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Discovery of Chlorophyll *d* in *Acaryochloris marina* and Chlorophyll *f* in a Unicellular Cyanobacterium, Strain KC1, Isolated from Lake Biwa

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Abstract

In this review, we described the biological characteristics of a cyanobacterium *Acaryochloris marina* and a unicellular cyanobacterium strain KC1 and the possible photosynthetic systems of the cells based on the physicochemical properties of chlorophylls. Strain KC1 as well as *Acaryochloris* spp. in addition to *Halomiclonema hongdechloris* should contribute the understanding of photosynthesis utilizing far red light.

Keywords: *Acaryochloris marina*; Chlorophyll *a*; Chlorophyll *a'*; Chlorophyll *d*; Chlorophyll *d'*; Chlorophyll *f*; Cyanobacteria; Pheophytin *a*; Strain KC1

Abbreviations: *A. marina*: *Acaryochloris marina*; Chl:chlorophyll; CCA: Complementary chromatic adaptation; CbCCA: Chlorophyll-based complementary chromatic adaptation; FR light: Far red light; *H. hongdechloris*: *Halomiclonema hongdechloris*; HPLC: High performance liquid chromatography; IC light: Incandescent light; P680: The primary electron donor of photosystem II; P700: The primary electron donor of Chl *a*-type photosystem I; P740: The primary electron donor of photosystem I in *A. marina*; Phe: Pheophytin; PS: Photosystem; RC: Reaction center; WF light: White fluorescent light

Introduction

A chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina* was accidentally discovered by Miyashita, one of the authors of this review. A colony of ascidians, *Lissoclinum patella* is a well known host of *Prochloron*. The *Prochloron* cells were squeezed out from the ascidians, inoculated in a seawater-based IMK medium. 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 found at the bottom of the wells. The microalga was ellipsoidal with 1-2 μm in length; smaller than *Prochloron* in ascidians (spherical with 10-30 μm in diameter). In December of 1993, the dominant pigment extracted from the microalga exhibited apparently the same retention time as that of Chl *b* on the reversed-phase HPLC elution profile. The absorption spectrum of the "Chl *b*-peak" was completely different from that of Chl *b* (Figure 1), but the same as that of Chl *d*. Here a new genus *Acaryochloris*, being unicellular cyanobacterium containing Chl *d* (Figure 1) as a major pigment, was established [1]. The molecular structure was confirmed by Mass and NMR analyses [2].

Chlorophyll *f* (Figure 1) was a new chlorophyll firstly reported by Chen et al. [3]. It was discovered in a methanolic extract of cells predominating in the enrichment culture of microalgae collected from Shark Bay stromatolites incubated under far red (FR) light. In the same period of time, a Chl *f*-producing cyanobacterium, strain KC1 (Figure 2A), was also discovered and isolated from freshwater environment by Miyashita. The discovery was also a fortunate accident, similar to that by Chen et al. in which the Chl *f*-producing cyanobacterium was a by-product during the hunting of chlorophyll *d*-producing cyanobacteria. Until recent years, Chl *d* was thought to be only detected in the cyanobacteria distributed in marine or salty lakes, since it had only been found in the cyanobacteria in the genus *Acaryochloris*, and the strains in *Acaryochloris* had only been isolated from saline environments but not from freshwater environments at all [4-7]. Actually, the strain *A. marina* MBIC11017 does not grow in freshwater media and requires sodium chloride for its growth at more than 1.5% (w/v) in the medium [2]. However, Chl *d* was detected in the sediment at the bottom of Lake Biwa, the largest freshwater lake in Japan [8]. The fact indicated that Chl *d*-containing microalgae exist in the freshwater lake. We collected algal mats and lake water from a shore zone of Lake Biwa. The samples were suspended in several media for freshwater algae, diluted and dispensed into cell culture plate or on agar plates. Those culture/agar plates were kept in an incubator with FR as the sole light source. Our attempt to isolate a Chl *d*-containing freshwater *Acaryochloris* sp. from Lake Biwa turned out to be a success (details will be reported elsewhere). After the isolation of freshwater *Acaryochloris* sp., Miyashita checked the culture/agar plates, which were left for a long time in the incubator with FR LED light, and found some cyanobacterial colonies that were different from those of *Acaryochloris* sp. in color; being dark-blue-green rather than yellow-green (Figure 2B). Morphological features of the cells were similar to those of *Acaryochloris* sp. in that the cell was unicellular, spherical to subspherical and aggregated (Figure 2A). Cells of strain KC1 were unicellular, coccoid to ovoid with 1.3-2.0 μm in diameter and 1.3-3.0 μm in length. The cells tended to form macroscopic colonies with extracellular mucilage in a liquid medium. We expected that the organism was a new Chl *d*-containing cyanobacterium which was closely related to the genus *Acaryochloris* phylogenetically, however, pigment analysis by means of HPLC showed that the cyanobacterium possessed no Chl *d* at all but Chl *a* as the major chlorophyll like the common cyanobacteria (Figure 3).

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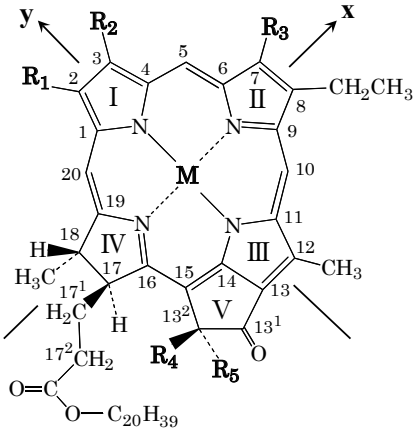

	M	R_1	R_2	R_3	R_4	R_5
Chl *a*	Mg	CH_3	$CH{=}CH_2$	CH_3	H	$COOCH_3$
Chl *b*	Mg	CH_3	$CH{=}CH_2$	CHO	H	$COOCH_3$
Chl *d*	Mg	CH_3	CHO	CH_3	H	$COOCH_3$
Chl *f*	Mg	CHO	$CH{=}CH_2$	CH_3	H	$COOCH_3$
Chl *a'*	Mg	CH_3	$CH{=}CH_2$	CH_3	$COOCH_3$	H
Chl *d'*	Mg	CH_3	CHO	CH_3	$COOCH_3$	H
Phe *a*	2H	CH_3	$CH{=}CH_2$	CH_3	H	$COOCH_3$

Figure1: Molecular structures of naturally occurring chlorophylls in oxygenic photosynthesis, according to the IUPAC numbering system.

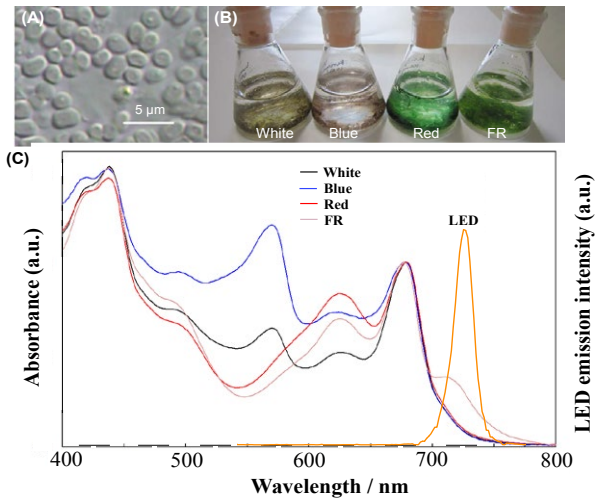

Figure 2: : Cell properties of strain KC1. (A) Cells grown under the white light, (B) color and (C) absorption spectra of the strain KC1 cells incubated under the different light conditions; white, blue, red and far red (FR). Isolated cells were cultured in BG-11 media using 50 mL or 100 mL conical flasks in stationary culture conditions, or using 100 mL flasks in aerobic culture conditions with aerating sterile air (0.05 L min^{-1}). All culturing flasks were incubated at 298 K. Monochromatic light sources were blue (MIL-B18), red (MIL-R18) and FR (MIL-IF18) LEDs (SANYO, Tokyo, Japan), and a light source of white light was the fluorescent light FL8N (Toshiba, Tokyo, Japan). Absorption spectra were measured by means of U-3900 spectrophotometer with φ60 integrating sphere (Hitachi, Tokyo, Japan) at room temperature. Cells were suspended in 20% polyethylene glycol (3,000 Da) aqueous solutions.

Nevertheless, an unusual chlorophyll was detected as a minor pigment, which showed typical two absorption peaks in the Soret (406 nm) and Q_Y (707 nm) regions in MeOH; they were clearly different from those of known chlorophylls. We concluded that the pigment was a new chlorophyll that should be named "Chl *f*". We started mass culture of the cells for chemical characterization such as detailed spectral properties, molecular mass and chemical structure. At around the same time, Chen et al. reported the discovery of Chl *f* [3], and then named the Chl *f* producing cyanobacterium *Halomicronema hongdechloris* [9]. The minor chlorophyll found in strain KC1 was also identified as Chl *f* by Mass and NMR analyses [10].

Both newly found chlorophylls, Chls *d* and *f*, have significant characteristics which can absorb light in the FR region. Those Chls must provide new insights on the photosynthesis especially on the oxygenic photosynthesis using FR light. In this review, we would like to summarize the biochemical and physicochemical characteristics of Chls *d* and *f* in addition to the biological properties of Chls *d*- and *f*-containing cyanobacteria. We also discuss the possible mechanism for oxygenic photosynthesis using FR light.

Absorption spectra

Chls *a, b, d* and *f*

Absorption spectra of Chls *a, b, d* and *f* in diethyl ether are shown in Figure 4. As compared to Chl *a*, Chl *b* shows red-shifted Soret bands and blue-shifted weak Q_Y bands, while the Q_Y bands of Chls *d* and *f* are intensified and shifted to longer wavelengths. The Q_X bands exhibit practically no intensity. The ratios of Soret/Q_Y band intensities show remarkable differences, 1.3 in Chl *a*, 2.8 in Chl *b*, 0.85 in Chl *d* and 0.65 in Chl *f*. The Soret band of Chl *f* is clearly split into two bands, most probably the so-called B-bands (longer wavelength) and η-bands (shorter wavelength). So one can easily distinguish them with little difficulty by their absorption spectra. We want to emphasize that one can easily distinguish Chl *f* from Chl *d* without spectrophotometer. As seen in Figure 5, Chl *f* looks blue-green as Chl *a*, while Chl *d* light-green as Chl *b*; the naked eye is often powerful for color judgment.

The inductive effects on the absorption wavelengths and intensities of Q_Y-bands of chlorophylls strongly depend on the nature and position of substituent(s) on the macrocycle, due to the presence of two different electronic transitions polarized in the x and y directions (the axes of transition moments are depicted in Figure1) [11-16]. Replacement of the electron-donating group, $-CH_3$, on ring II of Chl *a* by the electron-withdrawing group, -CHO, yielding Chl *b* (Figure 6), causes the blue-shift and significant intense reduction of the Q_Y-band. In contrast, replacement of $-CH_3$ on ring I of Chl *a* by -CHO, yielding to Chl *f*, causes the red-shift and intensity increase of the Q_Y-band. A similar phenomenon is clearly seen in Chl *d*, where $-CH{=}CH_2$ on ring I of Chl *a* is replaced with -CHO. These observations indicate that it is a general feature that substitution by the electron-withdrawing group on ring II causes the blue-shift and intensity reduction of the Q_Y-band and that the same substitution on ring I leads the opposite, namely, the red-shift and intensity increase of the Q_Y-band [10].

Pheophytin *a*

The Mg-free chlorophyll is called pheophytin (Phe). First of all, we emphasize that only Phe *a* (Figures 1 and 6) is present and functions in natural photosynthesis, and Phes *b, d* and *f* have not been found at all. In general, the more structured shape and red-shifted Soret band of Chls distinguish them from the corresponding Phes [10]. Removal of the central Mg increases the Soret and Q_X transition intensity, and

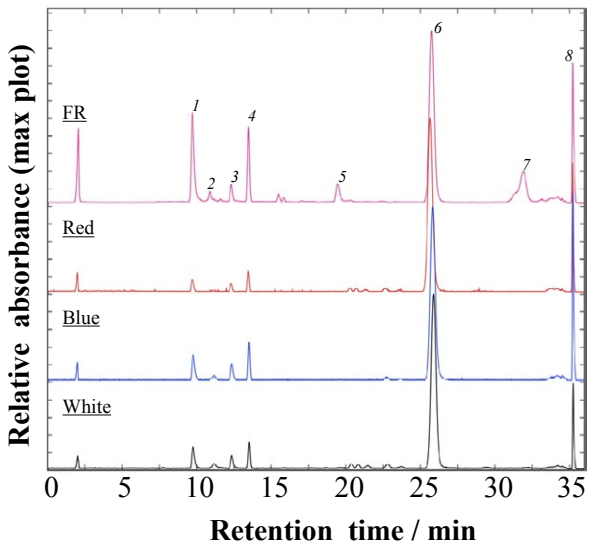

Figure 3: HPLC chromatograms of the methanol extracts from strain KC1 cells cultured under the different light conditions, far red (FR), red, blue and white. Chlorophylls and carotenoids were extracted with 100% methanol from strain KC1 cells and were injected into an ODS reversed phase HPLC column (Spherisorb 5 μm ODS2, 4.6 x 250 mm, Waters, UK). Pigments were eluted with water-methanol-acetone (0 to 10 min linear gradient 90% to 100% methanol, 10 to 30 min 100% methanol, 30 to 31 min linear gradient methanol to acetone, 31 to 35 min 100% acetone) at a flow rate of 1.0 mL min^{-1} at 298 K, and were monitored with a Waters 2996 PDA detector (350-800 nm). Chromatograms were normalized with the peak height of Chl *a* (peak 6). Peak 1: mixoxanthophyll, peak 2&3: unknown carotenoid, peak 4: zeaxanthin, peak 5: chlorophyll *f*, peak 6: chlorophyll *a*, peak 7 echinenone, peak 8: β-carotene.

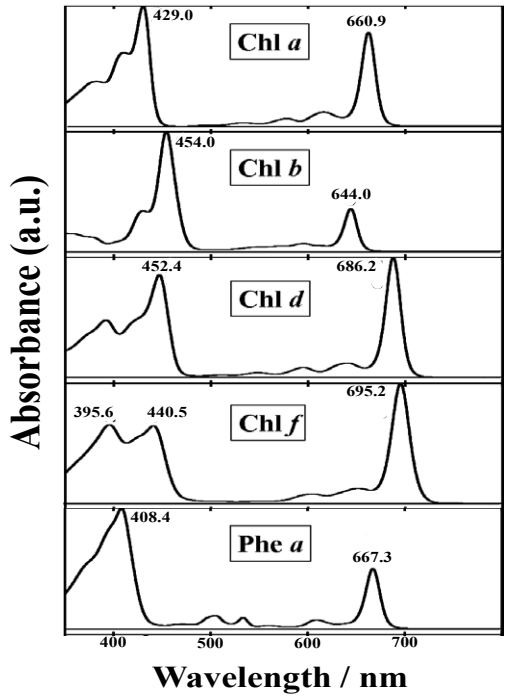

Figure 4: Absorption spectra of Chls *a, b, d, f* and Phe *a* in diethyl ether. Figure adapted from Kobayashi et al. (2013).

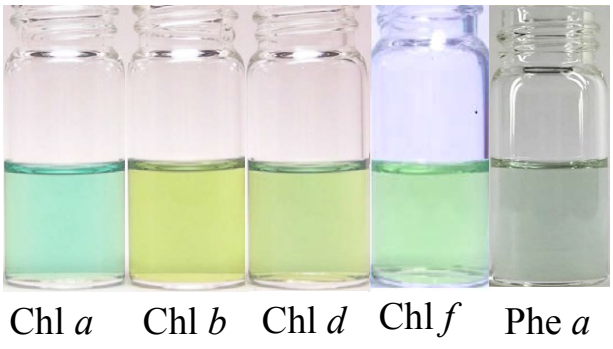

Figure 5: : Chlorophylls *a, b, d, f* and Phe *a* in diethyl ether.

hence the Soret/Q_Y-band ratio noticeably gets high, e.g., in diethyl ether Phe *a* shows the ratio of 2.0 (Figure 4). Pheophytin *a* has relatively strong and characteristic Q_X-bands in the region of 490-570 nm, and it is well resolved to the Q_X (0,0) and Q_X(1,0) transitions. The color of Phe *a* looks dark brownish-green (Figure 5), and hence the contamination of Phe *a* in a Chl sample is often noticeable even by the naked eye.

Minor but key chlorophylls in *Acaryochloris marina* and the strain KC1

Chl *a* and Chl *d'* in PS I of *Acaryochloris marina*

Although *A. marina* has Chl *d* as the dominant pigment, three minor chlorophylls, Chl *d'*, Phe *a* and Chl *a*, are present and function as key components in the reaction centers (RCs) of photosystem (PS) I and PS II [17]. Chlorophyll *d'* is the 13^2-epimer of Chl *d*, and Phe *a* is the Mg-free Chl *a* (Figure 6). Just as the Chl *a*/*a'* for P700 in the common cyanobacteria (Figure 7A) [18,19], the primary electron donor of PS I in *A. marina*, P740, was assigned to a Chl *d*/*d'* heterodimer (Figure 7B) on the basis of precise pigment analyses with HPLC [20-23], which was supported by Fourier-transformed infrared spectral study [24].

The primary electron acceptor, A_0, in PS I of *A. marina* is not Chl *d* but Chl *a* (Figure 7B) [25,26] as the common cyanobacteria (Figure 7A), supporting our hypothesis that Chl *a* or its derivative is a general feature of A_0 in the PS I-type RCs in [20], while the reason why Chl *a* functions as A_0 in the PS I-type RCs is still unclear.

The homology of PsaA and PsaB between *A. marina* and other cyanobacteria is low [27], which may reflect the replacement of almost all Chl *a* by Chl *d*, also Chl *a'* by Chl *d'*, in the PS I RC of *A. marina* [20-23]. The phylogenetic tree for PsaA/B shows that the branch length for the *Acaryochloris* is longer than the others, which means that the evolution rate of PsaA and PsaB in those cyanobacteria are faster than those in the common cyanobacteria [23]. The change of evolutionary rate in the protein with the same function is usually explained by the change of evolutional constraint of the protein. The reason of the low homology is inferred as the replacement of Chl *a* by Chl *d*.

Chl *a* and Phe *a* in PS II of *Acaryochloris marina*

Three models for the special pair in the PS II RC of *A. marina* has been presented: (1) a Chl *a* homodimer [17,28-32], (2) a Chl *a*/*d* heterodimer (Figure 7B) [21-23,33,34], and (3) a Chl *d* homodimer [35-38]. The Chl *a* homodimer model had been already denied, but there still remains controversy over two models, a Chl *a*/*d* heterodimer and a Chl *d* homodimer. To confirm the pigment arrangement in the PS II RC of *A. marina*, X-ray structural studies are strongly awaited.

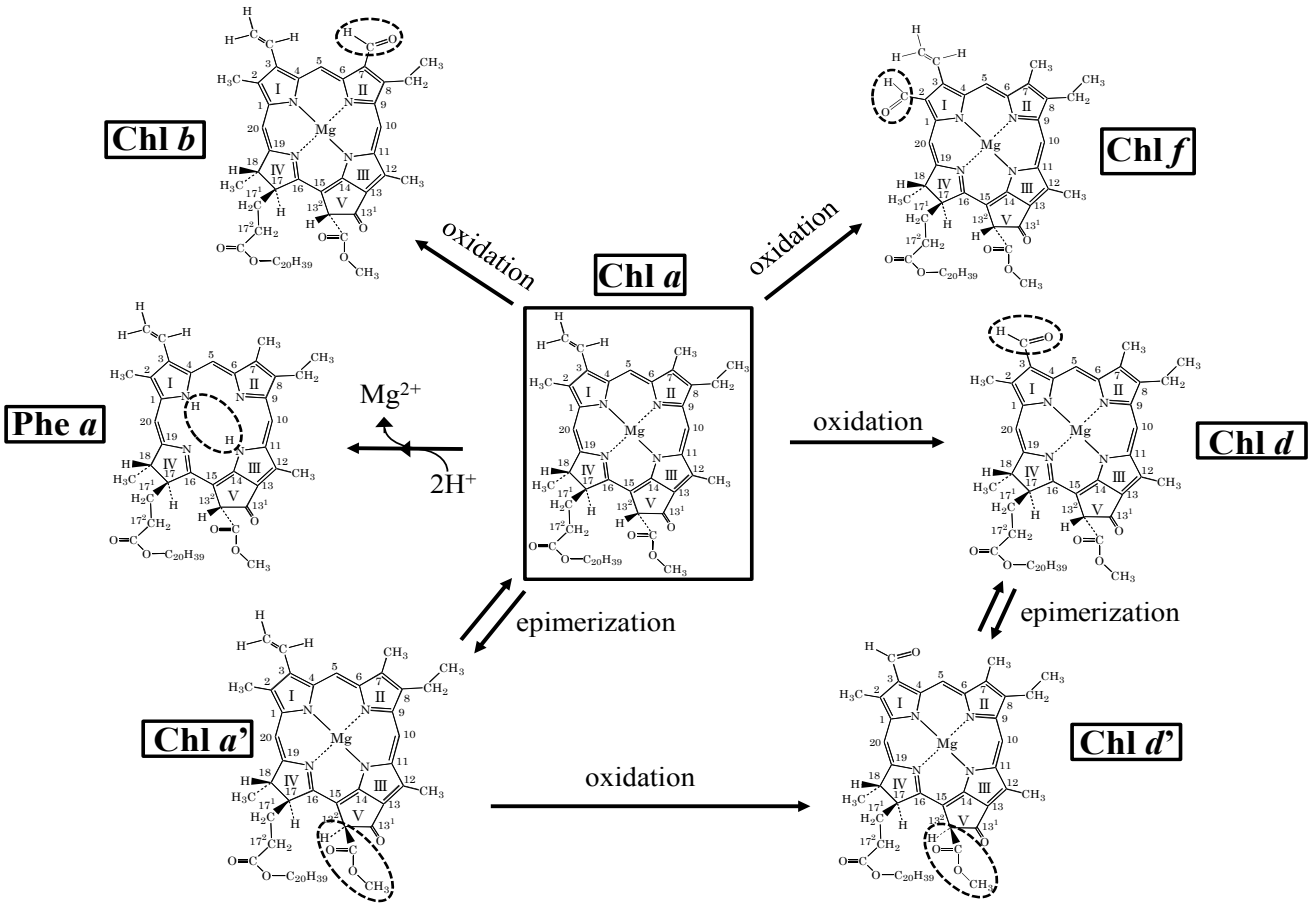

Figure 6: Chlorophyll *a* and its derivatives found in natural photosynthesis. Chlorophylls *a'* and *d'* function as the primary electron donors in PS I, Phe *a* as the primary electron acceptor in PS II, and Chl *b* as antenna. The function of Chl *f* has not yet been clarified.

Though Chl *d* is dominant in *A. marina*, pheophytin in *A. marina* is not *d*-type but *a*-type, namely, Phe *a* (Figure 7B), like the Chl *a*-type cyanobacteria (Figure 7A) [16,17,20,21,23,30,36,37,39,40]. It has not yet been clarified why *A. marina* uses Phe *a* as the primary electron acceptor in PS II. One of the reasons might be the use of a common electron acceptor, plastoquinone, which is supported in part by the fact that the reduction potential of Phe *d* (-0.63 V) in vitro is significantly less negative than that of Phe *a* (-0.75 V) [22], namely, Phe *d* is less favorable for reducing plastoquinone.

Chl *a*', Chl *f* and Phe *a* in the strain KC1

In the KC1 cells, Chl *a'* and Phe *a* are present as minor components as the common cyanobacteria, and Chl *f* is absent when incubated under white fluorescent (WF) light. Neither Chl *f'* nor Phe *f* is also detected at all. The results indicate that Chl *a'* and Phe *a* function as P700 and the primary electron acceptor of PS II, respectively, in the strain KC1 (Figure 7C) as in the common cyanobacteria (Figure 7A) [41] and that Chl *f* does not play as primary pair in PSI nor special pair in PSII. A small amount of Chl *f* is detected only when the KC1 cells are grown under FR LED light. It is of interesting to note that Chl *f* is not induced in the strain KC1 under WF light even if FR LED light is also used as additional light. The function of Chl *f* in energy storage is under debate, because uphill energy transfer is needed to deliver the excitation energy to Chl *a* molecules in the RC [42]. Chlorophyll *f* may function as not an electron transfer component but an antenna part (Figure 7C).

Light adaptation of *A. marina* and the strain KC1

The stoichiometric changes of Chl *d*/PS I, Chl *d*/PS II and PS I/PS II in the cells of *A. marina*

Pheophytin *a* and the epimers of Chls *a* and *d* are powerful indicators for determining the antenna size and the PS I/PS II stoichiometry, because two Phe *a* molecules are present only in PS II, and one molecule of Chl *a'* or *d'* is present only in PS I (see Figure 7).

The molar ratios of Chl *d*/*d'* and Chl *d*/Phe *a* in the cells grown under WF light (SWL in Figure 8) are 72 and 49, respectively, and these are 143 and 58 in the cells under the illumination of incandescent light (IC light; LWL in Figure 8) [39,41]. The ratio of Chl *d*/*d'* in the WF-cells is about half of that in IC-cells (Figure 8A), while the ratios of Chl *d*/Phe *a* in the WF- and IC-cells are almost the same (Figure 8B); the stoichiometries of Chl *d*/PS I, Chl *d*/PS II and PS I/PS II are calculated to be 72, 98 and 1.4 in the WF-cells, and 143, 116 and 0.8 in the IC-cells (Figure 8).

The content of Chl *a* in *A. marina* varies according to light conditions [30,31,39,41], the molar ratios of Chl *a*/*d'* and Chl *a*/Phe *a* in the WF-cells are 2.7 and 1.8, respectively, and these are 5.14 and 2.11 in the IC-cells; at least one molecule of Chl *a* is present in each RC [21,22,39]. It is of interest to note that as illustrated in Figure 8C the change of PS I/PS II stoichiometry is small, 1.4 and 0.8, compared to other cyanobacteria including the strain KC1.

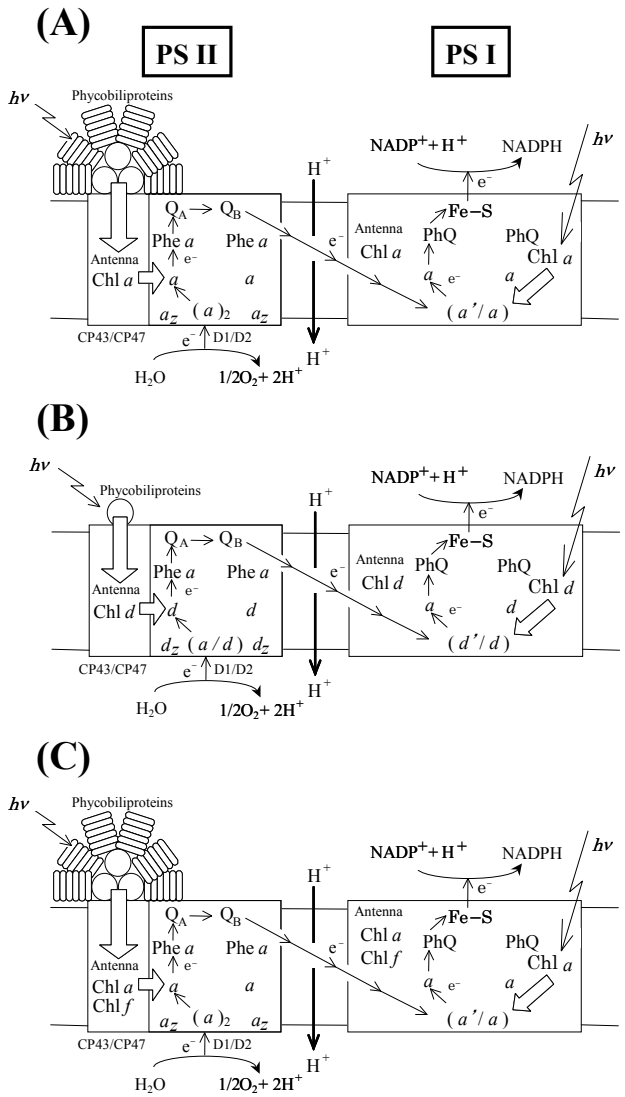

Figure 7: Models for pigment arrangement in photosystems of (A) typical cyanobacteria, (B) *A. marina* and (C) strain KC1 grown under far red LED light (FR light).

The stoichiometric changes of Chl *a*/PS I, Chl *a*/PS II and PS I/PS II in the strain KC1 cells

Drastic color change was observed in the cells of KC1 acclimated under different light conditions as seen in Figure 2B. As shown in Figure 2C, absorption spectra of the FR-cells show a clear shoulder over 700 nm, due to the presence of small amounts of Chl *f*. The absorption spectral changes observed in strain KC1 resemble those in *H. hongdechloris* cells grown under white light or red light [9].

Strain KC1 showed characteristic Chl-based complementary chromatic adaptation (CbCCA) in addition to the common complementary chromatic adaptation (CCA) that is well known in a part of cyanobacteria. The cells grown under WF light were blackish-green in color (Figure 2B). The cells grown under blue and red light showed reddish- and blue-green, respectively. It was identical to the common CCA, in which phycoerythrin absorbing light around 560 nm as its peak was increased under the blue light and decreased under red light (Figure 2C). On the contrary, phycocyanin absorbing light around 630 nm as its peak was increased under red light. In addition to those phycobiliprotein-based CCA, strain KC1 showed further and additional adaptation under FR condition. The cells grown under FR light looked green rather than blue-green of the cells grown under red light (Figure 2B). It was due to the decrease of phycocyanin, the increase of absorption around 450-500 nm, and the appearance of an additional absorption band around 720 nm (Figure 2C).

HPLC chromatograms of methanol-soluble pigments extracted from the cells grown under the each light condition showed that only the cells grown under FR light contained Chl *f* and accumulated echinenone (Figure 3). This result showed that appearance of extra absorption in the cell grown under FR light was due to the production of Chl *f* as an extra chlorophyll, which might make strain KC1 possible to perform photosynthesis under FR light as a sole light source.

The each content of Chl *a'*, Phe *a* and Chl *f* vs. Chl *a* is ca. 2.1%, 0.6% and 0% in the WF-cells, while 2.1%, 1.6% and 5.3% in the FR-cells; the stoichiometries of PS I/PS II were calculated to be 6.5 and 2.7, respectively, as shown in Figure 8C [41]. The molar ratios of Chl *f*/*a'* (Chl *f*/PS I), Chl *f*/Phe *a* (Chl *f*/PS II) and the stoichiometry of PS I/II in the FR-cells are 2.5 (2.5), 3.3 (6.6) and 2.7, respectively, suggesting that each PS I and PS II possesses ca. two Chl *f* molecules, which is supported in part by fluorescence experiments exhibiting the presence of Chl *f* in both PS I and PS II (S. Itoh, personal communication). The results also indicate that the positions permitting the insertion of Chl *f* are severely restricted in certain protein(s).

Chlorophyll *f* in the cells of strain KC1 was reversibly induced and destructed. The accumulation of Chl *f* in the cells grown under WF light was started after the transfer of cell under FR light, and reached a plateau within two weeks under the continuous light condition (Figure 9). The ratio of Chl *f*/Chl *a* increased from 0% to about 8% linearly. On the contrary, the loss of Chl *f* was rather faster than the accumulation, in which the ratio of Chl *f*/Chl *a* decreased from about 10% to about 1% within five days after the transfer of the cells from under FR to WF light. After sixth day, the content of Chl *f* decreased slowly and did not reach to zero for 2 weeks. The reason why Chl *f* was not completely disappeared under WF light for 2 weeks is possibly due to the self-shading effect by forming cell aggregates.

We had better pay attention that the emission spectrum of FR LED overlaps with the absorption spectra of the strain KC1 cells grown under white, blue or red light (Figure 2C), which is one indication that the KC1 cells in the absence of Chl *f* can absorb FR LED light by some Chl *a* molecules with longer wavelength absorption and that they may act as a trigger for Chl *f* biosynthesis. The other possibility of the trigger for Chl *f* induction might be a presence of photoreceptors like cyanobacteriochromes or phytochromes. Thus further studies are required to revel the molecular mechanism for Chl *f* induction and reduction.

Phylogenetic properties of a Chl *f*-containing cyanobacterium strain KC1

The SSU rRNA gene sequence of strain KC1 had 97.5% maximum identity (query coverage 99%, 1311/1344) with that reported as "*Aphanocapsa muscicola* 5N-04" [43]. Phylogenetic analysis based on the sequence indicated that strain KC1 formed a clade with some cyanobacteria including *Aphanocapsa muscicola* strains 5N-04 and VP3-03 [43] and *Acaryochloris* sp. JJ8A6 [44] which had a sister relationship to true *Acaryochloris*-clade (Figure 10). Strain KC1 was

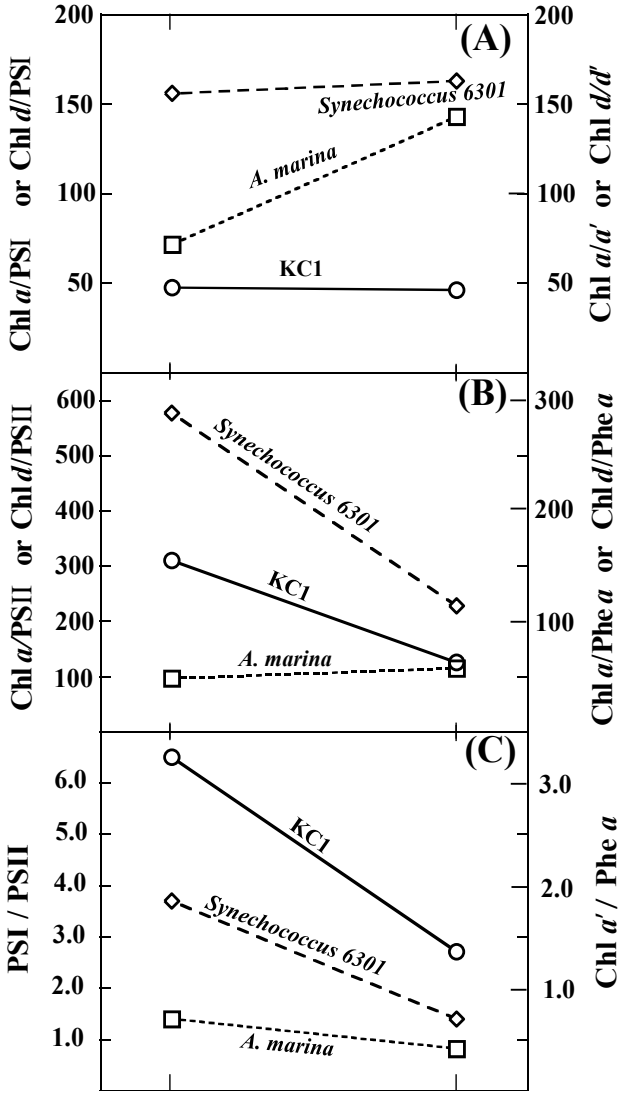

Figure 8: Light adaptation of *A. marina*, the strain KC1 and *Synechococcus*. Stoichiometries of Chl *a*/PS I and Chl *a*/PS II in Synechococcus (◊) and the strain KC1 (○) are calculated from the pigment molar ratios of Chl *a*/*a'* and Chl *a*/Phe *a*, respectively, on the basis of Chl *a'*/P700 = 1 and Phe *a*/P680 =2. Stoichiometries of Chl *d*/PS I and Chl *d*/PS II in *A. marina* (□) are calculated in a similar manner from the ratios of Chl *d*/*d'* and Chl *d*/Phe *a*. SWL (short wavelength light): white fluorescent light for *A. marina* and the strain KC1, yellow light for *Synechococcus*. LWL (long wavelength light): incandescent light for *A. marina*, far red LED light for the strain KC1, and red light for *Synechococcus*. Figure adapted from Akutsu et al. (2011).

both morphologically and phylogenetically different from the firstly reported Chl *f*-containing cyanobacterium, *H. hongdechloris* [9]. *H. hongdechloris* is filamentous and it has only 92% identity of SSU rRNA gene sequence with that of strain KC1. The taxonomical consideration of strain KC1 requires further consideration, since it is not suitable to assign strain KC1 as *Aphanocapsa* or *Acaryochloris* based on the characteristics discussed here.

Redox potentials of Chl *d* and Chl *f in vitro*

Oxidation potential, E_{ox}, of Chl *d* in acetonitrile is significantly higher than that of Chl *a* [22]. Chlorophyll *f* also has higher value than

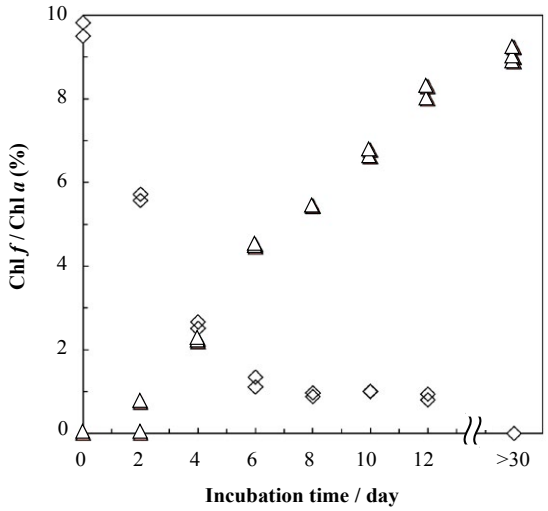

Figure 9: Time course of the Chl *f* content relative to Chl *a* in KC1. Cells were transferred from under the white light to the far red light (Δ) and from under the far red light to the white light (◊).

Chl *a* [10]. The order of E_{ox} values, Chl *b* (+0.94 V vs. SHE) > Chl *f* (+0.92 V) >> Chl *d* (+0.88 V) >> Chl *a* (+0.81 V) seen in Figure 11, is accounted for by invoking the inductive effect of substituent groups on the macrocycle, because the redox potentials of chlorophylls are sensibly affected by the nature of substituent groups on the conjugated π-electron system [10,22,45,46].

The -CHO substituent on Chls *b, d* and *f* (Figure 6) is an electron-withdrawing group (→CHO), and hence reduces the electronic density in their π-systems. The replacements of $-CH_3$ at C7 or C2 of Chl *a* by -CHO to yield Chl *b* or Chl *f* (Figure 6), respectively, cause the macrocycle to be electron poor, thus rendering the molecule less easily oxidized (Figure 11). Replacement of $-CH=CH_2$ at C3 of Chl *a* by -CHO to yield Chl *d* (Figure 6) makes the oxidation potential more positive than that of Chl *a* (Figure 11). Thus E_{ox} order becomes Chls *b, d, f* > Chl *a*, as mentioned above. When one pays attention to the group of $-CH_3$ at C7 of Chl *d* and the group of $-CH=CH_2$ at C3 of Chl *b* or C7 of Chl *f*, the $-CH_3$ moiety is more electron-donating ($\leftarrow CH_3$), thus making the macrocycle of Chl *d* more electron rich, and hence its oxidation potential less positive (Chls *b, f* > *d* > *a*). As expected from the inductive effect of substituent groups, Chls *b* and *f* will show the almost the same oxidation potentials, though a little higher oxidation potential of Chl *b* than that of Chl *f* by 20 mV in Figure 11 cannot be explained from the primitive way used here.

As seen in Figure 11, Phe *a* has terribly high oxidation potential of +1.14 V [22], which is in line with electron density decrease on the π-system by replacement of magnesium, Mg, with more electronegative hydrogen, H [15,46,47]. We should note that pheophytins have significantly higher oxidation potentials than the corresponding chlorophylls [10,22,46], but oxygenic photosynthesis uses Chl *a*, which has the lowest oxidation potential (Figure 11), even though higher oxidation potential is preferable to water splitting. The details of this mystery will be described elsewhere.

The redox potentials of chlorophylls are related to the energy levels of their molecular orbitals: the first oxidation potential is intimately related to the highest occupied molecular orbital (HOMO) and the first reduction potential to the lowest unoccupied molecular orbital

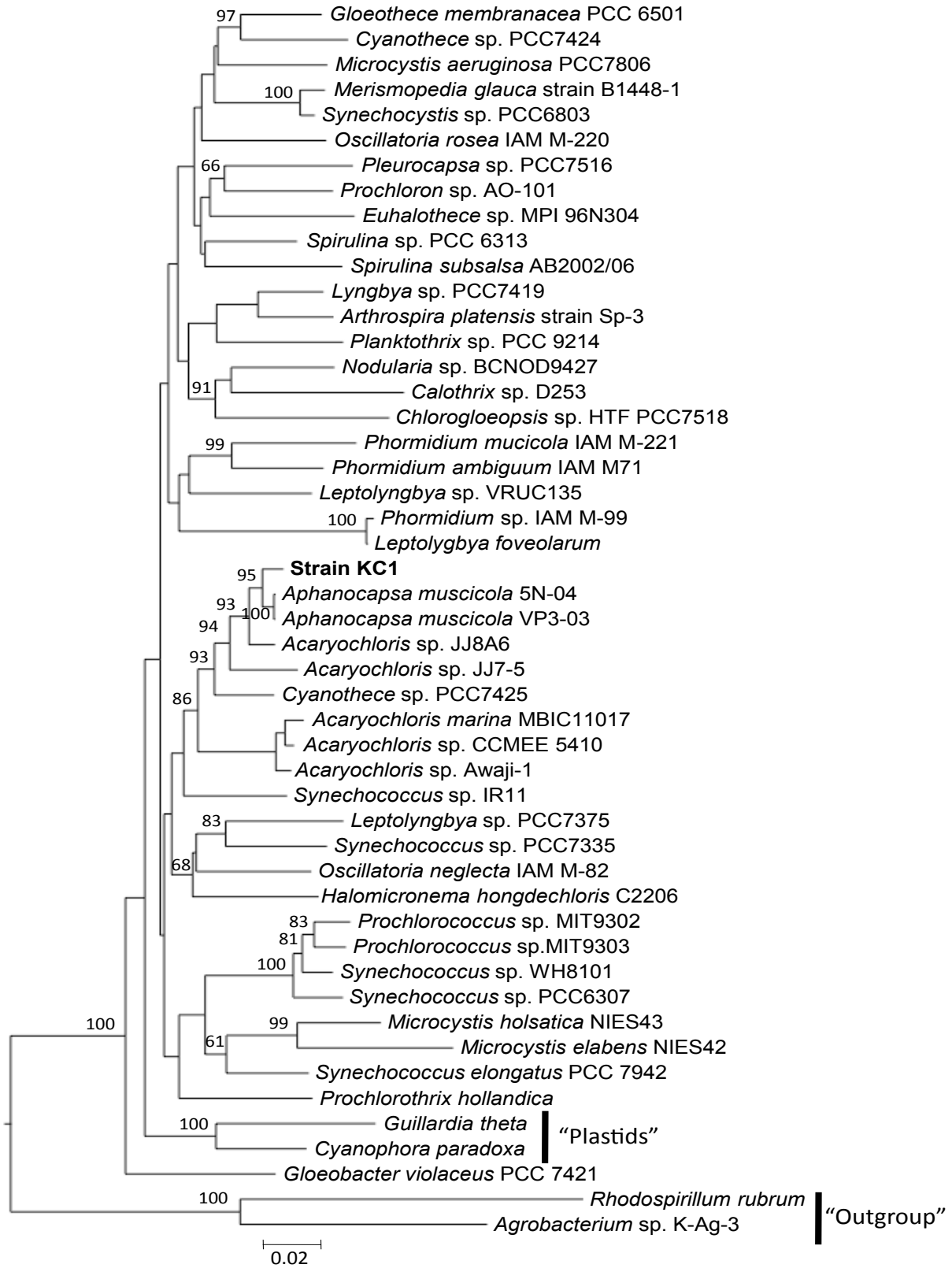

Figure 10: Phylogenetic position of a cyanobacterium strain KC1. The tree was constructed by Neighbor Joining method, using SSU rRNA partial sequence of approximately 1,212 bp. Numbers at each node shows bootstrap values and values less than 60% were not shown.

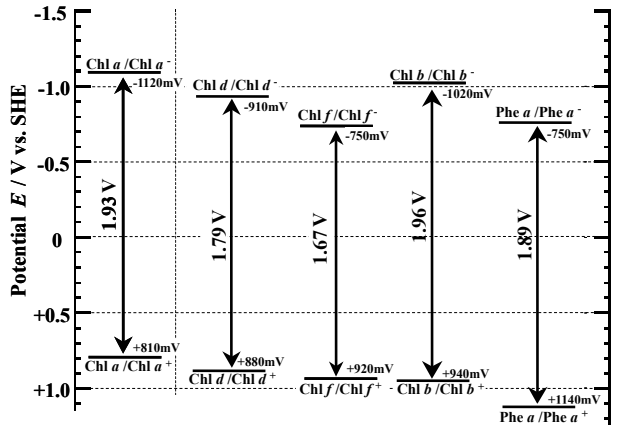

Figure 11: Redox potentials of Chls *a, b, d, f* and Phe *a* in acetonitrile. Figure adapted from Kobayashi et al. (2007, 2013).

(LUMO), and hence the redox potential difference seen in Figure 11 can be taken as an index for the Q_Y excitation energy, ΔE [15,46]. For example, ΔE for Chl *a* is 1.93 eV, which well corresponds to the Q_Y excitation wavelength of 661 nm for Chl *a* in Figure 4. Similarly, ΔE = 1.96 eV, 1.79 eV and 1.67 eV for Chls *b, d* and *f* also nicely correlate to the Q_Y peak wavelengths, 644 nm, 686 nm and 695 nm, respectively Pheophytin *a* also behaves in a similar fashion; 1.89 eV to 667 nm.

Evolution from Chl *a*-type cyanobacteria to *A. marina* and the strain KC1

Here we introduce our hypothesis about the evolution of *A. marina* and the strain KC1 from the Chl *a*-type cyanobacteria on the basis of the chlorophyll modification (Figure 6).

Since the Chl *a* → Chl *d* conversion occurs with ease under oxidative conditions [21,48], which supports in part the succession from the Chl *a*-type cyanobacteria to *A. marina*. Chlorophyll *f* is also produced from Chl *a* by oxidation, suggesting that Chl *f* also appeared after acquisition of Chl *a*. In contrast, spontaneous conversion of Chl *a* into Chl *b* has not yet been observed.

Chlorophyll *a'* and Chl *d'* are easily formed from Chl *a* and Chl *d*, respectively, by epimerization under weak basic conditions; these two primed chlorophylls, Chls *a'* and *d'*, function as the primary electron donor in PS I (Figure 7). Pheophytin *a* is also produced from Chl *a* with great ease under mild acidic conditions, and Phe *a* functions as the primary electron acceptor in PS II (Figure 7).

It is of interest to note that Chls *a', d, d', f* and Phe *a* are, so to speak, the secondary products from Chl *a*, but function as key components in natural oxygenic photosynthesis, while other possible artifacts, Phe *b, d, f* and Chls *b', f '* are not found in natural photosystems. We should emphasize again that only Chl *a* is the primary electron acceptor, A_0, in PS I with no exceptions.

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