

Unique photosystems in *Acaryochloris marina*

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Abstract A short overview is given on the discovery of the chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina* and the minor pigments that function as key components therein. In photosystem I, chlorophyll *d'*, chlorophyll *a*, and phylloquinone function as the primary electron donor, the primary electron acceptor and the secondary electron acceptor, respectively. In photosystem II, pheophytin *a* serves as the primary electron acceptor. The oxidation potential of chlorophyll *d* was higher than that of chlorophyll *a* in vitro, while the oxidation potential of P740 was almost the same as that of P700. These results help us to broaden our view on the questions about the unique photosystems in *Acaryochloris marina*.

Keywords *Acaryochloris marina* · Chlorophyll *a* · Chlorophyll *d* · Chlorophyll *d'* · Cyanobacteria · Pheophytin *a*

Abbreviations

BChl	Bacteriochlorophyll
Chl	Chlorophyll
HPLC	High performance liquid chromatography
PBP	Phycobiliproteins

P680	The primary electron donor of photosystem II
P700	The primary electron donor of Chl <i>a</i> -type photosystem I
P740	The primary electron donor of photosystem I in <i>A. marina</i>
Phe	Pheophytin
PS	Photosystem
RC	Reaction center

Introduction

A chlorophyll *d*-dominated cyanobacterium, *Acaryochloris marina*, was discovered by Miyashita, one of the authors of this review. In August 1993, while visiting Palau for two days, he came across one colony of ascidians, *Lissoclinum patella*, as a gift from Drs. M. Dionisio-sese and T. Maruyama. *L. patella* is a well-known host of *Prochloron*, a symbiotic cyanobacterium containing chlorophyll (Chl) *a* and Chl *b* (Fig. 1) without phycobiliproteins (PBP), but no one had ever succeeded in culturing *Prochloron*. The *Prochloron* cells were squeezed out from the ascidians, inoculated in a seawater-based IMK medium in a 24-well tissue culture plate, and then brought to Japan.

Though the *Prochloron* cells divided one or two times, they died within a few weeks, and the samples were left as it was. More than one month later, small yellowish-green colonies like green algae were unexpectedly found at the bottom of the wells. The microalga was ellipsoidal with 1–2 μm length; smaller than *Prochloron* in ascidians (spherical with 10–30 μm diameter). Miyashita assumed the yellowish-green microalga to be a free-living stage of *Prochloron*, and tried to incubate it in a fresh seawater-based medium, where the microalga grew well.

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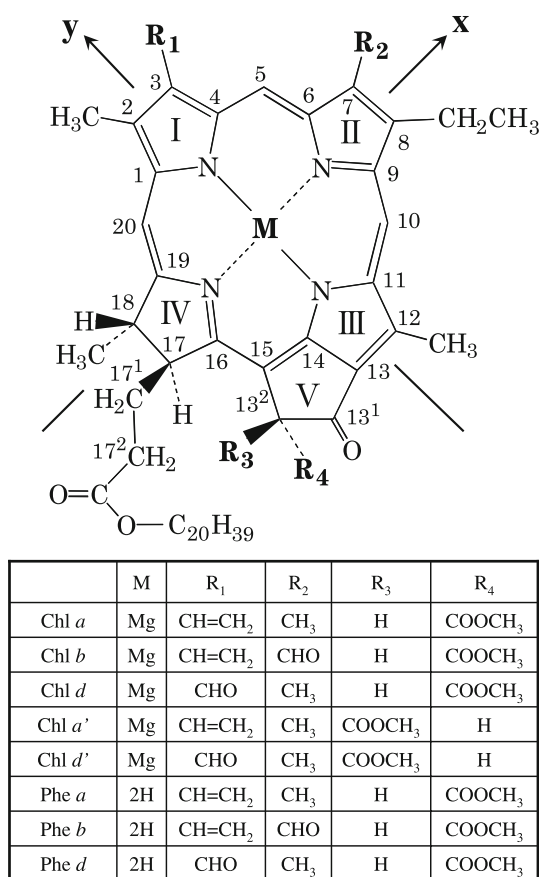


Fig. 1 Molecular structure and carbon numbering of chlorophylls, according to the IUPAC numbering system

In December 1993, pigment analysis of the alga was performed. The dominant pigment exhibited apparently the same retention time as that of Chl *b* on the reversed-phase HPLC elution profile. Trace amounts of Chl *a*, zeaxanthin, and magnesium 3,8-divinylphaeoporphyrin *a*₅ monoethyl ester were also detected. At that point, there was no doubt that the microalga should be a free-living *Prochloron*, but a big surprise awaited Miyashita.

The absorption spectrum of the “Chl *b*-peak” was of an unknown feature, and completely different from that of Chl *b* (Fig. 2). A few days later, the same spectrum was found in a book “Photosynthetic Pigments of Algae” (K. S. Rowan); the pigment was Chl *d*! NMR analysis confirmed the judgment (Miyashita et al. 1997). Here, a new genus, *Acaryochloris*, being unicellular cyanobacterium containing Chl *d* (Fig. 1) as a major pigment, was established (Miyashita et al. 1996) and has opened a new window for the photosynthesis science.

Later, in a red seaweed, *Ahnfeltiopsis flabelliformis*, Murakami found small patches on the thalli surfaces, a cyanobacterium-like prokaryotic epiphyte containing Chl *d*, while Chl *d* was absent inside the thalli (Murakami et al. 2004). Miyashita cut the thalli into small pieces, and

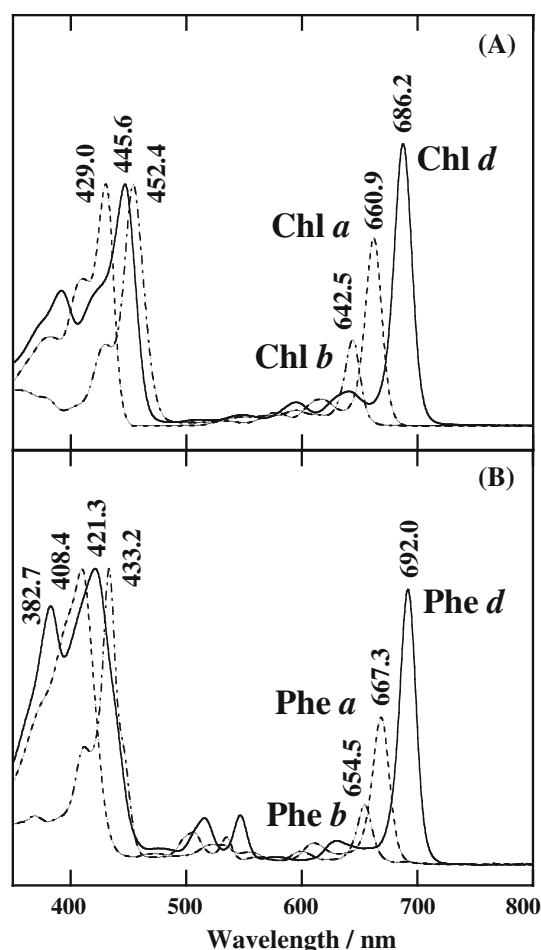


Fig. 2 Absorption spectra of (A) Chl *a*, *b*, *d* and (B) Phe *a*, *b*, and *d* in diethyl ether

then put them in a tissue culture plate with a seawater-based medium. Familiar yellowish-green colonies appeared at the bottom of the well. The molecular phylogenetic analysis showed the cells were of an *Acaryochloris* sp. Epiphytic *Acaryochloris* sp. was found in several red algae around Awaji Island, Japan (Ohkubo et al. 2006). This finding showed that Chl *d* detected in red algae was due to epiphytic *Acaryochloris* sp., and the genus *Acaryochloris* was the only organism that synthesizes Chl *d*.

Minor but key components in *Acaryochloris marina*

Though *A. marina* has Chl *d* as the major pigment, a few minor chlorophyllous pigments, Chl *d'*, Phe *a*, and Chl *a* (Fig. 1), are also present and function as key components in the reaction centers (RCs) of photosystem (PS) I and PS II (Akiyama et al. 2001). The content of Chl *a* varies according to light conditions (Boichenko et al. 2000; Akiyama et al. 2004; Mimuro et al. 2004), but at least one molecule of Chl *a* seems to be present in each RC (Akiyama et al. 2004; Kobayashi et al. 2005, 2007).

Photosystem I of *A. marina*

Chl *d'*, Chl *a* and phylloquinone in PS I

Chl *d'*, the 13²-epimer of Chl *d* (Fig. 1), was always detected in *A. marina* as a minor component, while Chl *a'* was absent (compare Fig. 3A with B) (Akiyama et al.

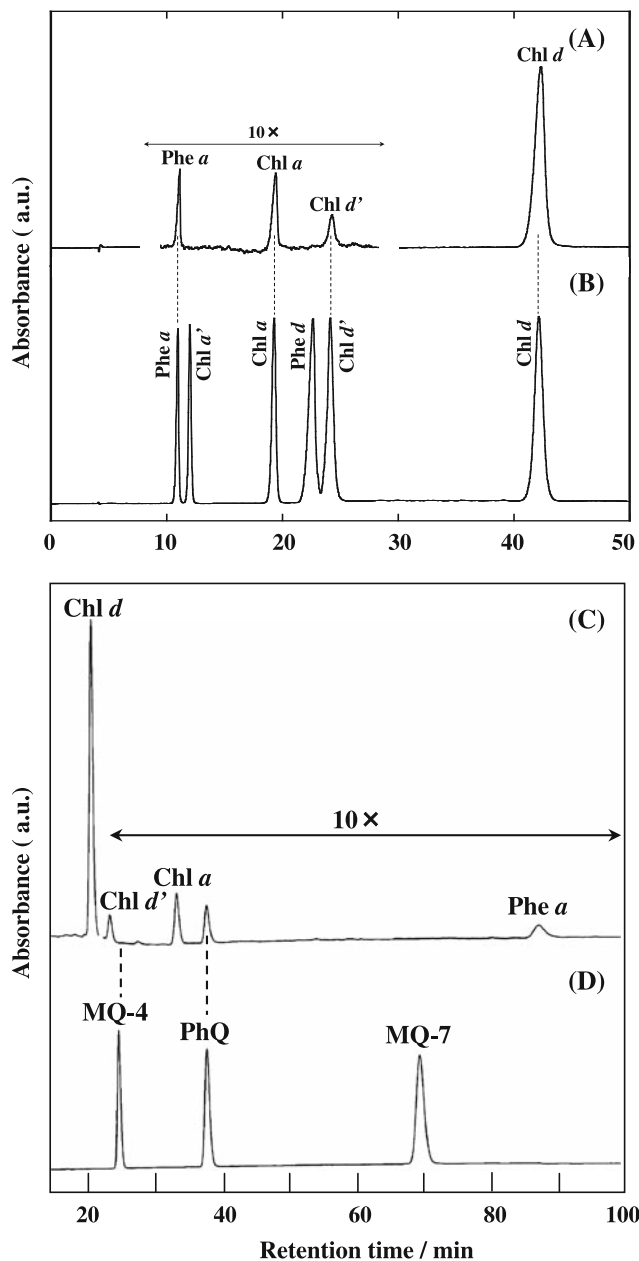


Fig. 3 (A, B) Normal-phase and (C, D) reversed-phase HPLC profiles for (A, C) acetone/methanol extracts of *A. marina*; (B) a mixture of authentic Phe *a*, Phe *d*, Chl *a'*, Chl *a*, Chl *d'*, and Chl *d*; and (D) a mixture of authentic MQ-4, PhQ and MQ-7. Eluents of normal-phase and reversed-phase HPLC are n-hexane/2-propanol/methanol (100/0.7/0.2, v/v/v) and ethanol/methanol/water (86/14/3, v/v/v), respectively. Detection wavelength is (A, B) 650 nm and (C, D) 248 nm

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), then a homodimer of Chl *d'* (Akiyama et al. 2001), and finally a Chl *d/d'* heterodimer (Fig. 4A) (Akiyama et al. 2002, 2004; Kobayashi et al. 2005, 2007), just like the Chl *a/a'* for P700 in other cyanobacteria and higher plants (Fig. 4C) (Kobayashi et al. 1988; Jordan et al. 2001): a dimer model for P740 was supported by FTIR spectroscopy (Sivakumar et al. 2003). The homology of PsaA and PsaB between *A. marina* and other cyanobacteria is low (Swingley et al. 2008), which may reflect the replacement of Chl *a* by Chl *d*, also Chl *a'* by Chl *d'*, in the PS I RC of *A. marina* (Fig. 4A), although the physiological significance of Chl *d'* in P740, as well as Chl *d'* in P700, is not yet clear. PsaA and PsaB in *Acaryochloris marina* as well as *Prochlorococcus* sp. have lower homology to those in common cyanobacteria. The phylogenetic tree for PsaA/B (Fig. 5) (Miyashita et al. unpublished data) shows that the branch length for the *Acaryochloris* (▼) as well as

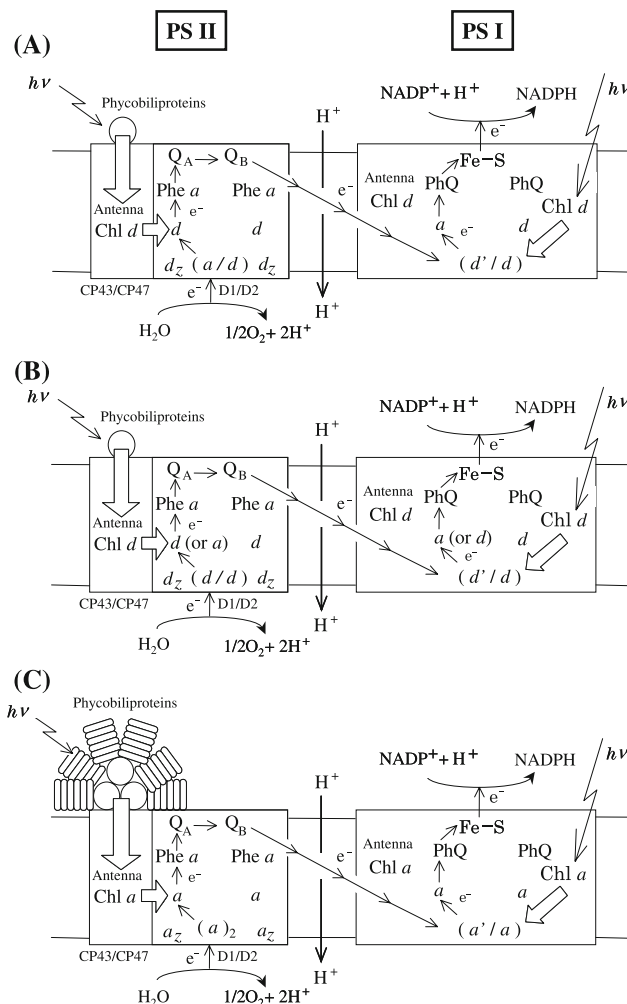


Fig. 4 Models for pigment arrangement in photosystems of (A, B) *A. marina* and (C) typical cyanobacteria

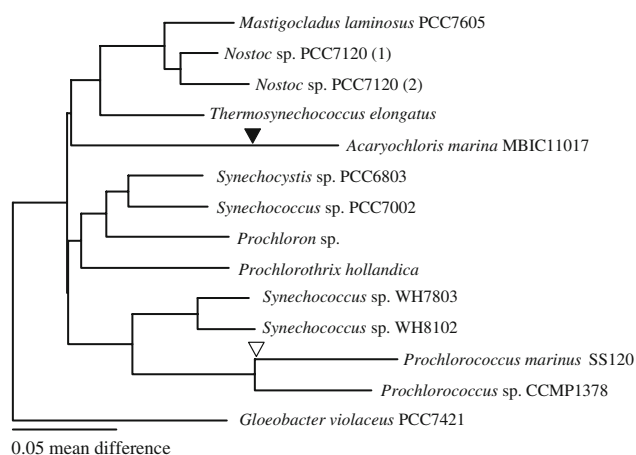


Fig. 5 Phylogenetic tree of cyanobacterial PS I core proteins. The amino acid sequences of PsaA and PsaB were combined. After eliminating the ambiguous alignment regions and gap-containing sites, the tree was inferred using neighbor-joining method (mean difference) with a total 1186 amino acid sites

Prochlorococcus (∇) were longer than the others. The long branch means that the evolution rates of PsaA and PsaB in those cyanobacteria are faster than those in common cyanobacteria. The change of evolutionary rate in the protein with the same function is usually explained by the change of evolutionary constraint of the protein. The reason of the low homology is inferred as the replacement of Chl *a* by Chl *d* and divinyl-chlorophyll *a*, respectively.

It is interesting to note that the primary electron acceptor, A_0 , in PS I of *A. marina* is not Chl *d* but appears to be Chl *a* (Fig. 4A), which was first shown by laser photolysis experiment (Kumazaki et al. 2002), and then supported by flash-induced spectral analysis (Itoh et al. 2007). The results support our hypothesis that Chl *a* or its derivative is a general feature of A_0 in the PS I-type RCs (see Fig. 2 in Akiyama et al. 2002), though the reason why Chl *a* functions as A_0 in the PS I-type RCs is still unclear, and the role and localization of Chl *a* in PS I of *A. marina* remain controversial (Fig. 4B) (Tomo et al. 2008). Further investigation is required to settle these questions.

The secondary electron acceptor, A_1 , in *A. marina* was identified as phylloquinone (PhQ) (Fig. 4A) by HPLC, absorption, and mass analyses of cells (Ohashi et al. 2007, 2008a), and then supported by HPLC and absorption analyses of PS I (Tomo et al. 2008). The quinone from *A. marina* cells showed the same retention time as the authentic PhQ (Fig. 3C, D), and the same result was recently reported in PS I of *A. marina*. The molar ratio of quinone/Chl *d'* was 2/1 both in cells (Fig. 3C) (Ohashi et al. 2007, 2008a) and in PS I (Tomo et al. 2008), indicating that two molecules of the quinone are present per PS I (Fig. 4A, B). The quinone exhibited the same absorption spectrum as the PhQ standard (Ohashi et al. 2007, 2008a;

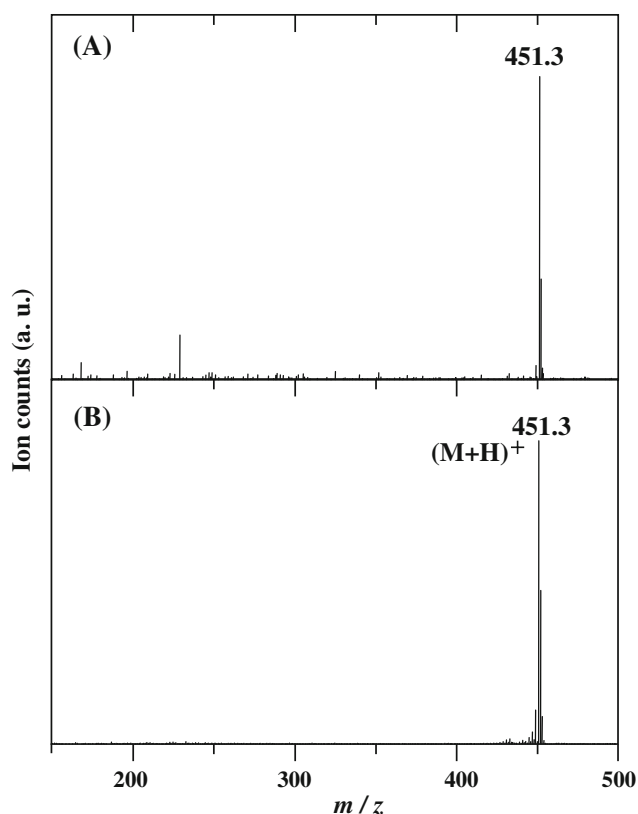


Fig. 6 APCI-mass spectra of (A) A_1 quinone of *A. marina* and (B) authentic PhQ

Tomo et al. 2008), indicating the presence of naphthoquinone framework in A_1 . The typical APCI-mass spectrum of the quinone is illustrated in Fig. 6A; the quinone showed a molecular ion peak $[M + H]^+$ at m/z 451.3, which was the same value as the authentic PhQ ($C_{31}H_{46}O_2$; mol wt. = 450.7, Fig. 6B). We thus concluded that A_1 of *A. marina* is PhQ.

Redox potentials of Chl *d* and P740

The midpoint potential E_m for P740 in PS I of *A. marina* was initially reported as +335 mV (Hu et al. 1998), being significantly lower than that of P700 (ca. +430 mV) in most Chl *a*-type PS Is (Ke 2001; Nakamura et al. 2005; Kato et al. 2008) (Fig. 7). Based on this, Chl *d* was supposed to have an oxidation potential E_{ox} lower than that of Chl *a*. The longer wavelength of the Chl *d* Q_Y -band (Fig. 2A) also appeared to support the view that Chl *d* is oxidized more easily than Chl *a* (see Fig. 1 in Ohashi et al. 2008b).

However, the E_{ox} value of Chl *d* in acetonitrile was found to be higher than that of Chl *a* (Kobayashi et al. 2007). The E_{ox} value order, Chl *b* (+0.93 V vs. SHE) > Chl *d* (+0.88 V) > Chl *a* (+0.81 V), is accounted

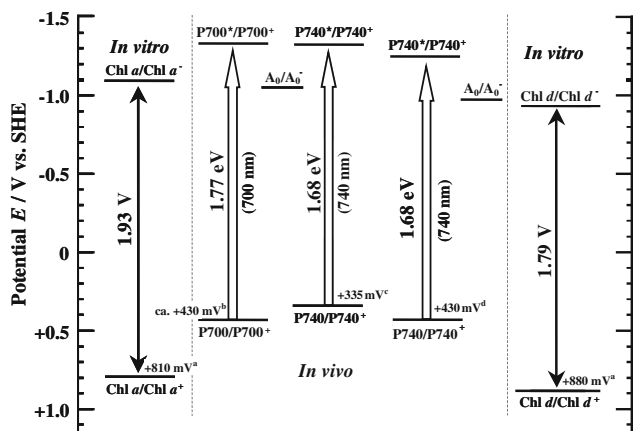


Fig. 7 Redox potentials of Chl *a* and Chl *d* in vitro, and P700 and P740 in vivo. **a** Kobayashi et al. 2007; **b** Nakamura et al. 2005; Kato et al. 2008; **c** Hu et al. 1998; **d** Benjamin et al. 2007; Telfer et al. 2007; Tomo et al. 2008

for by invoking the inductive effect of substituents on the conjugated π -electron system on the macrocycle.

The pheophytins showed the same E_{ox} order; Phe *b* (+1.25 V) > Phe *d* (+1.21 V) > Phe *a* (+1.14 V) (Kobayashi et al. 2007). The significantly higher potentials than those of the corresponding Chls are in line with electron density decrease on the π -system by replacement of magnesium with more electronegative hydrogen (Fig. 1). As a result, the E_{ox} of Chl *a* is the lowest among the Chls and Phe (see Table 1 in Kobayashi et al. 2007).

Recently, the E_m of P740 was re-examined and found to be +430 mV (Benjamin et al. 2007; Telfer et al. 2007) and +439 mV (Tomo et al. 2008), being much higher than the initial report (+335 mV) and almost equal to the Chl *a*-type P700 values of other cyanobacteria (Fig. 7), which is supported by the ENDOR analyses showing the location of spin density between P700⁺ and P740⁺ was almost same (Mino et al. 2005; Itoh et al. 2008). Significant differences in a few amino acids around P740 and P700 might also yield similar E_m values. The difference in the oxidation potential shifts between Chl *a*/Chl *a*⁺ \rightarrow P700/P700⁺ and Chl *d*/*d*⁺ \rightarrow P740/P740⁺ in Fig. 7, suggests that the interaction between the special pair chlorophylls (Chl *d* and *d*') of P740 might be stronger than that between Chl *a* and *a*' of P700.

The stronger interaction between Chl *d* and *d*' of P740 than Chl *a* and *a*' of P700 is partially supported by absorption spectral properties. In diethyl ether, the wavelengths of Q_Y maximum of Chl *a* and Chl *d* are 661 nm and 686 nm, respectively (Fig. 2A) (Kobayashi et al. 2006), and hence the excitation energy shifts caused by dimerization (Chl *a* \rightarrow P700 and Chl *d* \rightarrow P740) are roughly calculated to be 0.11 eV and 0.13 eV, respectively.

In purple bacterial special pairs, the energy shift of 0.13 eV is also calculated for BChl *a* (771 nm) \rightarrow P840,

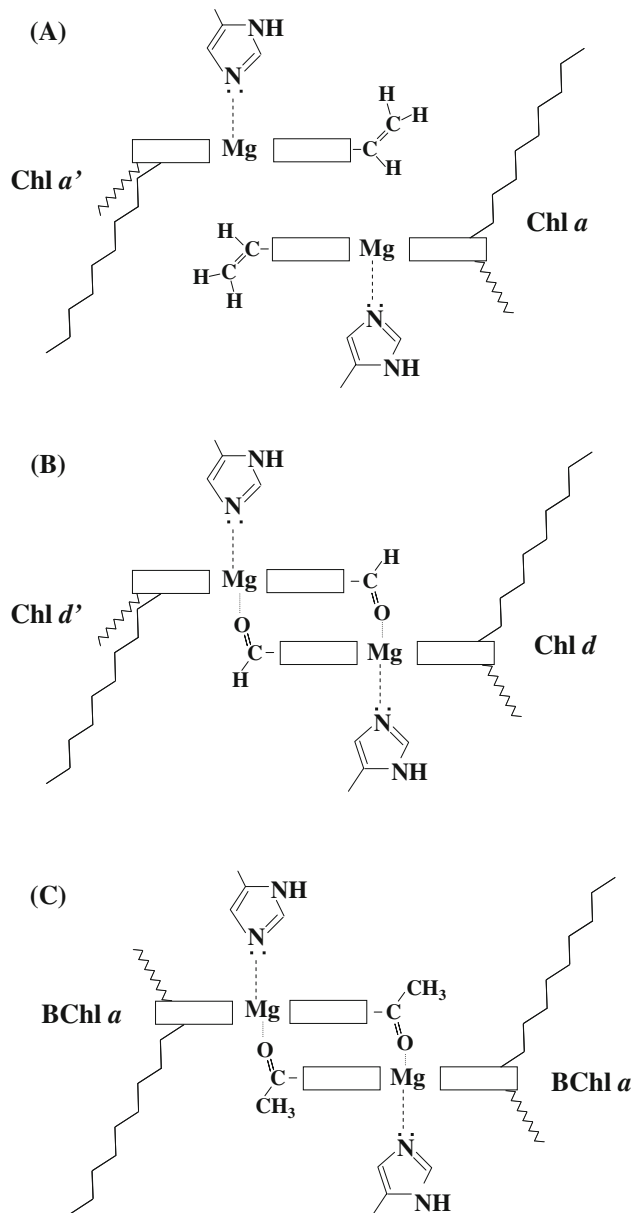


Fig. 8 Schematic illustrations of special pairs of (A) P700 in Chl *a*-type PSI, (B) P740 in *A. marina* PS I, and (C) P870 in purple bacteria

and a much larger shift of 0.28 eV for BChl *b* (796 nm) \rightarrow P970, suggesting that similarly stronger interaction is present in both P740 and P840, and much stronger interaction is present in P970. In this context, the interaction between two BChl *g*' molecules in P798 of heliobacteria (Kobayashi et al. 1991) is presumed to be very weak, since the energy shift for BChl *g*' (767 nm) \rightarrow P798 is significantly small, ca. 0.07 eV.

It is well known that the special pair consists of two (bacterio)chlorophyll molecules that are axially coordinated by two histidine residues from the protein subunits (Fig. 8). In the case of the purple bacterial special pair,

both BChl *a* and BChl *b* molecules have acetyl group at C3 of ring I, which are in direct contact with the Mg atoms of the other BChl in the special pair (see Fig. 8C) (Deisenhofer et al. 1984); the average distance between rings I of (BChl *a*)₂ is 3.5 Å (Allen et al. 1987) and (BChl *b*)₂ is closer to 3 Å (Deisenhofer et al. 1984). Chl *d* and *d'* in P740 have formyl group at C3 of ring I (Fig. 1), and hence coordination of formyl group to Mg is also expected (Fig. 8B). In contrast, the coordination of vinyl group at C3 of ring I of Chl *a* and *a'* to Mg was not observed in P700, where the vinyl group was configured as avoiding steric hindrance against the macrocycle of the other Chl (Fig. 8A) (Jordan et al. 2001). The weak interaction expected between two BChl *g'* molecules in P798 may be due to the presence of vinyl group at the same position, like Chl *a* and *a'* in P700. Further support comes from the interplanar distance of 3.6 Å in P700 (Jordan et al. 2001), being longer than 3–3.5 Å in P840 and P970. A slightly stronger interaction is hence expected to exist in the special pair Chls *d/d'* in P740 than the Chls *a/a'* in P700, and other experimental results are expected to confirm the hypothesis.

The similarity of oxidation potentials, being around +430 mV for both P700 and P740, may be required for the use of a common electron donor, plastocyanin, for P700⁺ and P740⁺. The redox potential of plastocyanin is known to be affected by the amino acids around the active site, which are also responsible for binding to PS I. These amino acids are highly conserved among *A. marina*, other cyanobacteria, and higher plants (NCBI data base), and hence the redox potential of plastocyanin in *A. marina* seems to be similar in other cyanobacteria (Benjamin et al. 2007; Tomo et al. 2008). If this is the case, the lower reducing power of P740* may affect the energetics of electron flow on the acceptor side (Benjamin et al. 2007), and thus the redox potential of A₀, Chl *a* (Fig. 4A) or Chl *d* (Fig. 4B), is expected to be lower than that in other cyanobacteria (see Fig. 7). Direct redox potential measurements for the electron transport components in vivo are strongly needed.

Photosystem II of *A. marina*

Phe *a* and Chl *a* in PS II

In the PS II RC of *A. marina*, the primary electron acceptor is not Phe *d* but Phe *a* (see Fig. 3A, C and 4A, B) (Akiyama et al. 2001, 2002, 2004; Mimuro et al. 2004; Kobayashi et al. 2005; Razeghifard et al. 2005; Chen et al. 2005; Tomo et al. 2007). It has not been clarified yet why *A. marina* uses Phe *a* instead of Phe *d*. One of the reasons might be the use of a common electron acceptor, plastoquinone, for pheophytin, because the reduction potential of

Phe *d* (−0.63 V) was found to be significantly more positive than that of Phe *a* (−0.75 V) in acetonitrile (Kobayashi et al. 2007).

Identity of the special pair in PS II

The nature of the special pair in the PS II RC has been controversial, and three models have been proposed; (1) a Chl *a* homodimer, (2) a Chl *a/d* heterodimer (Fig. 4A), and a Chl *d* homodimer (Fig. 4B). The Chl *a* dimer model was first proposed on the basis of the origin of delayed fluorescence by Mimuro et al. (1999), and was supported by the same method (Mimuro et al. 2000, 2004; Akimoto et al. 2006), the estimated value of the redox potential (Boichenko et al. 2000), and the pigment stoichiometry analysis (Akiyama et al. 2001, 2002, 2004). The Chl *a/d* heterodimer model (Fig. 4A) was first proposed on the pigment stoichiometry analysis by Kobayashi et al. (2005), and was supported by the redox potential measurement of Chls *a* and *d* (Kobayashi et al. 2007), the flash-induced spectral experiment (Schlodder et al. 2007), and its theoretical analysis (Renger and Schlodder 2008). The Chl *d* homodimer model (Fig. 4B) was first proposed on the spectral analysis by Itoh et al. (2001), and was supported by the similar methods (Itoh et al. 2008; Nieuwenburg et al. 2003; Tomo et al. 2007) and pigment stoichiometry (Chen et al. 2005).

The Chl *a* homodimer model is most unlikely, because the minimum ratio of Chl *a*/Phe *a* in *A. marina* whole cells grown under low-light condition was 2/2 (Mimuro et al. 2004), indicating only one Chl *a* molecule is located in the PS II RC (Kobayashi et al. 2005) on the basis of Phe *a*/PS II = 2, PS I/PS II = 1 (Akiyama et al. 2001), and the presence of one Chl *a* molecule as an active A₀ in PS I (Kumazaki et al. 2002). The model was also excluded by a quantitative explanation of the experimental data (Renger and Schlodder 2008).

It should be interesting to note that Itoh et al. (2007) and Schlodder et al. (2007) observed very similar difference spectra in the Q_Y region with main bleaching around 727 nm, but their interpretations are completely different; the former assumed that the main bleaching arose from both a homodimeric Chl *d* special pair (Fig. 4B) designated by them as P727 and an electrochromic shift due to the accessory Chl *d*, while the latter inferred that the bleaching was solely caused by the electrochromic shift of the accessory Chl *d* and that the special pair is either a Chl *a* homo- or a Chl *a*/Chl *d* heterodimer (Fig. 4A). Tomo et al. (2007), however, reported a significantly different spectrum with main bleaching around 713 nm, and they assumed that the bleaching was derived from a homodimeric Chl *d* special pair (Fig. 4B), and named it P713. The difference between 727 nm and 713 nm may originate in

seen in D1. The evolutionary constraint on the tryptophan residue was changed in D2 of *Acaryochloris*, indicating a substitution of the D2-side special pair Chl *a* (P680) with Chl *d* in the PS II RC of *A. marina* (Fig. 4A), although this idea has not been widely accepted yet.

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