*, soluble cytochrome c_2 is an immediate electron donor to $(BChl)_2^+$ (Fig. 2). Although cytochrome c_2 has been suggested to be bound weakly to the RC complex under some conditions (Dutton *et al.* 1975), the bound form of this cytochrome can be easily removed from the membrane by salt

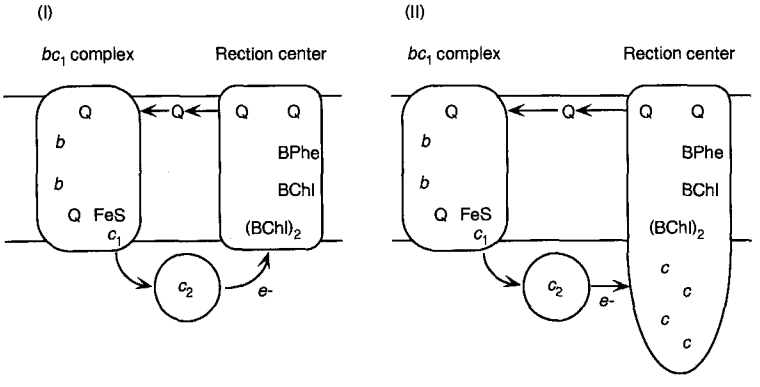


Fig. 2. Schematic representation of the cyclic electron transfer system and two types of reaction center in purple bacteria. (I), reaction center without the bound-cytochrome c subunit; (II), that with the subunit. Q, quinone; *b*, cytochrome *b* heme; FeS, iron-sulfur cluster; *c*, cytochrome *c* heme; Bphe, bacteriopheophytin.

washing.

In the case of the RC with the tightly bound cytochrome subunit, soluble cytochrome c_2 works as an electron donor to the bound cytochrome heme. In *C. vinosum* cytochrome *c*-551, a water-soluble cytochrome, which is suggested to be related to cytochrome c_2 (Dickerson *et al.* 1976), was shown to donate electrons to photooxidized cytochrome *c*-555 within milliseconds (Van Grondelle *et al.* 1977). Cytochrome c_2 in *Rp. viridis* was also shown to be a donor to the membrane-bound cytochrome *c*-558 in a membrane preparation (Shill and Wood 1984). In this respect, the tightly bound cytochrome *c* does not seem to be an alternative to the water-soluble cytochrome c_2, but instead appears to be an additional component in the electron path from soluble cytochrome c_2 to $(BChl)_2$ (Fig. 2).

Evolutionary relationships between two types of RC in purple bacteria

We have investigated the evolutionary origins of the two types of RC and the role of the bound cytochrome subunit. The types of RC in many non-sulfur purple bacteria and their placement in the phylogenetic tree have been shown (Dutton and Prince 1978, Matsuura and Shimada 1986, Fukushima *et al.* 1988, Matsuura and Shimada 1990, Nagashima *et al.* 1993a), and are summarized in Fig. 3 in terms of the presence or absence of the cytochrome subunit, together with the type of soluble cytochrome c_2 (Dickerson 1980). The phylogenetic tree was based on the 16S rRNA sequence (Woese 1987).

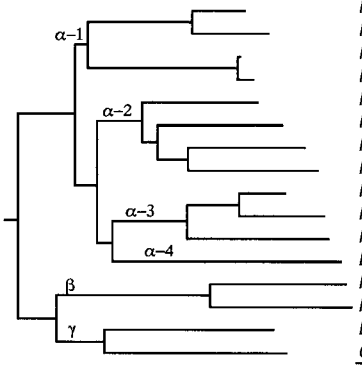
Rb. sphaeroides, Rb. capsulatus, Rs. rubrum and Rp. palustris possess RCs without tightly bound cytochromes and have been used extensively in studies of photosynthesis, partly because they are easy to grow and maintain in laboratory culture. All these species grow well anaerobically in photosynthesis and aerobically in respiration. There are many other purple photosynthetic bacteria whose growth conditions are more restricted to anaerobic conditions or aerobic conditions.

Both types of RCs were widely distributed in the alpha subclass, and all the species in the beta and gamma subclasses have RCs with the bound cytochrome subunit. The bound cytochrome subunits of all species are presumed to have similar characteristics, i.e., four hemes per RC with a wide range of midpoint potentials, rapidly oxidizable by the oxidized $(BChl)_2$, and reducible by soluble cytochrome c_2 in the periplasmic space. In the comparison of the three-dimensional structure of the RC complexes of *Rp. viridis* and *Rb. sphaeroides*, the bound cytochrome subunit is found to be attached supplementarily to the L and M subunits at the periplasmic side of the complex (Deisenhofer *et al.* 1985, Allen *et al.* 1987). These observations and the phylogenetic relationship (Fig. 3) suggest that the ancestral purple bacteria had an RC with the cytochrome subunit and that the subunit was independently lost in at least four lines in each subgroup of the alpha subclass (Matsuura and Shimada 1990).

Direct and indirect electron transfer

RC complexes isolated from species that possess the bound cytochromes usually retain the tightly bound cytochrome subunit. An exception is *Rubrivivax gelatinosus* (formaly called *Rhodocyclus gelatinosus*) which belong to the beta subclass of purple bacteria (Fukushima *et al.* 1988). In *Rv. gelatinosus*, the cytochrome subunit has been shown to be lost during the preparation of RCs. RC complexes isolated from the membranes of *Rv. gelatinosus* have no cytochromes, while whole cells and membrane preparations do have cytochromes bound to the photosynthetic RC. Fukushima *et al.* (1988) isolated RC-B870 pigment-protein complexes from *Rv. gelatinosus* in which the cytochrome subunit was retained. This led us to examine the effect of removing the cytochrome subunit from the RC complex on the electron transfer from cytochrome c_2 (Matsuura *et al.* 1988a).

In the RC-B870 complex, which consists of six polype-



	RC bound cyt. c subunit	Cyt. c_2 type
Rs. rubrum	-	L
Rs. photometricum	+	L
Rs. molischianum	+	M
Rs. fulvum	+	M
Rm. vannielii	+	M
Rp. viridis	+	M
Rp. acidophila	+	M
Rp. palustris	-	L(M)
Rb. sphaeroides	-	L
Rb. capsulatus	-	L
Ro. denitrificans	+	L
Er. longus	-	?
Rv. geratinosus	+	S
Rc. tenuis	+	S
Ec. halophila	+	(S)
Ch. vinosum	+	(S)

Fig. 3. Phylogenetic relationships of purple bacterial species and the presence of RC-bound cytochrome *c* subunit and the type of cytochrome c_2. The phylogenetic tree is based on 16S rRNA (Woese 1987). This figure was redrawn from Matsuura and Shimada (1990).

ptides including a bound cytochrome subunit, cytochrome *c* from horse heart or cytochrome c_2 from *Rv. gelatinosus* was oxidized by the photo-oxidized $(BChl)_2$ indirectly via cytochrome *c*-555 in the complex. In the RC core complex composed of two polypeptides, the oxidized $(BChl)_2$ was reduced directly by the soluble cytochromes. Direct electron transfer from soluble cytochrome c_2 to the isolated RC core complex is probably an artificial rather than natural, physiological pathway. However, the rate of direct electron transfer to the oxidized $(BChl)_2$ is as fast as that from the soluble cytochrome to the cytochrome *c*-555 bound to the RC-B870 complex. This suggests that the direct pathway from soluble cytochrome c_2 to the $(BChl)_2$ would also be possible in vivo if the bound cytochrome subunit were lost by mutation (Matsuura *et al.* 1988a).

As described in the previous section, we have suggested that, according to phylogenetic analysis, the RCs of these species may have evolved by the mutational loss of a bound cytochrome subunit (Matsuura and Shimada 1990). The results of the biochemical removal of the subunit (Matsuura *et al.* 1988a) lend support to this hypothesis by showing that artificial removal of the bound cytochrome does not markedly change the rate of electron transfer from cytochrome c_2 to the photo-oxidized $(BChl)_2$ in RCs of *Rv. gelatinosus*. If the response of *Rv. gelatinosus* is applicable to other species, it can be suggested that mutational loss of the bound cytochrome subunit from the RC would not result in serious risk to survival, since direct electron transfer between cytochrome c_2 and the RC $(BChl)_2$ is possible and is of comparable efficiency. A study with the method of sight-directed mutagenesis in *Rv. gelatinosus* is under investigation to examine this possibility.

Comparative studies of genes for the RC in purple bacteria

In order to study the variation of RC structures in purple bacteria further, we have made comparative analyses of the gene structure of the RC polypeptides (Nagashima *et al.* 1993b, Nagashima *et al.* 1994; Nagashima, K.V.P., Hiraishi, A., Shimada, K. and Matsuura, K., in preparation). Genes coding for subunits of the RC complex form the *puf* operon, together with genes coding for the alpha and beta subunits of the light-harvesting 1 (LH1) complex (Fig. 4). The nucleotide sequence of the *puf* operon has been determined in several species belonging to the alpha subclass of purple bacteria (Williams *et al.* 1983, Kiley *et al.* 1984, Youvan *et al.* 1984, Michel *et al.* 1986). The order of the genes in the *puf* operon is conserved, from the upstream, *puf*B, *puf*A, *puf*L, and *puf*M which code for the beta and alpha subunits of LH1, and the L and M subunits of the RC, respectively (Fig. 4). In the species having the cytochrome subunit in the RC, a gene for this subunit, *puf*C, is found immediately downstream of *puf*M (Michel *et al.* 1986). The gene coding for the H subunit has been shown to be coded in the *puh* operon, about 40 kb upstream from the *puf* operon in *Rb. capsulatus* (Youvan *et al.* 1984), and some 30 kb away in *Rb. sphaeroides* (Lee *et al.* 1989).

We determined the nucleotide sequence of the *puf* operon of *Rv. gelatinosus* (Nagashima *et al.* 1994), a photosynthetic bacterium belonging to the beta subclass of purple bacteria. The operon contains two unknown open reading frames in addition to five photosynthetic genes which have been reported in species belonging to the alpha subclass. As described above, detergent treatment of the RC-LH1 complex easily dissociates the cytochrome subunit of the RC of *Rv. gelatinosus* from the LM core (Fukushima *et al.* 1988), but has no such effect in *Rp. viridis*. Comparison of the deduced amino acid sequences of the cytochrome subunits of *Rv. gelatinosus* and *Rp. viridis* revealed that a significant number of residues is deleted in *Rv. gelatinosus*. These deletions occur mainly in the region of the attachment site to the M subunit of the LM core proteins, suggesting that the interaction of the cytochrome subunit with the core pro-

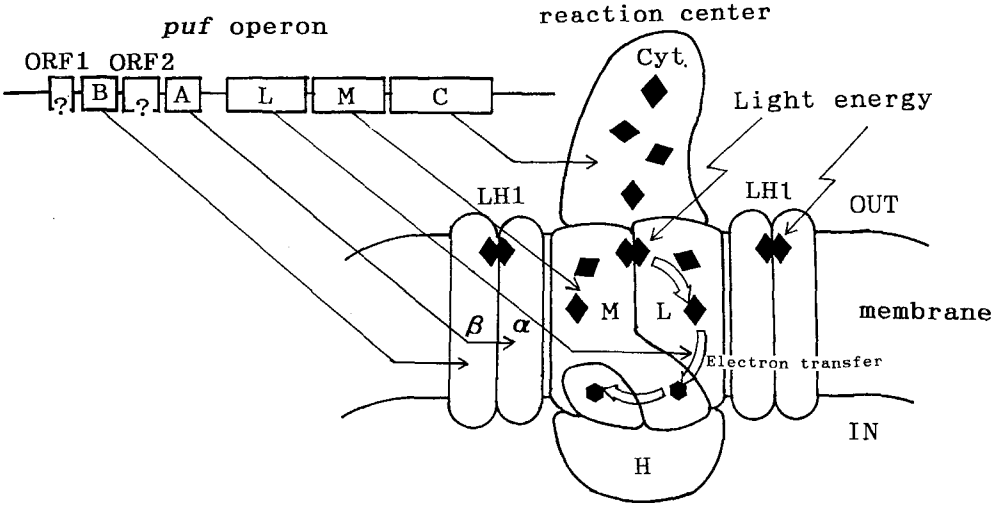


Fig. 4. A diagram of the RC-LH1 complex and its gene arrangement in *Rv. gelatinosus*. Closed symbols represent heme, bacteriochlorophyll, bacteriopheophytin, and quinone.

teins in *Rv. gelatinosus* is different from that in *Rp. viridis* (Nagashima *et al*. 1994). In *Rv. gelatinosus*, this subunit may be tilted more to the M side than in *Rp. viridis*. This is consistent with the report by Nitschke *et al*. (1992) that suggests the orientations of heme planes in *Rv. gelatinosus* are different from those in *Rp. viridis*. Presumably, this difference accounts for the easy dissociability of the cytochrome subunit from the LM core in *Rv. gelatinosus*.

Possible horizontal RC gene transfer in purple bacteria

By analyzing nucleotide sequences of the LH1 alpha, M, L and cytochrome subunits, we suggested the possibility of horizontal transfer of the genes for photosynthetic apparatus from ancestral *Rs. rubrum* to ancestral *Rv. gelatinosus* (Nagashima *et al*. 1993b, Nagashima *et al*. 1994). The primary structure of RC genes in *Rv. gelatinosus* shows considerable similarity to those of purple bacteria in the alpha subclass. The deduced amino acid sequences of RC proteins of *Rv. gelatinosus* showed the highest homology to those of *Rs. rubrum*. *Rv. gelatinosus* was classified to the alpha subclass in the phylogenetic trees constructed from the amino acid sequences, which does not match the phylogenetic trees based on 16S rRNA and soluble cytochrome c_2. This discrepancy is explained by the hypothesis of horizontal transfer of the *puf* photosynthetic genes from the purple bacteria in the alpha subclass, probably an ancestor of the Rhodospirillum species to an ancestor of *Rv. gelatinosus*. The ancestral Rhodospirillum may have possessed a bound cytochrome subunit in the RC, and the subunit was probably lost in *Rs. rubrum* but not in *Rv. gelatinosus*. The cytochrome subunit in *Rv. gelatinosus* showed a partial loss of amino acids in the subunit polypeptide, which probably resulted in the weak association to the RC core polypeptides.

Function of the RC-bound cytochrome subunit

In all species in purple bacteria which possess a cytochrome subunit with four hemes examined to date except *Rs. molischianum*, two are high-potential with alpha-band peaks at long wavelengths (555-559 nm) and two are low-potential hemes with peaks at short wavelengths (550-554 nm) (Dutton and Prince 1978, Matsuura and Shimada 1990). We have shown that the RC-bound cytochrome *c* subunit of *Rs. molischianum* have one high potential heme (Em of 390 mV, alpha-band peak at 558 nm) and three low-potential hemes (Em values below 100 mV, alpha-band peaks at 552 nm) (Nagashima *et al*. 1993a). The finding in *Rs. molischianum* suggests that the second high-potential heme is not necessarily essential for the RC-bound cytochromes in purple bacteria. The indispensable role of the bound cytochromes in the RC complexes in those species of purple bacteria remains to be answered. Thus far we have shown that the bound cytochrome subunit is not necessary merely to allow electron transfer from cytochrome c_2 to the $(BChl)_2$ in *Rv. gelatinosus* (Matsuura *et al*. 1988a).

Electron transfer in RC and membrane potential relationships

After the understanding of the three-dimensional crystal structure of the RC of *Rp. viridis* (Deisenhofer *et al*. 1985), precise studies on the structure function relationship in RC became possible. The electron transfer system of *Rp. viridis* has been extensively investigated, but the relationship between electron transfer and the generation of membrane potential has not been fully understood. Dracheva *et al*. (1988) measured membrane potential changes by incorporating the RC into liposomes and sticking them to one side of a collodion film placed between two compartments equipped with electrodes. They showed that the extent of the membrane potential generated by the photo-oxidation of high-potential hemes and the special pair was approximately proportional to the distance of electron movement in the RC complex.

In *Rs. molischianum* (Nagashima *et al*. 1993a) the kinetic measurements of photo-oxidations of hemes suggested that the highest potential heme is at the nearest position as in the case of *Rp. viridis*. However, if the extent of membrane potential determined by carotenoid band shift is proportional to the distance of electron movement, the heme with the highest potential, *c*-558, is assumed to be located in the most distant position from $(BChl)_2$. We have proposed a model (Nagashima *et al*. 1993a) in which the magnitude of the membrane potential depends not only on the distance of electron movement across the membrane but also on the dielectric property in the cytochrome subunit, which can be greatly affected by the distance of the redox centers from the protein-aqueous interface. According to this model, the highest potential heme, *c*-558, is estimated to be proximal to the special pair and to the aqueous surface, so that the largest electrostatic potential change occurs upon its photo-oxidation.

Cytochrome bc Complexes

Mitochondrial bc_1 complex driven by the RC of purple bacteria

The cytochrome *bc* complex is a central component of respiratory and photosynthetic electron flow in a variety of eukaryotic and prokaryotic systems. It contains two cytochrome b, a cytochrome *c*, a Rieske-type iron-sulfur cluster, and two bound quinones. There are remarkable similarities between complexes from taxonomically diverse species. The cytochrome bc_1 complex is found in the mitochondrial inner membrane, and is involved in the delivery of reducing equivalents from the substrate dehydrogenases to cytochrome *c*, and then to molecular oxygen. In the photosynthetic bacterial membrane, the complex operates with the RC complex to constitute a light-activated, cyclic electron transfer system (Fig. 2). In spite of differences in the ultimate sources of the reductant and oxidant, the complexes are essentially identical in terms of redox components and function.

It has been shown that the isolated mitochondrial bc_1

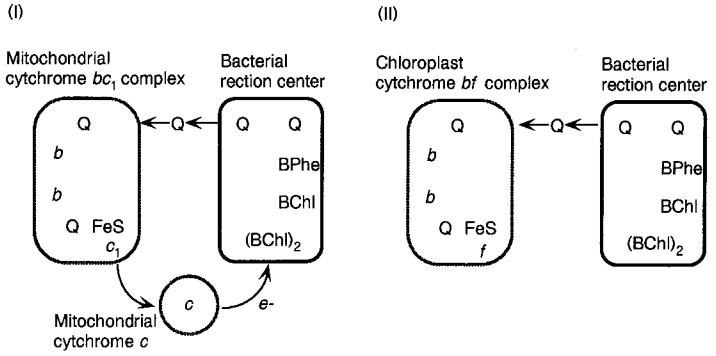


Fig. 5. Difference in electron transfer pathways between two hybrid electron transfer systems with bacterial reaction center and mitochondrial bc_1 complex (I) and chloroplast *bf* complex (II).

complex behaves like the bacterial complex, when activated by flash illumination in a hybrid mixture in detergent solution containing the isolated RC complex from *Rb. sphaeroides* and cytochrome *c* (Packham *et al.* 1980, Matsuura *et al.* 1981, Matsuura and Dutton 1981, Matsuura *et al.* 1983, Moser *et al.* 1986). The three proteins in the hybrid system formed a cyclic electron transfer system (Fig. 5, I) which, under a variety of experimental conditions, displays light-induced kinetic behavior similar to that found in vivo with the photosynthetic bacterial membranes of *Rb. sphaeroides* (Fig. 2). Flash-excitation of the RC resulted in the reduction and/or oxidation of cytochrome b in the bc_1 complex within milliseconds. The system also revealed the presence of functional quinones associated with the bc_1 complex.

The similarity of mitochondrial complexes to photosynthetic complexes in bacteria supports the hypothesis of close evolutionary relationship. *Paracoccus denitrificans*, an aerobic bacterium, is known to share origins close to mitochondria, and to the photosynthetic bacterium, *Rhodobacter sphaeroides*. In this sense, the hybrid system is a kind of reunion of previously separated components into different organisms.

The chloroplast bf complex driven by the RC of purple bacteria

The *bc* complex analog in chloroplasts contains cytochromes b_6 and *f*, and functions between photosystems II and I in linear electron flow from water to $NADP^+$, accepting electrons from plastoquinol and delivering them to plastocyanin; and in cyclic electron flow around photosystem I. In the latter case, the electron donor is still uncertain, although it may well be a plastoquinol; and again the terminal electron acceptor is plastocyanin. Using the isolated cytochrome *bf* complex, Prince *et al.* (1982) reported the construction of another hybrid system with isolated RCs from *Rb. sphaeroides*. The RCs can indeed deliver electrons to the cytochrome *bf* complex, although only under high ambient redox potential conditions. The reduction reactions occur only after the second single turnover flash. These results clearly showed that the *bf* complex functions in such a way that a two-electron redox couple, probably a quinone, is capable of reducing both cytochrome b_6 and cytochrome *f*, the latter via the Rieske iron-sulfur cluster, in a coupled reaction where both electrons must leave the two-electron carrier. Cytochrome b_6 is thus reduced in a manner analogous to "oxidant-induced reduction".

It is noteworthy that in the case of the chloroplast *bf* complex (Prince *et al.* 1982), the complex makes good "contact" only at the reducing end of the purple bacterial RC (Fig. 5, II). In the case of the mitochondrial bc_1 complex (Fig. 5, I), however, two routes of cytochrome b reduction seem possible (Matsuura and Dutton 1981). In the first, reduction caused by electron delivery from the reducing end of the RC. In the second, oxidant-induced reduction takes place, which requires the existence of quinol molecules in the system and the oxidation of the quinol through the oxidizing end of the RC. The latter did not occur in the chloroplast system because the oxidizing end of the RC was not functionally connected to the *bf* complex, even in the presence of plastocyanin and cytochrome *c*. This probably reflects that the chloroplast *bf* complex is more distantly related phylogenetically to the RC of purple bacteria than the mitochondrial complex (Fig. 1).

Cytochrome bf complexes in cyanobacteria

The electron transfer reactions induced by the oxidation of the *bf* complex were studied in intact cells of cyanobacteria (Matsuura *et al.* 1988c) partly because they were not observed in the hybrid system. The effect of ambient redox potentials on electron transfer through the cytochrome bc_1 complex has been useful in studies in chromatophore vesicles of *Rb. sphaeroides* and the hybrid systems described above. The rate of electron transfer through the complex and the pattern of redox changes of the two cytochrome *b* moieties after a flash are dependent on the redox states of ubiquinone and cytochrome *b* prior to illumination. In the cyanobacterial cells, the redox environments of the photosynthetic electron-transfer system was controlled by the respiratory electron influx to the plastoquinone pool and the efflux from the pool. While controlling the redox environments, we measured the

flash-induced redox changes in cytochrome *b*-563 in intact cells of Synechocystis PCC 6714 (Matsuura *et al.* 1988c).

The results clearly indicated that the flash-induced kinetics of oxidation and reduction of cytochrome b-563 in the intact cells are variable and depend on the redox steady state of the electron transfer system, controlled by the influx and efflux of electrons in the respiratory system. The observations were essentially the same as those obtained in chromatophores of purple bacteria and the hybrid system with mitochondrial and bacterial complexes. Thus, the mechanism of electron transfer through the cytochrome *bf* complex in cyanobacteria seems essentially the same as that through the cytochrome bc_1 complex in purple photosynthetic bacteria or mitochondria.

Cytochrome c_2

Cytochrome c_2 and plastocyanin

As described above, there are many similarities between the electron transfer system in chloroplasts and photosynthetic bacteria. Another example is the functional equivalence between plastocyanin in chloroplasts and cytochrome c_2 in purple photosynthetic bacteria. These proteins transfer electrons from cytochrome *bc*-type complexes to photochemical RC complexes. In chloroplasts of higher plants, plastocyanin transfers electrons from cytochrome *f*, in the cytochrome *bf* complex, to P700, the RC chlorophyll in photosystem I. In purple photosynthetic bacteria, soluble cytochrome c_2 transfers electrons from cytochrome c_1, in the cytochrome bc_1 complex, to the special pair of bacteriochlorophyll ($(BChl)_2$) in the RC complex.

Although plastocyanin and cytochrome c_2 have different prosthetic groups, copper or heme, they are both water-soluble peripheral membrane proteins with molecular weights of about 10 kDa. Their physiological reactants, P700 in the photosystem I RC complex and $(BChl)_2$ in the bacterial RC complex, resemble each other in their functions, as do cytochrome *f* in the cytochrome *bf* complex and cytochrome c_1 in the cytochrome bc_1 complex. Furthermore, in some eucaryotic algae and cyanobacteria under conditions of copper deficiency, plastocyanin can be replaced by c-type cytochromes (Bohner *et al.* 1980, Sandmann and Boger 1980), which seem to be evolutionally homologous to cytochrome c_2.

Factors affecting the rate of photosynthetic electron transfer through the water-soluble peripheral membrane proteins of plastocyanin and cytochrome c_2, were compared between spinach chloroplasts and *Rb. sphaeroides*, a photosynthetic bacterium (Matsuura and Itoh 1985). In spinach chloroplasts, the rate of flash-induced oxidation of cytochrome *f* was highly dependent on the salt concentration in the suspending medium. The salt effect was similar to that on the reaction rate between P700 in thylakoid fragments and externally added plastocyanin. In intact cells of *Rb. sphaeroides*, however, in which cytochrome c_2 is located in the periplasmic space exposed to the outer ionic environment, the rate of cytochrome c_1 oxidation via cytochrome c_2 was almost independent of the salt concentration. This independence was in contrast to the strong dependence on salt concentration of reactions between isolated RCs and cytochrome c_2. This suggests that plastocyanin reacts via collision with the photosystem I RC and cytochrome *bf* complex in a manner that is controlled by the surface electrostatic potential. Cytochrome c_2, on the other hand, reacts with the bacterial RC and the cytochrome bc_1 complex, perhaps by forming a complex prior to activation of the RC.

The observed difference in the salt effect between the reactions through plastocyanin and cytochrome c_2 is apparently not due to differences in the prosthetic groups. One possible explanation is the difference in the environment of the proteins under physiological conditions: plastocyanin functions in a constant internal ionic environment, while cytochrome c_2 exists in the periplasmic space exposed to an external environment having a wide variety of ionic conditions, and, further, the bacteria may

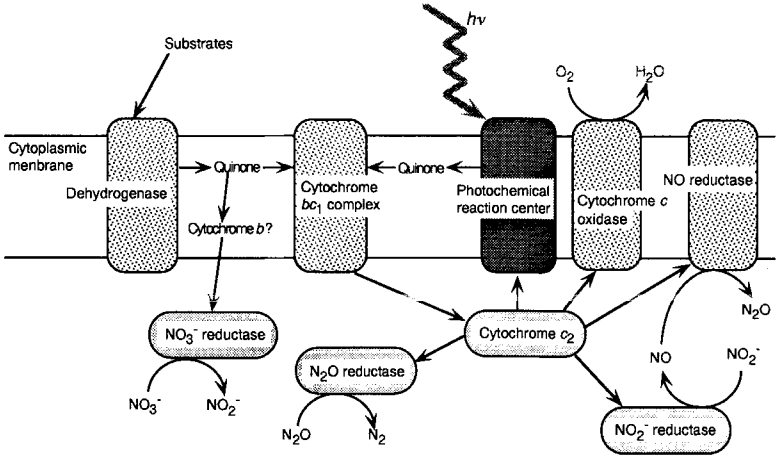


Fig. 6. Electron transfer pathways for denitrification and its relationship to photosynthetic and respiratory systems in *Rb. sphaeroides* forma sp. *denitrificans*.

become adapted to the changing environment (Matsuura and Itoh 1985).

Key role of Cytochrome c_2 in branched electron transfer system of purple bacteria

The denitrifying phototrophic bacterium *Rb. sphaeroides* forma sp. *denitrificans* utilizes the energy of denitrification for growth in the dark and under illumination. The organism was reported by Satoh *et al.* (1986) as the first photosynthetic organism found to use anaerobic respiration as an energy source. Electron transfer systems for both denitrification and photosynthesis are synthesized under anaerobic conditions in the presence of NO_3^- (Itoh *et al.* 1988). The anaerobic growth rate is accelerated by NO_3^- under light-limiting conditions, and the presence of NO_3^- lowers the synthesis of pigments for photosynthesis (Itoh *et al.* 1988).

Some of the redox components for photosynthesis in *Rb.sphaeroides* are common to those for denitrification. The cyclic electron transfer system of photosynthesis consists of the RC, the cytochrome bc_1 complex, cytochrome c_2, and ubiquinone (Fig. 2). The components other than the RC have also been shown to participate in electron transfer for denitrification (Itoh *et al.* 1989a, Itoh *et al.* 1989b). The release of nitrogen gas from NO_3^- includes four enzymatic steps, i.e., the reductions of NO_3^-, NO_2^-, NO, and N_2O. Figure 6 presents an outline of the entire electron transfer system for denitrification in *Rb. sphaeroides* forma sp. *denitrificans* (Itoh *et al.* 1989b). The main characteristics of the system are as follows : (i) ubiquinone, the cytochrome bc_1 complex, and cytochrome c_2 are involved in the electron transfer pathways for denitrifications as common components with those for O2 respiration and photosynthesis; (ii) three of the terminal enzymes for denitrification, NO_3^-, NO_2^-, and N_2O reductases, are periplasmic proteins and all four terminal reduction reactions take place in periplasmic space ; (iii) the electron transfer pathways for the three terminal enzymes, NO_2^-, NO, and N_2O reductases, branch at the level of cytochrome c_2.

The physiological consequences of the involvement of the cytochrome bc_1 complex which reduces cytochrome c_2 in the electron pathway from substrates to NO_2^-, NO, and N_2O are that the redox energy for the electron transfer is coupled to the generation of an electrochemical gradient of protons across the membrane. An additional consequence is that the reduction is under the control of the high-energy state of the membrane. These effects were directly shown by Itoh *et al.* (1989b) as the N_2O-induced carotenoid band shift and the CCCP-sensitive inhibitory effect of illumination on the N_2O reduction.

Kinetic differentiation of cytochrome c_2

When cells of the denitrifying phototrophic bacterium *Rb. sphaeroides* forma sp. *denitrificans* were grown anaerobically under illumination in the presence of NO_3^-, the content of RCs per cellular protein was less than that in cells grown photosynthetically without NO_3^- under the same light intensity (Matsuura *et al.* 1988b). The contents of cytochrome c_1 and c_2 that work in both photosynthetic and denitrifying electron transport systems were almost constant, and were independent of the presence of NO_3^- during growth. Consequently, the ratio of cytochrome c_1 and c_2 to the RC was more than 3 : 1 in the photo-denitrifying cells, but approaches 1 : 1 in the photosynthetic cells under light-limiting conditions. In spite of the excess of cytochromes c_1+c_2 over the RC in the photo-denitrifying cells, all of them were oxidized by illumination within a few hundred milliseconds in the presence of antimycin.

When glycerol was added to increase viscosity in the periplasm, biphasic oxidation of cytochromes c_1+c_2 was apparent in the photo-denitirifying cells with repetitive flashes (Matsuura *et al.* 1988b). The fast-phase oxidation, which took place instantaneously after the first and second flashes, showed a similar pattern to the oxidation in the light-limiting photosynthetic cells. The rate of the slow-phase oxidation was sensitive to viscosity and appeared to reflect a diffusion-controlled second-order reaction between cytochrome c_2 and the RC. The biphasic oxidation of cytochromes c_1+c_2 suggests that these cytochromes exists in the photo-denitrifying cells as two different pools in relation to the RC. While cytochrome c_2 and the cytochrome bc_1 complex are common components in photosynthesis, respiration, and denitrification, the kinetic differentiations suggest that the cytochrome system may be heterogeneously located on the membrane for each electron transfer system (Matsuura *et al.* 1988b).

Concluding Remarks

Comparative studies of electron transfer systems have become more useful than before for elucidating the principal mechanisms and evolution of the energy conserving systems. This was resulted from the advancements of related fields and techniques ; especially, the clarification of bacterial phyla, precise knowledge of three-dimensional structure of proteins, and accumulation of sequence information of genes. When these pieces of new information is combined with biochemical and biophysical measurements of the function in various organisms, understanding of the systems has improved greatly.

By studying the components of the photosynthetic cyclic electron transfer systems comparatively, we have shown many similarities and some variations in corresponding components in purple bacteria and in other bacteria, chloroplasts, and mitochondria. The components and the system in purple bacteria seem to be simpler than those in other organisms. This suggests that the photosynthetic cyclic electron transfer system of purple bacteria may retain old characteristics of membrane enegy-conserving electron transfer systems of the common ancestor of eubacteria, chloroplasts, and mitochondria.

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