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REVIEW

An overview on chlorophylls and quinones in the photosystem I-type reaction centers

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Abstract Minor but key chlorophylls (Chls) and quinones in photosystem (PS) I-type reaction centers (RCs) are overviewed in regard to their molecular structures. In the PS I-type RCs, the prime-type chlorophylls, namely, bacteriochlorophyll (BChl) *a′* in green sulfur bacteria, BChl *g′* in heliobacteria, Chl *a′* in Chl *a*-type PS I, and Chl *d′* in Chl *d*-type PS I, function as the special pairs, either as homodimers, (BChl a')$_2$ and (BChl g')$_2$ in anoxygenic organisms, or heterodimers, Chl *a*/*a′* and Chl *d*/*d′* in oxygenic photosynthesis. Conversions of BChl *g* to Chl *a* and Chl *a* to Chl *d* take place spontaneously under mild condition in vitro. The primary electron acceptors, A_0, are Chl *a*-derivatives even in anoxygenic PS I-type RCs. The secondary electron acceptors are naphthoquinones, whereas the side chains may have been modified after the birth of cyanobacteria, leading to succession from menaquinone to phylloquinone in oxygenic PS I.

Keywords Bacteriochlorophyll *g* · Chlorophyll *a* · Green sulfur bacteria · Heliobacteria · Menaquinone · Photosystem I

Abbreviations

BChl	Bacteriochlorophyll
BPhe	Bacteriopheophytin
Chl	Chlorophyll
HPLC	High performance liquid chromatography
MQ	Menaquinone
P700	The primary electron donor of photosystem I
P740	The primary electron donor of PS I in *A. marina*
P798	The primary electron donor of heliobacteria
P840	The primary electron donor of green sulfur bacteria
P870	The primary electron donor of purple bacteria
P960	The primary electron donor of bacteriochlorophyll *b* containing purple bacteria
Phe	Pheophytin
PhQ	Phylloquinone
PS	Photosystem
RC	Reaction center

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Introduction

Photosynthetic reaction center (RC) complexes are classified into two types, photosystem (PS) I-type (FeS-type) and PS II-type (Phe-Q type), depending on the electron transfer components (Fig. 1). Anoxygenic photosynthetic bacteria contain only one type of RC, whereas cyanobacteria contain both types. Heliobacteria and green sulfur bacteria possess PS I-type RCs, and purple bacteria and green filamentous bacteria have PS II-type RCs (Xiong et al. 2000; Ke 2001a).

Chlorophylls (Chls) and bacteriochlorophylls (BChls) (Fig. 2) are the main photosynthetic pigments (Scheer 2006). Chl *a* (Fig. 2a) is present in cyanobacteria, and their derivatives are present also in the RCs of heliobacteria (Ke 2001b) and green sulfur bacteria (Scheer 2006), functioning as the primary electron acceptors: 8^1-OH-Chl *a* esterified with farnesol in heliobacteria (Van de Meent et al. 1991) and Chl *a* esterified with Δ2,6-phytadienol (Chl $a_{\Delta 2,6PD}$) in green sulfur bacteria (Figs. 1, 2a; Kobayashi et al. 2000). BChl *a* (Fig. 2c) is present in purple bacteria, green filamentous bacteria, and green sulfur bacteria, while heliobacteria possess a unique pigment, BChl *g* (Fig. 2b).

Other two types of chlorophylls are functional as key cofactors in the RCs; one is prime-type Chl and BChl, i.e., Chl *a′*, Chl *d′*, BChl *a′* and BChl *g′*, and the other is Mg-free Chl or BChl, i.e., pheophytin (Phe) *a* or bacteriopheophytin (BPhe) *a* and BPhe *b* (Figs. 1, 2; Kobayashi et al. 2006a). The prime-type Chls or BChls form the special pair in the PS I-type RCs, while Phe *a*, BPhes *a* and *b* are the primary electron acceptor in the PS II-type RCs (Fig. 1). These pigments are minor, but key components for the charge separation and subsequent electron transfer processes in photosystems (Kobayashi et al. 2006a).

Heliobacteria have two molecules of BChl *g′* per P798 (Kobayashi et al. 1991a). Since the RC consists of two identical subunits (Liebl et al.1993), P798 is considered to be a (BChl g')$_2$ homodimer (Figs. 1, 3; Kobayashi et al. 1991a, b, 1992, 2006a; Kobayashi 1996).

For green sulfur bacteria, the special pair in the RC is named P840 (Fig. 1). The homodimeric RC-core contains 16 molecules of BChl *a*-type pigments, and two of them are BChl *a′* forming P840 (Figs. 1, 3; Kobayashi et al. 1999, 2006a).

Gloeobacter violaceus is assigned to an early branched species in the phylogenetic tree of cyanobacteria based on the 16S rRNA sequence (Nakamura et al. 2003) and thus to a connecting site between the anoxygenic photosynthesis and oxygenic photosynthesis in the phylogenetic tree. *G. violaceus* has Chl *a′* and Phe *a* as minor pigments (Mimuro et al. 2005). A heterodimer of Chl *a* and *a′* functions as P700 (Fig. 3), and Phe *a* as the primary electron acceptors in PS II, as for typical cyanobacteria.

Acaryochloris marina has a unique pigment, Chl *d* (Fig. 2a; Miyashita et al. 1996, 1997; see review by Ohashi et al. 2008a), and is included in the clade of cyanobacteria (Miyashita et al. 1997; Murakami et al. 2004). The species differentiation to this organism will be accompanied by the appearance of Chl *d*. *A. marina* has Chl *d′* (Fig. 2a) and Phe *a* as minor but key pigments (Akiyama et al. 2001). Like the heterodimer of Chl *a*/*a′* as P700, a heterodimer of Chl *d*/*d′* functions as P740 (Fig. 3; Akiyama et al. 2002, 2004; Kobayashi et al. 2005; Ohashi et al. 2008a).

The electron acceptor A_1 in oxygenic PS I has been identified as phylloquinone (PhQ, Fig. 4a) in the mid 1980s (Takahashi et al. 1985; Schoeder and Lockau 1986). However, it is not known whether a quinone analogous to A_1 in oxygenic PS I-type RCs exists and acts in heliobacterial and/or green sulfur bacterial RCs as an electron acceptor between A_0 and F_x, although several groups report that menaquinone (MQ, Fig. 4b) species are present and function as A_1 in their RCs.

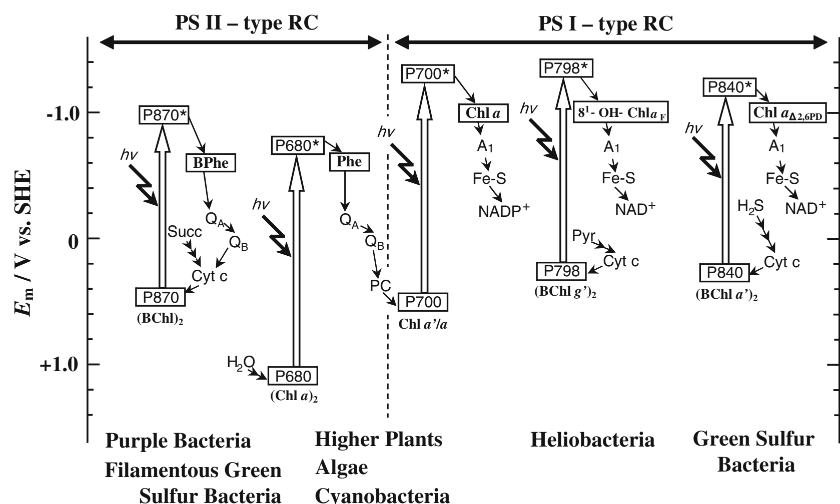
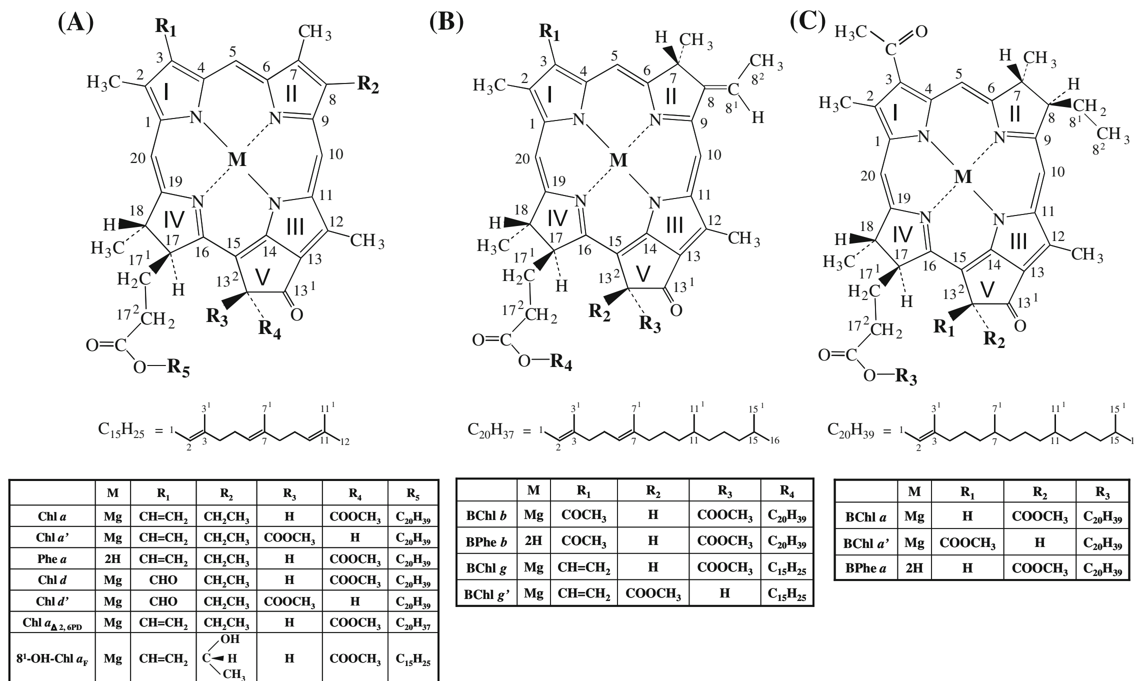


Fig. 1 Comparison of photosynthetic electron transport between the PS I- and PS II-type RCs in terms of the redox potentials. For simplicity, some primary electron donors, P960, P850, P865, and P740 are omitted. P960 and P850 are for BChl *b*-type and Zn-BChl *a*-type purple bacteria; P865 is for green filamentous bacteria. P740 is the primary electron donors of PS I of *A. marina*. Adapted from Kobayashi et al. (2006a)



	M	R_1	R_2	R_3	R_4	R_5
Chl *a*	Mg	$CH{=}CH_2$	CH_2CH_3	H	$COOCH_3$	$C_{20}H_{39}$
Chl *a'*	Mg	$CH{=}CH_2$	CH_2CH_3	$COOCH_3$	H	$C_{20}H_{39}$
Phe *a*	2H	$CH{=}CH_2$	CH_2CH_3	H	$COOCH_3$	$C_{20}H_{39}$
Chl *d*	Mg	CHO	CH_2CH_3	H	$COOCH_3$	$C_{20}H_{39}$
Chl *d'*	Mg	CHO	CH_2CH_3	$COOCH_3$	H	$C_{20}H_{39}$
Chl $a_{\Delta 2,6PD}$	Mg	$CH{=}CH_2$	CH_2CH_3	H	$COOCH_3$	$C_{20}H_{37}$
8^1-OH-Chl a_F	Mg	$CH{=}CH_2$	C(OH)(H)(CH_3)	H	$COOCH_3$	$C_{15}H_{25}$

	M	R_1	R_2	R_3	R_4
BChl *b*	Mg	$COCH_3$	H	$COOCH_3$	$C_{20}H_{39}$
BPhe *b*	2H	$COCH_3$	H	$COOCH_3$	$C_{20}H_{39}$
BChl *g*	Mg	$CH{=}CH_2$	H	$COOCH_3$	$C_{15}H_{25}$
BChl *g'*	Mg	$CH{=}CH_2$	$COOCH_3$	H	$C_{15}H_{25}$

	M	R_1	R_2	R_3
BChl *a*	Mg	H	$COOCH_3$	$C_{20}H_{39}$
BChl *a'*	Mg	$COOCH_3$	H	$C_{20}H_{39}$
BPhe *a*	2H	H	$COOCH_3$	$C_{20}H_{39}$

Fig. 2 Molecular structures and carbon numbering of (**a**) Chls and Phes, (**b**) BChl *b*, BPhe *b*, BChl *g* and BChl *g*′, and (**c**) BChl *a*, BChl *a*′ and BPhe *a*, according to the IUPAC numbering system

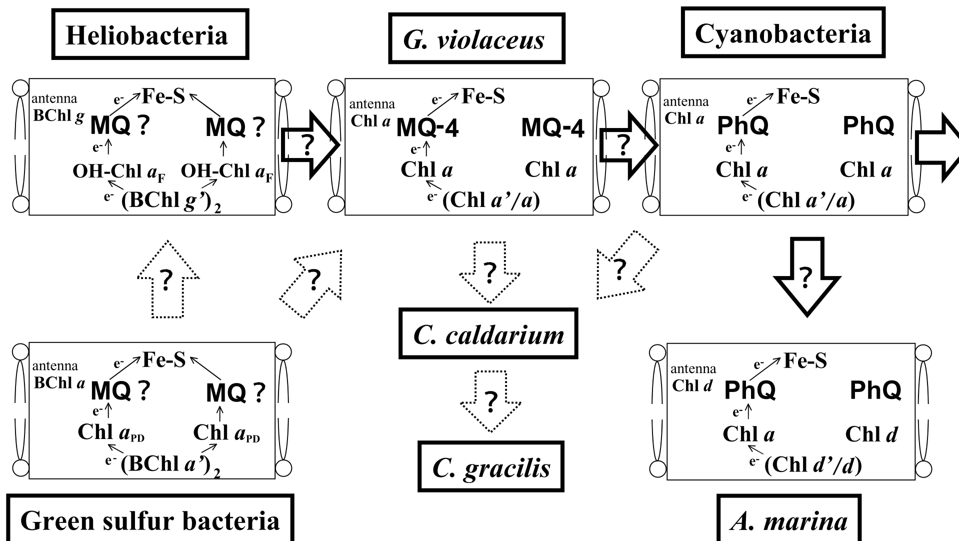


Fig. 3 Schematic arrangement of chlorophylls and quinones in the PS I-type RCs. Our hypothesis about the evolution of the PS I-type RCs based on their molecular modification is designated with solid arrows

As mentioned above, A_1 in oxygenic PS I is PhQ, however, MQ was recently reported to function as A_1 in a primitive unicellular red alga *Cyanidium caldarium* (Yoshida et al. 2003), then in a primitive cyanobacterium *G. violaceus* (Mimuro et al. 2005), and a marine centric diatom *Chaetoceros gracilis* (Ikeda et al. 2008). It has been shown that *A. marina* uses PhQ as A_1 (Ohashi et al. 2008a). These findings indicate that the side chains of A_1 were modified after the birth of cyanobacteria.

Key chlorophylls in the PS I-type RCs

Heliobacteria

Heliobacterium chlorum was first isolated in 1981 from a soil sample collected in front of the Biology Department, Indiana University using an incorrectly prepared culture medium for other anoxygenic bacteria (Gest 1994). Later, several other species were isolated from rice fields, hot

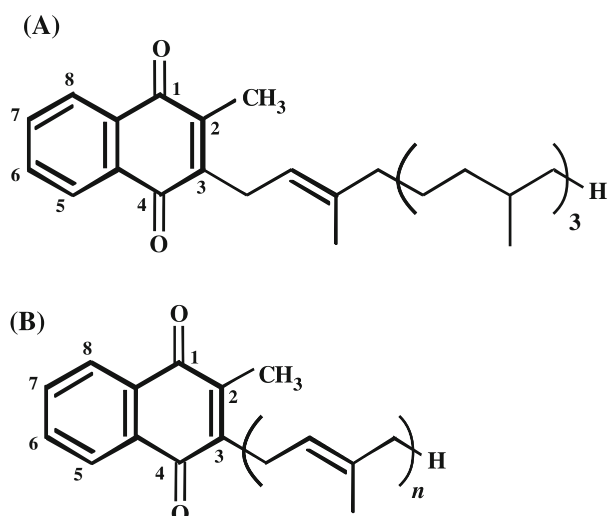


Fig. 4 Molecular structures of and carbon numbering (**a**) PhQ and (**b**) MQ-*n*

springs and the banks of soda lakes (see review by Neerken and Amesz 2001).

A new bacteriochlorophyll, BChl *g*, was discovered in this bacterium (Brockmann and Lipinski 1983). BChl *g*, like BChl *b*, contains an 8-ethylidene group on ring II (Fig. 2b). Isomerization (intramolecular proton transfer) of BChl *g* on ring II easily took place to yield Chl *a* esterified with farnesol, Chl a_F (Fig. 2a; Brockmann and Lipinski 1983; Michalski et al. 1987; Kobayashi et al. 1998a), suggesting that BChl *g* is one of the likely candidates for the ancestor of Chl *a* in oxygenic photosynthesis, whereas similar reactions were observed in BChl *b*, but yielded 3-acetyl-Chl *a* (Steiner et al. 1983; Kobayashi et al. 1998b). Heliobacteria are phylogenetically close to cyanobacteria than the other groups of anoxygenic photosynthetic bacteria (Xiong et al. 2000; Blankenship 1992), this also supports one of the evolutional hypotheses that Chl *a* arose from BChl *g*, although the evolution from anoxygenic photosynthetic organisms to cyanobacteria is a matter of controversy (Wu et al. 2009), and further investigation is needed.

Reversible photobleaching at 798 nm was ascribed to the primary electron donor, P798 (Fuller et al. 1985). P798 was initially assumed to be a BChl *g* dimer on the basis of optical and ESR measurements (Prince et al. 1985; Fischer 1990). The positive charge on $P798^+$ was found to be delocalized over the two BChl *g* molecules (Prince et al. 1985; Rigby et al. 2001). Later a small amount of BChl g' (Fig. 2b) was found in *Hb. chlorum* and *Heliobacillus mobilis* (Kobayashi et al. 1991a), where the molar ratio of BChl g/g' was about 36/2 (Fig. 5a). This ratio, when combined with the BChl *g*/P798 ratio of 35–40 (Van de Meent et al. 1990), yields a BChl g'/P798 ratio of 2. P798

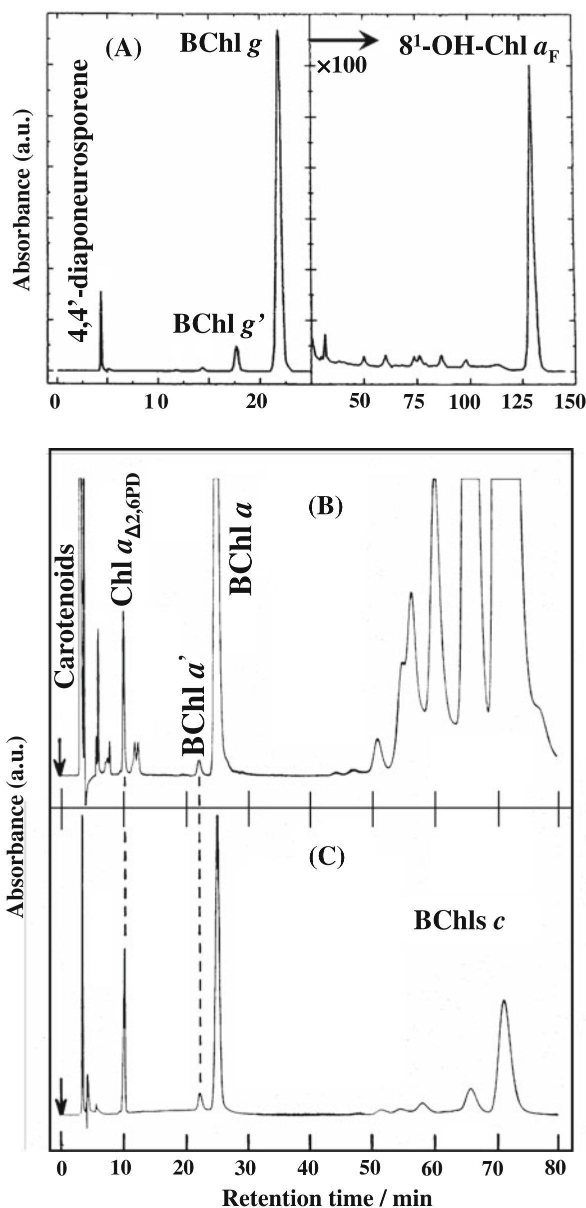


Fig. 5 Normal-phase HPLC profiles for extracts of (**a**) *Hb. chlorum* cells, and *Chl. tepidum* (**b**) cells and (**c**) RC. Detection wavelength is 400 nm. Adapted from Van de Meent et al. (1991) and Kobayashi et al. (2000)

was hence proposed to be a $(\text{BChl } g')_2$ homodimer (Figs. 1, 3; Kobayashi et al. 1991a, b), supported in part by the fact that the RC consists of two identical subunits (Liebl et al. 1993). The homodimeric RC proteins may provide a symmetrical environment for P798.

The primary electron acceptor, A_0, in heliobacteria shows a bleaching at around 670 nm (Nuijs et al. 1985a; Fuller et al. 1985), which was initially considered to be due to a BChl *c* or Chl *a*-like pigment. Later, the molecular structure has been identified to be 8^1-OH-Chl *a* esterified with farnesol (Fig. 2a; Van de Meent et al. 1991). Two

molecules of 8^1-OH-Chl *a* per P798 are present in both cells (Fig. 5a) and the RC complexes (Van de Meent et al. 1991; Neerken et al. 2000). Interestingly, excitation of 8^1-OH-Chl *a* gives rise to an alternative primary reaction not involving excited P798 (Neerken et al. 2000; Neerken and Amesz 2001), suggesting that 8^1-OH-Chl *a* in the excited state would function as initiator of the photochemical reaction from P798 A_0* to $P798^+$ A_0^-. Indeed, excitation of 8^1-OH-Chl *a* resulted in the generation of a significantly larger amount of $P798^+$ than by direct excitation of BChl *g*.

Green sulfur bacteria

Photobleaching at about 840 nm in a green sulfur bacterium, *Chloropseudomonas ethylicum*, was first observed by Sybesma and Vredenberg (1963), and the primary donor was named P840. The homodimeric nature of P840 was proposed by Olson et al. (1977), in agreement with a later finding that *psc*A is the sole gene coding for the RC of green sulfur bacteria and that the RC has a homodimeric structure made up of two identical core proteins (Büttner et al. 1992a, b; see review by Hauska et al. 2001). This contrasts with the finding of the two genes, *psa*A and *psa*B, coding for the heterodimeric RC core of Chl *a*-type PS I.

The RC contains 16 BChl *a*-type molecules (Permentier et al. 2000; Hauska et al. 2001), hence the size is substantially smaller than its counterpart of Chl *a*-type PS I with *ca.* 90 Chl *a* molecules. Two of the 16 pigments are BChls *a′* (see Fig. 5b, c) forming a homodimeric P840 (Figs. 1, 3; Kobayashi et al. 1992, 2000). Since also in P798 of heliobacteria, two of the BChl *g*-type pigments are BChl *g′*, 13^2-epimers seem to be a general feature of the special pair in the FeS-type RCs (Figs. 1, 3; Kobayashi et al. 2006a).

The midpoint potential of P840 was estimated to be around +0.24 V (Fowler et al. 1971; Prince and Olson 1976), which is almost the same as that of P798, ca. +0.23 V (Prince et al. 1985), but is by ca. 0.15 V more negative than the special pair of $(BChl\ a)_2$ in the RC of purple bacteria (see Fig. 1). The redox potential difference reflects the earliest steps in photoreactions (Kobayashi et al. 1999, 2006b).

The A_0 in the RC of green sulfur bacteria showed a bleaching at around 670 nm and was initially assumed to be BPhe *c* (Nuijs et al. 1985b). Braumann et al. (1986) first isolated the A_0 from *Prosthecochloris aestuarii*, where it was designated BChl 663 after its absorption maximum wavelength in vitro and was considered to be a lipophilic form of BChl *c*. Later, BChl 663 was supposed to be an isomer of Chl *a* (Van de Meent et al. 1992). Finally, it was identified as Chl *a* esterified at $C17^3$ with Δ2,6-phytadienol (Fig. 2a; Kobayashi et al. 2000). Four molecules of BChl 663 are present in the RC, and two of them functioning as A_0 (Figs. 1, 3) and the other two being accessory pigments (Permentier et al. 2000). In general, it appears that the A_0 molecules in the PS I-type RCs have been Chl *a*-derivatives (Fig. 1), suggesting that the PS I-type RCs are derived from a common ancestor. Like heliobacteria, evidence has been obtained for a direct pathway of charge separation from excited BChl 663 not involving excited antenna BChls (Neerken et al. 1999; Hauska et al. 2001).

The question whether the homodimeric RCs of heliobacteria and green sulfur bacteria are totally symmetric is still open, although both branches in the homodimeric RCs are supposed a priori to be equivalently active (see Fig. 3). The hypotheses would be supported by circumstantial evidence of nonlocalized cation radical distributions on two BChl molecules of the special pairs upon their photooxidation, through ENDOR and FTIR spectroscopy for heliobacterial RC (Nabedryk et al. 1996; Rigby et al. 2001), as well as for green sulfur bacterial RC (Rigby et al. 1994; Nabedryk et al. 1996).

Cyanobacterial PS I

Gloeobacter violaceus

In 1972, *G. violaceus* was isolated from the surface of a limestone rock collected in the Kernwald in Switzerland by Rippka et al. (1974). *G. violaceus* is a unicellular cyanobacterium of unusual structure; it lacks thylakoid membranes in cytoplasm, and the photosystems are located on the cytoplasmic membrane. *G. violaceus* is assigned to an early-branched species in the phylogenetic tree of cyanobacteria based on the 16S rRNA sequence (Nakamura et al. 2003) and thus to a connecting site between the anoxygenic photosynthesis and oxygenic photosynthesis in the phylogenetic tree.

Gloeobacter violaceus contains Chl *a* as a major pigment (Rippka et al. 1974) and two minor pigments, Phe *a* and Chl *a′*, as shown in Fig. 6a (Mimuro et al. 2005; Ohashi et al. 2008a). The pigment composition is identical to that in a typical cyanobacterium *Synechocystis* sp. (Fig. 6b). In both *G. violaceus* and typical cyanobacteria, a heterodimer of Chl *a* and *a′* functions as P700 in the heterodimeric PS I RC (Fig. 3), and Phe *a* as the primary electron acceptor in PS II; namely, no chlorophyll modification might have taken place during biological evolution from *G. violaceus* to typical cyanobacteria.

Acaryochloris marina

In 1993, a chlorophyll *d*-dominated cyanobacterium *A. marina*, was discovered by Miyashita (Miyashita et al.

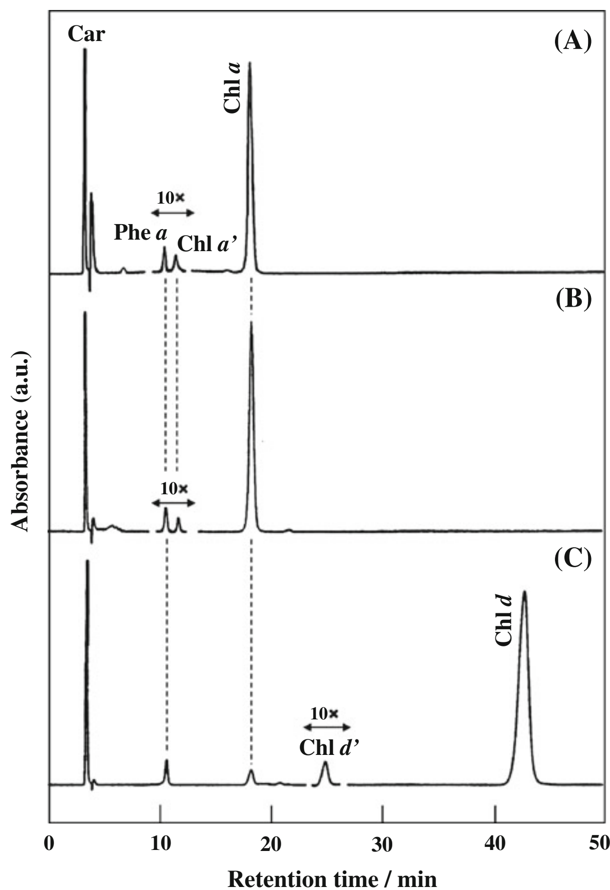


Fig. 6 Normal-phase HPLC profiles for acetone/methanol extracts of (**a**) *G. violaceus*, (**b**) *Synechocystis* sp. PCC 6803, and (**c**) *A. marina*. Detection wavelength is 425 nm. Modified from Ohashi et al. (2008a, b)

1996, 1997; see review by Ohashi et al. 2008a). Later, in a red seaweed *Ahnfeltiopsis flabelliformis*, small patches were found on the thalli surfaces, a cyanobacterium-like prokaryotic epiphyte containing Chl *d*, while Chl *d* was absent inside the thalli; the cells were identified as an *Acaryochloris* sp. (Murakami et al. 2004). Epiphytic *Acaryochloris* sp. was then found in several red algae (Ohkubo et al. 2006). Recently, Kashiyama et al. (2008) demonstrated that Chl *d* synthesis is widespread in oceanic and lacustrine environments covering a range of temperatures and salinities.

A major pigment in *A. marina* is Chl *d* (Fig. 2a). Chl *d'* and Phe *a* are present as minor components, while neither Chl *a*′ nor Phe *d* is present (Fig. 6c; Akiyama et al. 2001). P740, the primary electron donor of PS I in *A. marina*, was initially proposed to be a homodimer of Chl *d* (Hu et al. 1998), later a homodimer of Chl *d*′ (Akiyama et al. 2001), and finally a Chl *d*/*d*′ heterodimer (Fig. 3; Akiyama et al. 2002, 2004; Kobayashi et al. 2005; Iemura et al. 2008; Ohashi et al. 2008a), just like the Chl *a*/*a*′ for P700 (Figs. 1, 3). Therefore, 13^2-epimers seem to be a general feature of the special pair in the FeS-type RCs (Figs. 1, 3), although the reason is not clarified yet why these special pairs are made of 13^2-epimers of Chls *a*, *d* and BChls *a*, *g*. The primary electron acceptor, A_0, in PS I of *A. marina* is Chl *a* (Kumazaki et al. 2002), supporting our hypothesis that the use of Chl *a*-derivatives is also a general feature of A_0 in the PS I-type RCs (Figs. 1, 3; Van de Meent et al. 1991; Kobayashi 1996; Kobayashi et al. 1999, 2000).

Unique conversion of chlorophylls

BChl *g* to Chl *a*

Heliobacteria is one of the candidates for the ancestor of the Chl *a*-type PS I RC (Michalski et al. 1987; Beer-Romero et al. 1988; Kobayashi et al. 1998a; Xiong et al. 2000). Heliobacteria is phylogenetically close to cyanobacteria, even though the order of branching has been hypothesized variously. For example, Xiong et al. (2000) proposed the order from heliobacteria to cyanobacteria. On the other hand, Cavalier-Smith (2006) proposed the order from cyanobacteria to heliobacteria, and Mulkidjanian et al. (2006) also proposed the lineage from ancient cyanobacteria to heliobacteria.

A large difference is seen in the antenna pigment and the primary electron donor between heliobacterial and cyanobacterial PS I; pigments in antenna and P798 of heliobacteria are BChl *g* and (BChl g')$_2$, respectively, (Kobayashi et al. 1991a,b), and those in antenna and P700 of cyanobacterial PS I are Chl *a* and Chl *a*/*a*′, respectively (see Fig. 3). Though the molecular structures of BChl *g*/*g*′ and Chl *a*/*a*′ are seemingly quite different, the isomerization (intramolecular hydrogen transfer) of BChl *g*/*g*′ could lead to Chl *a*/*a*′. Such an isomerization actually occurs spontaneously under weakly acidic conditions in vitro where pheophytinization of BChl *g* does not occur (Kobayashi et al. 1998a). This novel conversion of BChl *g* to Chl *a* is not a redox reaction but an intramolecular hydrogen transfer, and proceeds without such side reactions as allomerization. Further, the product, Chl *a*, is energetically much more stable than BChl *g*, so that the reverse reaction is almost impossible. These facts may support one of the hypotheses that heliobacteria might be a likely candidate for the ancestor of the Chl *a*-type PS I RC, although detailed phylogenetic analyses are still required.

It is of interest to note that conversion from BChl to Chl seen in vitro is not used in chlorophyll biosynthesis, but the reversed conversion from Chl to BChl is used, and that an evolutionary older photosynthetic bacteria use BChl and plants use Chl, namely, the direction is from BChl to Chl.

Chl *a* to Chl *d*

The biosynthetic pathway of Chl *d* in *A. marina* has not yet been elucidated, while the molecular structure of Chl *d* suggests that Chl *d* might be oxidatively biosynthesized from Chl *a*, as is the case with Chl *b*. An oxidative cleavage of a C=C double bond of a vinyl group of Chl *a* at ring I ($-CH{=}CH_2$ to $-CHO$) should take place during the divergent pathway to *A. marina*, resulting in the advantage of utilizing longer wavelength red light that cannot be efficiently absorbed by Chl *a*-type algae in colonial ascidians. Though Chl *d*-derivatives were artificially synthesized from Chl *a*-derivatives by the Mironov group (1994, 1996, 2004), the reaction requires both a special metal complex (e.g., OsO_4) and a strong oxidant (e.g., H_5IO_6), and thus is highly unlikely to occur in nature.

Recently, we came across the formation of Chl *d* from Chl *a* catalyzed by papain in aqueous organic solvents at room temperature (Kobayashi et al. 2005; Koizumi et al. 2005; Okada et al. 2009). Quite recently, the Chl *a* to Chl *d* conversion has been performed when Chl *a* was incubated with extracts of several vegetables; papaya (skin), cucumber (skin), haricot (bean), Japanese radish (leaf), scallion (leaf), and white radish (leaf) (Itoh et al. 2009; details will be discussed elsewhere). These findings suggest that the Chl *a* to Chl *d* conversion is not a very difficult reaction in nature, and will provide a new insight into the unsolved question as to the birth of Chl *d* in photosynthesis.

Homodimeric and heterodimeric special pairs in PS I-type RCs

As shown in Figs. 1 and 3, prime-type chlorophylls function as the primary electron donors in the PS I-type RCs. BChl g' constitutes P798 as a (BChl $g')_2$ homodimer in heliobacteria and BChl a' constitutes P840 as a (BChl $a')_2$ homodimer in green sulfur bacteria, while Chl a' constitutes P700 as a Chl a/a' heterodimer, and Chl d' constitutes P740 as a Chl d/d' heterodimer in *A. marina* (Fig. 3).

The C13^2-stereoisomers, e.g., Chl *a* and a', are hardly distinguishable by absorption spectra in organic solvents (Watanabe et al. 1984; Kobayashi et al. 2006b). However, small but significant differences in the absorption spectra between BChl *g* and g' were found in an HPLC eluent (*n*-hexane:2-propanol:methanol = 100/0.8/0.4, v/v/v), where marked differences were noted in the Q_X-band and slight differences in the Soret bands (Kobayashi et al. 1991a: Kobayashi 1996). Similar differences were observed in the absorption spectra of BChl a/a' and BChl b/b' (Fig. 7; Takahashi et al. 2005). There is also an effect on the Q_Y-band, but this is much smaller and barely discernible in Fig. 7.

Similar optical differences are seen in benzene (Kobayashi et al. 2006b), but not in diethyl ether or acetone (Takahashi et al. 2005). Interestingly, the corresponding pheophytins, BPhes a/a', b/b' and g/g', are not distinguishable by absorption spectra, suggesting that central metal, Mg, of BChls should play a key role in the optical deference observed there. The differences are found to be related to a higher proportion of 6-coordinated Mg in the 'normal' BChls in the presence of extraneous nucleophiles, while the 'prime' BChls are almost exclusively 5-coordinated under the same conditions (Takahashi et al. 2005; Kobayashi et al. 2006b).

The Q_X-band of BChl *a* (Evans and Katz 1975; Callahan and Cotton 1987; Kania and Fiedor 2006; Fiedor et al. 2008) and metal-substituted BChl *a* (Hartwich et al. 1998; Noy et al. 2000) is sensitive to the coordination state of the central metal, shifting to the red with an increasing number of ligands. As an example, for BChl *a*, 6-coordination (two axial ligands) is dominant in pyridine, THF, 1-butanol, 1-propanol, ethanol and methanol, while the pigment is 5-coordinated (single axial ligand) in DMF, 2-propanol, diethyl ether, acetone and acetonitrile (Fiedor et al. 2008).

As mentioned above, the differences seen between the C13^2-stereoisomers in Fig. 7 are also related to a higher proportion of 6-coordinated Mg (two axial ligands) in the 'normal' BChls, whereas the 'prime' BChls are almost exclusively 5-coordinated (only one axial ligand) (Takahashi et al. 2005; Kobayashi et al. 2006b). These explain why the difference appears only in limited solvents (there are few suitable ligands in HPLC eluent or benzene, thus emphasizing the differences in ligation strength), and disappeared in the corresponding BPhes. The extra ligand for 'normal' BChls is methanol in our HPLC eluent, and probably water in benzene (Kobayashi et al. 2006b).

The presence of two coordinatively unsaturated sites in axial positions of (B)Chls determines the interaction of (B)Chls with the environment. The coordination of nucleophilic amino acid residues, histidine (His) in most cases, to the central metal Mg serves to bind (B)Chls to proteins. The axial coordination of central Mg in (B)Chls is of great structural and functional importance for photosynthetic chlorophyll proteins, and hence the axial ligation of (B)Chls has long been a subject of extensive studies (Katz et al. 1968; Evans and Katz 1975; Cotton et al. 1978; Cotton and Van Duyne 1981; Clarke et al. 1982; Brereton and Sanders 1983; Krawczyk 1989; Takahashi et al. 2005; Kania and Fiedor 2006; Kobayashi et al. 2006b, Fiedor et al. 2008); both 5- and 6-coordinations are possible, depending on the environment, as mentioned above. However, 5-coordination is preferred in vivo (Yeates et al. 1988; Deisenhofer and Michel 1989; Ermler et al. 1994), while 6-coordination is also observed in photosynthetic antenna LH 1 (Fiedor 2006).

It is well known that the special pair consists of two (bacterio)chlorophyll molecules axially coordinated by two histidine residues from the protein subunits (Fig. 8); two histidine residues bind strongly to the central Mg atoms with distances of 1.9 Å in *Rp. viridis* and 2.3 Å in *Rb. sphaeroides* (Lancaster et al. 1995). In the purple bacterial special pairs, both BChl *a* and BChl *b* molecules have acetyl group at C3 of ring I (Fig. 2b, c), and some interaction between the acetyl carbonyl oxygen and the partner's Mg is expectable (Iemura et al. 2008: Ohashi et al. 2008a). However, Mg of (B)Chls is usually 5-coordinated in vivo, and such an acetyl C=O···Mg interaction seems to be generally unrecognized.

In the case of the special pair of *Rp. viridis*, two hydrogen bonds are clearly observed for two acetyl groups (Deisenhofer and Michel 1989; Lancaster et al. 1995), where one is with His and the other with a tyrosine residue (Tyr). Both acetyl groups are nearly coplanar to the corresponding macrocycles (Fig. 8b; see also Fig. 7(top) in Lancaster et al. 1995). However, when one watches the special pair of *Rb. sphaeroides* closely, the conformation of one acetyl group not forming such a hydrogen bond with amino acid residues looks vertical to the macrocycle, while the other acetyl carbonyl oxygen forming a hydrogen bond with His is nearly coplanar to the macrocycle like in *Rp. viridis* (Fig. 8a, see also Fig. 7 (bottom) in Lancaster et al. 1995; Fig. 2 in Robotham and O'Malley 2008), suggesting the presence of a weak interaction between Mg and the hydrogen bond free acetyl C=O.

It is of interest to note that the conformation of the acetyl groups forming a hydrogen bond with His or Tyr in the special pairs of both *Rb. sphaeroides* (Fig. 8a) and *Rp. viridis* (Fig. 8b) is not vertical but nearly coplanar to the macrocycle, indicating also the presence of some very much weak interaction between acetyl C=O and Mg, although such an interaction has not yet been widely accepted. If such a weak interaction might be absent, the conformation of these acetyl groups should be vertical to the macrocycle due to the hydrogen bonds between acetyl C=O and His(or Tyr). We hence propose the idea that such an interaction might be present in these special pairs, where the strength is very weak, because acetyl C=O is known to be a poor ligand to Mg (Evans and Katz 1975; Kania and Fiedor 2006), and hence such an interaction is usually disregarded.

In contrast, Chl a' and Chl *a* in P700 are known to be 5-coordinated (Jordan et al. 2001); a histidine residue coordinates with Mg as the 5th-ligand from the face of the macrocycle, where a long phytol chain is absent (Fig. 8e). The coordination of vinyl groups at C^3 of ring I of Chls a' and *a* to Mg is absent in P700, and each vinyl group is configurated so as to avoid steric hindrance against the macrocycle of the other Chl (Fig. 8e; Jordan et al. 2001; Iemura et al. 2008: Ohashi et al. 2008a).

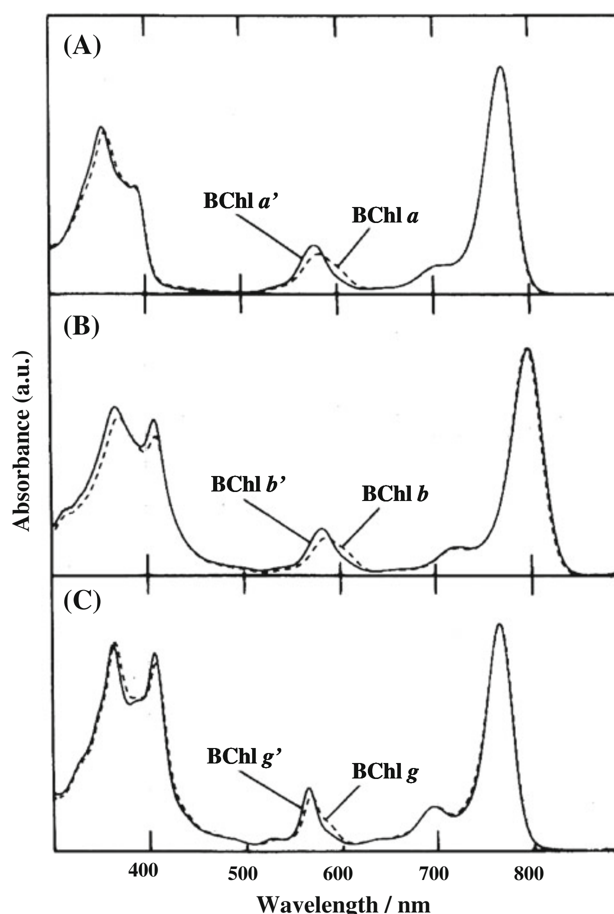


Fig. 7 Absorption spectra of (**a**) BChl a/a', (**b**) BChl b/b', and (**c**) BChl g/g' in hexane/2-propanol/methanol (100/2/0.3, v/v/v). The Q_Y-band maxima are arbitrarily scaled to a common height. Adapted from Takahashi et al. (2005)

In this sense, the special pair of BChl g' in the heliobacterial RC is also assumed to be 5-coordinated, and the 5th-ligand (histidine residue) should coordinate with Mg from the excellently flat face where both a long farnesol chain and a methoxycarbonyl moiety are absent (Fig. 8c). Note that Chls *a*, a' and BChl g' have not an acetyl group but a vinyl group on ring I and that the vinyl group is highly unlikely to coordinate with Mg as the 6th-ligand (Fig. 8c, e; Iemura et al. 2008: Ohashi et al. 2008a). Therefore, the interaction between the special pair (B)Chls in P798 (Fig. 8c) and P700 (Fig. 8e) should be weaker than those in P870 (Fig. 8a) and P960 (Fig. 8b).

The very weak interaction in P798 and P700 is partly supported by the significantly small energy shifts in going from BChl g' (767 nm) to P798 and from Chl a/a' (661 nm) to P700, namely, 0.07 eV and 0.11 eV as shown in Table 1 (Iemura et al. 2008; Ohashi et al. 2008a). Among the PS I-type special pairs, these shifts are the smallest and the second smallest in Table 1, suggesting that the distance between the two BChl g' molecules in P798

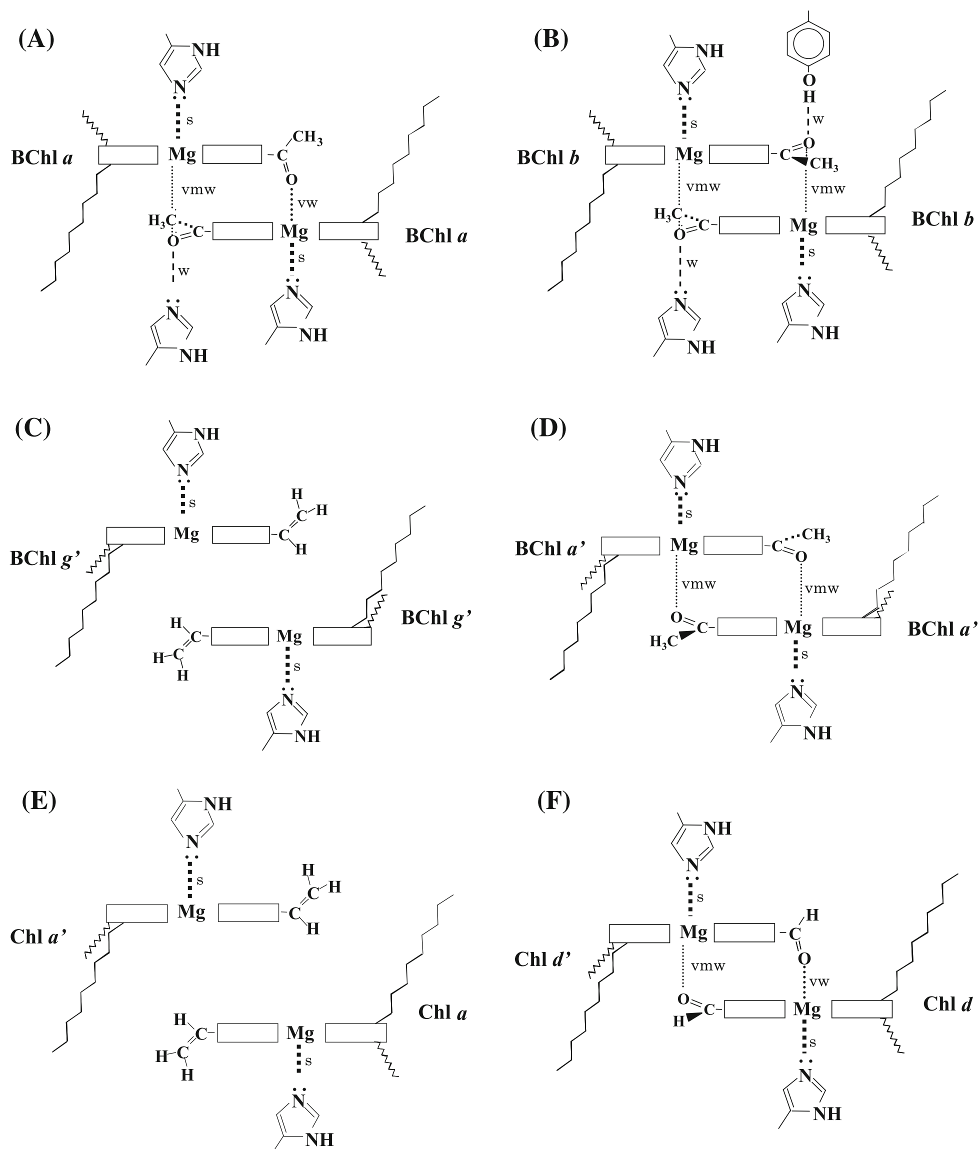


Fig. 8 Schematic illustration of special pairs of (**a**) P870, (**b**) P960 in purple bacteria, (**c**) P798 in heliobacteria, (**d**) P840 in green sulfur bacteria, (**e**) P700, and (**f**) P740 in oxygenic PS I. *s* strong, *w* weak, *vw* very weak, *vmw* very much weak. For simplicity, hydrogen bonds of the C=O groups at rings IV and V with amino acid residues are omitted. P870, P960, and P700 are illustrated based on the crystal structures (Lancaster et al. 1995; Jordan et al. 2001). P798, P840 and P740 are our proposed models. Modified from Ohashi et al. (2008a) and Iemura et al. (2008)

(Fig. 8c) is the longest, and that between Chl a' and Chl *a* in P700 (Fig. 8e) is the second longest.

Note that the excitation energy shift by dimerization of two Chl *a* molecules (661 nm) forming P680 is only 0.06 V, which is the smallest in Table 1. In P680, the special pair Chls *a* has a very long interplanar distance (4 – 5 Å), and are regarded as almost monomeric (Zouni et al. 2001; Kamiya and Shen 2003). The corresponding distance in P700 is 3.6 Å and a dimeric structure is clearly seen (Jordan et al. 2001). These facts indicate that the interplanar distance between two BChl g' molecules in P798 is estimated to be around 4 Å, longer than that in P700 but slightly shorter than that in P680. To substantiate our speculation, a crystallographic study for the heliobacterial RC is awaited.

As might have been expected, the energy shift from BChl a' (771 nm) to P840 (0.13 eV) in green sulfur bacteria is smaller than that from BChl *a* (771 nm) to P870 (0.18 eV) in BChl *a*-type purple bacteria, but significantly larger than that from BChl g' (767 nm) to P798 (0.07 eV) in heliobacteria (Table 1). This strongly suggests that some interaction between acetyl C=O and Mg might be present in the special pair BChl a' molecules in P840 (Fig. 8d). However, the interaction in P840 (Fig. 8d) should be weaker than that in P870 (Fig. 8a), because BChl a' prefers 5-coordination to 6-coordination, as mentioned above.

In view of this, two types of −C=O···Mg interactions in P740 of *A. marina* are expected; one is a somewhat strong interaction between −C=O of Chl d' and Mg of Chl *d*, and the other is a weaker one between −C=O of Chl *d* and Mg of Chl d' (Fig. 8f). This speculation is partially supported by absorption spectral properties. The Q_Y maxima of Chl *a* and Chl *d* in diethyl ether are 661 and 686 nm, respectively, (Kobayashi et al. 2006b), and hence the excitation

Table 1 The Q_Y absorption wavelengths and the transition energy maxima of special pair chlorophylls and primary electron donors

	λ_{max}/nm	$h\nu_{max}$/eV	Δ/eV
Heliobacteria			
BChl *g*′	767[a]	1.62[a]	0.07
P798	798	1.55	
Cyanobacteria and higher plants			
Chl *a*	661[a]	1.88[a]	0.06
P680	680	1.82	
Chl *a*, *a*′	661[a]	1.88[a]	0.11
P700	700	1.77	
A. marina			
Chl *d*, *d*′	686[a]	1.81[a]	0.13
P740	740	1.68	
Green sulfur bacteria			
BChl *a*′	771[a]	1.61[a]	0.13
P840	840	1.48	
Purple bacteria			
BChl *a*	771[a]	1.61[a]	0.18
P870	870	1.43	
BChl *b*	791[a]	1.57[a]	0.28
P960	960	1.29	

[a] In diethyl ether

energy shifts caused by dimerization, Chl *a*/Chl *a*′ to P700 and Chl *d*/*d*′ to P740, are calculated to be roughly 0.11 and 0.13 eV, respectively, (Table 1; Iemura et al. 2008; Ohashi et al. 2008a), namely, the interaction between Chls *d*′ and *d* in P740 of *A. marina* is slightly but significantly stronger than that between Chls *a*′ and *a* in P700.

Further support comes from the interplanar distance of 3.6 Å in P700 (Jordan et al. 2001), being slightly but significantly longer than those in P870 and P960; the distance between rings I of $(\text{BChl } a)_2$ is 3.5 Å (Allen et al. 1987) and $(\text{BChl } b)_2$ is closer to 3 Å (Deisenhofer et al. 1984). The interplanar distance in P740 is expected to be somewhat shorter than that in P700 and almost the same as that in P870. To confirm our rough estimation, a crystallographic study for the PS I RC of *A. marina* is anticipated.

Existence and identity of quinone molecules as A_1

Heliobacteria

The electron acceptor A_1 in PS I was identified as phylloquinone (PhQ, Fig. 4a) in the mid 1980s (Takahashi et al. 1985; Schoeder and Lockau 1986), and its function as a bridge between A_0 and F_x was extensively documented (Brettel 1997; Itoh et al. 2001). However, for many years there has been considerable controversy concerning the nature and role of such an electron acceptor in PS I (Powls and Redfearm 1969; Brettel 1997). Similar controversies now apply to the RCs of heliobacteria and green sulfur bacteria.

It is not known whether a quinone analogous to A_1 in PS I acts in heliobacteria as an acceptor between A_0 and F_x. Menaquinones-7,8,9, and 10 (Fig. 4b) have been identified as the quinones in *Hb. chlorum* (Hiraishi 1989), whereas only MQ-7 has been identified in green sulfur bacteria (Powls and Redfearm 1969), in addition to the *Chlorobium* quinone and 1′-hydroxymenaquinone-7 (Powls and Redfearm 1969).

The presence of MQ in the RC of *Hc. mobilis* was first reported by Trost and Blankenship (1989), and then photoaccumulation of semiquinone was observed in the RCs of *Hc. mobilis* and *Hb. chlorum* (Brok et al. 1986; Trost et al. 1992; Muhiuddin et al. 1999). However, no corresponding spectral change in the UV region was observed (Brettel et al. 1998), and MQ can be extracted without affecting charge separation to FeS centers (Kleinherenbrink et al. 1993). Evidence regarding the existence of a functional quinone in the heliobacterial RC has been scarce and is still controversial (see reviews by Neerken and Amesz 2001 and Oh-oka 2007). The nature and contents of MQ species in heliobacteria are summarized in Table 2.

Recently, Miyamoto et al. (2008) detected a new type of ESP signal, ascribable to the $\text{P798}^{+}\text{A}_1^{-}$ state, in the RC core complex of *Heliobacterium modesticaldum*. This finding appears to confirm the presence of MQ as a photoreducible cofactor in the heliobacterial RC (Fig. 3), while its reaction mechanism is somewhat different from that of PhQ functioning as A_1 in PS I, in terms of molecular orientation and/or distance (Miyamoto et al. 2008). We have recently identified the quinone in this RC as well as those in *Heliobacterium fasciatum* RC and *Hb. mobilis* cells (Ohashi et al., unpublished data).

Green sulfur bacteria

An equivalent to the electron acceptor A_1 in oxygenic PS I, which is bound PhQ acting between A_0 and F_x, is not required in the RC of green sulfur bacteria (Hauska et al. 2001). Originally an equivalent role of MQ-7 (Redfearm and Powls 1968; Powls and Redfearm 1969; Collins and Jones 1981) as A_1 in the RC of green sulfur bacteria was considered (Hauska 1988) on the basis of semiquinone radical photoaccumulation in membranes (Nitschke et al. 1987) when electron transfer to the iron-sulfur centers was blocked, as was supported by Kjær et al. (1998) and Muhiuddin et al. (1999). Kjær et al. (1998) found that the RC of *Chlorobium vibrioforme* contains 1.7 molecules of MQ-7 and concluded that MQ-7 is analogous to PhQ in PS I on the basis of EPR measurement. Takaichi and Oh-oka (1999) also reported the presence of approximately one MQ-7 molecule in the photoactive RC of *Chlorobium*

Table 2 MQ species and stoichiometries of MQ/P798 and MQ/P740 in the RCs of heliobacteria and green sulfur bacteria

	MQ-6/P	MQ-7/P	MQ-8/P	MQ-9/P	MQ-10/P	Ref.
Hb. chlorum						
	[c]	[c]	[c]	[c]	[c]	Brok et al. (1986)
	0%[a,b]	1%[a,b]	15%[a,b]	79%[a,b]	4%[a,b]	Hiraishi (1989)
	[c]	[c]	[c]	[c]	[c]	Muhiuddin et al. (1999)
Hb. modesticaldum						
	[c]	[c]	[c]	[c]	[c]	Miyamoto et al. (2008)
Hb. mobilis						
	0	0	~0.1	~1.1	~0.1	Trost and Blankenship (1989)
Chl. limicola						
	[c]	[c]	[c]	[c]	[c]	Nitschke et al. (1987)
	[c]	[c]	[c]	[c]	[c]	Muhiuddin et al. (1999)
Chl. vibrioforme						
	0	1.7	0	0	0	Kjær et al. (1998)
Chl. thiosulphatophilium						
	0[a]	A[a]	0[a]	0[a]	0[a]	Redfearm and Powls (1968)
	0[a]	B[a]	0[a]	0[a]	0[a]	Powls and Redfearm (1969)
Chl. tepidum						
	0	0.9[d]	0	0	0	Takaichi and Oh-oka (1999)
	0	0.6–1.2[e]	0	0	0	Kusumoto et al. (1999)

A ca. 50 m moles per mole of chlorophyll

B ca. 70 m moles per mole of chlorophyll

[a] Cells or membranes

[b] Percentage of total peak area of HPLC

[c] Quinone was detected, but the chemical identity and content were not described

[d] Quinone was detected, but no description whether functional or not

[e] Quinone was detected, but not functional as A_1

tepidum. The nature and contents of MQ species are summarized in Table 2.

However, the essential role of MQ as A_1 has been challenged, because preparations completely devoid of MQ but capable of electron transfer to the FeS centers were obtained (Oh-oka et al. 1993; Frankenberg et al. 1996; Hager-Braun et al. 1997; Permentier et al. 2000). Further, Kusumoto et al. (1999) found no evidence that MQ acts as A_1 in their RC, although one MQ molecule remained per RC. Thus the question as to the existence of quinone and the function as an electron acceptor between A_0 and F_x in the RC of green sulfur bacteria has not been settled.

Gloeobacter violaceus and *Acaryochloris marina*

It is well established that A_1 in PS I is PhQ (Fig. 4a) in oxygenic photosynthetic organisms. Though PhQ was not detected in PS I particles of *G. violaceus*, a peak corresponding to MQ-4 (Fig. 4b) was clearly observed by reversed-phase HPLC analysis (Mimuro et al. 2005). The quinone exhibited the same absorption spectrum as an authentic MQ-4 sample (Mimuro et al. 2005). The stoichiometry of MQ-4/Chl *a′* is 2/1, indicating that two MQ-4 molecules are present in PS I, since one Chl *a′* molecule is present per P700 in PS I (Fig. 3). These results strongly suggest that A_1 of the PS I RC in *G. violaceus* is MQ-4 (Mimuro et al. 2005).

In contrast, the quinone of *A. marina* showed the same retention time as PhQ in *Synechocystis* sp. and the authentic PhQ by reversed-phase HPLC analysis (Ohashi et al. 2008a, b). The purified quinone from *A. marina* exhibited the same absorption spectrum as the PhQ standard (data not shown), indicating the presence of naphthoquinone framework (Fig. 4). The molar ratio of PhQ/Chl *d′* in *A. marina* is 2/1, indicating that A_1 in *A. marina* is PhQ (Ohashi et al. 2008a, b).

Succession of quinones: MQ to PhQ

Our results show that the molecular modification of quinones, A_1, did not occur during the evolution from anoxygenic heliobacteria into an early-diverging oxygenic

cyanobacterium, e.g., *G. violaceus*, and that the molecular conversion of MQ to PhQ took place most probably after the birth of cyanobacteria (see Fig. 3). This hypothesis indicates that optimization of photosystems might be delayed from the biological evolution.

At least three species, a primitive cyanobacterium *G. violaceus*, a primitive unicellular red alga *C. caldarium* (Yoshida et al. 2003), and a marine centric diatom *C. gracilis* (Ikeda et al. 2008) have been reported to use MQ-4 as A_1. As regards the electron transfer, use of MQ instead of PhQ as A_1 poses no problem, because their redox properties are almost the same. These algae seem to witness an ancient event that for a while after the birth of oxygenic photosynthesis, MQ was widely used by ancestral cyanobacteria, and some algae, like *C. caldarium* and *C. gracilis* (Fig. 3). However, one still cannot exclude the possibility that MQ found in *C. caldarium* and *C. gracilis* is due to the horizontal gene transfer.

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