MECHANISMS OF ACIDO-TOLERANCE AND CHARACTERISTCS OF PHOTOSYSTEMS IN AN ACIDO- AND THERMO-PHILIC RED ALGA, *Cyanidium caldarium*

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Abstract

In this chapter, we describe the mechanisms of acido-tolerance in an acido- and thermo-philic red alga, *Cyanidium caldarium*. In spite of the extremely acidic environments it inhabits, the intracellular pH of *Cyanidium* cells is kept neutral by pumping out the protons previously leaked into the cells according to the steep pH gradient. The H^+ pump is driven by the plasma membrane ATPase, utilizing intracellular ATP produced by both oxidative phosphorylation and cyclic photophosphorylation via photosystem I. We also describe the characteristics and function of the two photosystems, Photosystem I (PSI) and II (PSII) in *Cyanidium caldarium* in comparison with those of cyanobacteria, other eukaryotic algae and higher plants, based on the crystal structures of the two complexes reported so far.

1. Introduction

Cyanidium caldarium is a primitive, unicellular red alga found in acidic hot springs throughout the world (Allen, 1959; Doemel and Brock, 1971). The alga is an obligate autotroph and has a discrete nucleus, mitochondria and a large single chloroplast but no vacuoles (Enami et al., 1975). The alga can grow at pH values as low as 0 (Allen, 1959), and the pH optimum for growth is between 1.0 and 4.0. No cell division occurs above pH 5 (Doemel and Brock, 1971). The extremely acidophilic nature of *C. caldarium* is a common feature of the Cyanidiaceae, a red algal group comprising six species, namely, *Galdieria sulphuraria, Galdieria partita, Galdieria*

daedala, Galdieria maxima, Cyanidium caldarium and *Cyanidioschyzon merolae* (Albertano et al., 2000; Ciniglia et al., 2004*)*. Among these, the complete genome of *G. sulphuraria* and *C. merolae* has been sequenced (Weber et al., 2004; Matsuzaki et al., 2004). *C. merolae* is distinguished from others by the lack of a rigid cell wall, which enables its cells to be broken down simply by freeze-thawing. *Galdieria* is a facultative heterotroph, which contrasts to the other two groups *Cyanidium* and *Cyanidioschyzon* that are obligate autotrophy (Weber et al., 2007). Like most of other species of Cyanidiaceae (Weber et al., 2007), *C. caldarium* is moderately thermophilic (grows at temperatures around 42-45 ºC). This makes its proteins rather thermostable, which facilitates its use as a model organism for studies on photosynthetic pigment-protein complexes of red algae. Here, we describe the mechanisms of acido-tolerance in *C. caldarium* based on studies carried out in our group as well as by other researchers. We also describe the characteristics of the two photosystems, PSI and PSII, of *C. caldarium*, and compare them with those of cyanobacteria, other eukaryotic algae and higher plants.

2. Mechanisms of acido-tolerance in *Cyanidium caldarium*

In spite of the extremely acidic environment, the intracellular pH of *Cyanidium* cells is considered to be neutral from the following indirect lines of evidence. (1) Photosynthetic oxygen evolution of cell-free preparations of the alga, supplied with phenyl-*p*-benzoquinone as electron acceptor, is optimal at pH 7 and inactive at pH 3 (Enami and Fukuda, 1975). (2) Most of the soluble proteins of *Cyanidium* are as acid labile as proteins of ordinary, non-acidophilic algae (Enami, 1978). (3) When *Cyanidium* cells were disrupted by a French pressure cell in distilled water, alkalization of the cell suspension from pH 5.9 to 6.5 was observed (Enami et al., unpublished data). These facts suggest that, in spite of the extracellular low pH environment, the intracellular pH of *Cyanidium* is maintained neutral. Thus, *Cyanidium* is expected to have a mechanism to keep the intracellular pH neutral against the steep pH gradient across the plasma membrane.

2.1. ACTIVE H^+ EFFLUX AGAINST THE STEEP pH GRADIENT ACROSS THE PLASMA MEMBRANE

Active H^+ efflux, depending on the supply of oxygen or light, was found to be indispensable for *Cyanidium* to maintain a neutral intracellular pH (Kura-Hotta and Enami, 1981; 1984). When the pH of the cell suspension was measured using a glass electrode, alkalization of the medium (an indication for H^+ influx into the cell) was observed at acidic pH in the dark when respiratory activity was inhibited by adding respiratory poisons (e.g. rotenone or antimycin) or by

introducing pure nitrogen. The extent of H^+ influx increased as the pH of the medium decreased (Kura-Hotta and Enami, 1984). This indicates that H^+ leak passively into the cell according to the steep pH gradient across the plasma membrane when there is no energy supply available. The pH of the medium, previously alkalized under anaerobic conditions, returned to the initial level upon re-aeration of the cell suspension, suggesting that active H^+ efflux becomes functional again. Excess H^+, accumulated in the cell during anaerobic incubation, can now be pumped out.

Such a dependence of active H^+ efflux has not only been found for oxygen supply but also for light (Kura-Hotta and Enami, 1981). Given an acidic environment, illumination of cells caused a significant pH decrease of the medium, indicating H^+ efflux from the cells. Both, rate and extent of H^+ efflux from the cells increased as the pH of the medium was lowered, suggesting an increased activity of proton pumps at lower pH. The H^+ efflux was not affected by addition of DCMU, an inhibitor of photosystem II (PSII) electron transfer. Also, its action spectrum corresponded with the absorption spectrum of chlorophyll *a* but not with that of phycocyanin (Kura-Hotta and Enami, 1981), indicating that the light-induced H^+ efflux is driven by photosystem I (PSI).

The light-induced H^+ efflux was revealed to be an active H^+ transport system depending on intracellular ATP produced by cyclic photophosphorylation via PSI (Enami and Kura-Hotta, 1984). Triton X-100 was found to act as an effective uncoupler in intact *Cyanidium* cells without breaking down the steep pH gradient across the plasma membrane (Enami and Fukuda, 1977). Triton X-100 significantly reduced the intracellular ATP levels, stimulated the oxygen-evolving activity with phenyl-*p*-benzoquinone as electron acceptor, and completely inhibited the light-induced H^+ efflux (Enami and Kura-Hotta, 1984). Inhibition of the light-induced H^+ efflux by Triton X-100 correlated well with the depression of light-induced ATP synthesis. Furthermore, the light-induced H^+ efflux was completely inhibited by diethylstilbestrol, a specific inhibitor of plasma membrane ATPase, without any changes in the intracellular ATP level (Enami and Kura-Hotta, 1984). This indicates that the plasma membrane ATPase is responsible for active H^+ efflux in *C. caldarium*.

On the basis of the results described above, it was concluded that the active H^+ efflux is functioning to maintain the intracellular pH constant against passive H^+ influx according to the steep pH gradient across the plasma membrane. This active H^+ efflux is driven by the plasma membrane ATPase of *C. caldarium*, utilizing intracellular ATP produced by oxidative phosphorylation during respiration and cyclic photo-phosphorylation via PSI (Kura-Hotta and Enami, 1981; Kura-Hotta and Enami, 1984; Enami and Kura-Hotta, 1984).

2.2. MEASUREMENT OF INTRACELLULAR pH IN *CYANIDIUM* CELLS BY ^{31}P-NMR

The conclusion stated above, is based on the observation of the external pH changes of the cell suspension. However, it is important to also determine the intracellular pH of *Cyanidium* cells under various conditions to confirm a passive H^+ influx and active H^+ efflux across the plasma membrane. Phosphorus-31 nuclear magnetic resonance (^{31}P-NMR), developed by Moon and Richards (1973), is a powerful technique to measure the intracellular pH of intact cells, and has been applied to a variety of cells such as higher plants (Roberts et al., 1981), *Rhodopseudomonas sphaeroides* G1C (Akutsu et al., 1986), *Synechococcus* strain Y-7c-s (Kallas and Dahlquist, 1981), and *Chlorella vulgaris* (Mitsumori and Ito, 1984).

Thus, we determined the intracellular pH of *Cyanidium* cells as a function of external pH by ^{31}P-NMR (Enami et al., 1986). Figure 1 shows the ^{31}P-NMR spectra of *Cyanidium* cells incubated at external pH of 3.0 under aerobic (1) and anaerobic (2) conditions at 40ºC for 1h in the dark. Note that the three peaks derived from ATP clearly appeared in cells cultivated under aerobic conditions but almost disappeared under anaerobic conditions. The chemical shift value of inorganic phosphate (Pi) was increased by 0.89 ppm upon anaerobic treatment. The chemical shift of Pi signals caused by pH was used to calculate the intracellular pH of *Cyanidium* as a function of external pH. Cells incubated under aerobic conditions in the dark or anaerobic conditions in the light were found to maintain their intracellular pH within a narrow range (pH 6.8 to 7.0) even when the external pH was changed from pH 1.2 to 8.4. In contrast, the intracellular pH of cells incubated under anaerobic conditions in the dark was acidified as the external pH decreased. Upon either re-aeration or illumination of cells, the intracellular pH returned to the neutral range. In accordance with these results, the intensities of NMR signals for intracellular ATP decreased in cells incubated under dark anaerobic conditions where the intracellular pH was acidified due to a lack of energy supply. On the other hand, intracellular ATP was maintained at a high level in cells incubated under aerobic conditions in the dark as well as in the light or under anaerobic conditions in the light, i.e. under conditions where the intracellular pH was kept constant (Enami et al., 1986). These results indicate that when ATP production by either respiration or photosynthesis is suppressed, passive transport of protons into the cells results in an intracellular acidification. If ATP production is functional, cells are able to pump out protons and maintain their intracellular pH at a constant, physiological range.

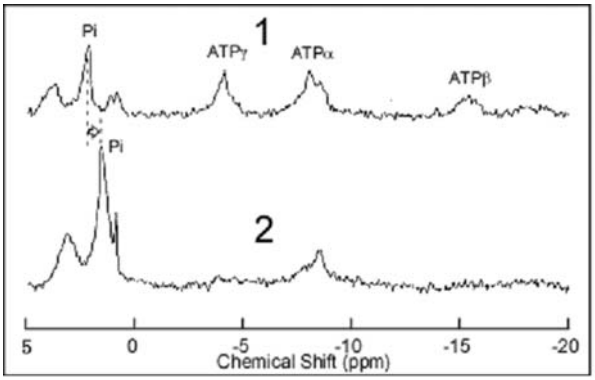

Figure 1. ^{31}P-NMR spectra of *Cyanidium* cells incubated under aerobic (1) or anaerobic (2) condition.

2.3. CHARACTERIZATION OF PLASMA MEMBRANE H^+-ATPase IN *CYANIDIUM* CELLS

As described above, the intracellular pH of *Cyanidium* is considered to be maintained neutral by active H^+ efflux mediated by a plasma membrane H^+-ATPase. We have cloned and sequenced the corresponding gene, encoding the plasma membrane H^+-ATPase from *C. caldarium* (Ohta et el., 1997). The ORF comprises 2,865 bp and encodes a polypeptide of 955 amino acids with a predicted molecular mass of 105,371 Da. The deduced amino acid sequence was more homologous to those of plasma membrane H^+-ATPases from higher plants (86.5% similarity and 54.1% identity) than to that from the salt-tolerant green alga *Dunaliella bioculata* (75.1% similarity and 40.1% identity). The C-terminal region of plasma membrane H^+-ATPase from higher plants has been reported to serve as an autoinhibitory domain, important for regulation of its activity (Wolf et al., 1995). A homologous sequence, corresponding to the autoinhibitory domain, however, was not found in the C-terminal region of the plasma membrane H^+-ATPase from *Cyanidium*. This may be a characteristic feature of the red algal plasma membrane H^+-ATPase as it has the distinctive function to pump out H^+ against the steep pH gradient across the plasma membrane. A homologous plasma membrane H^+-ATPase (98.5% similarity and 90.1% identity) was found in another acido- and thermo-philic primitive red alga, *Cyanidioschyzon merolae* 10D, which also did not contain the regulatory/auto-inhibitory domain (Matsuzaki et al., 2004). Like *Cyanidium*, *Cyanidioschyzon* also inhabits acidic hot springs.

In preliminary experiments, we found ATP hydrolysis in cell-free preparations of *Cyanidium*. This enzyme activity exhibited typical characteristics of a plasma membrane H^+-ATPase; namely, a pH optimum around pH 6 and specific inhibition by vanadate, a specific inhibitor of plasma membrane H^+-ATPase (Enami et al., unpublished data). Furthermore, the Michaelis constant (Km) for ATP hydrolysis was found to be significantly varied by pH: The $Km_{(ATP)}$ value

was about 1 mM at pH 6 and about 12 mM at pH 7, indicating that the affinity of the enzyme for ATP differed over 10-fold between pH 6 and pH 7. We estimated that the intracellular ATP concentration in *Cyanidium* is about 1 mM (Enami et al., unpublished data) and it is therefore likely that the plasma membrane H^+-ATPase is responsible for active H^+ efflux at pH 6, but does not function at pH 7. Thus, the intracellular pH is maintained constant around pH 7 via regulation of the substrate affinity of plasma membrane H^+-ATPase (Enami et al., unpublished data).

2.4. MECHANISMS OF ACIDO-TOLERANCE IN *CYANIDIUM* CELLS

In order to interpret the results described above, a model for the mechanisms of acido-tolerance in *Cyanidium* cells is illustrated in Figure 2.

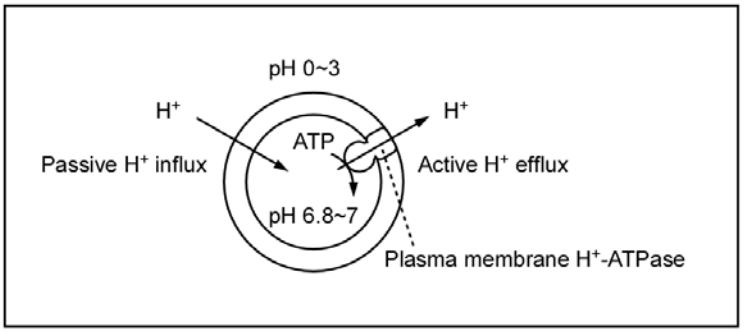

Figure 2. Schematic representation for the mechanism of acido-tolerance in *Cyanidium* cells.

In *Cyanidium* cells grown in extremely acidic environments, H^+ passively leak into the cells due to the steep pH gradient. The H^+ leaked into the cells are pumped out against the steep pH gradient by a plasma membrane ATPase to maintain the intracellular pH in a physiological range. The plasma membrane ATPase of *Cyanidium* and other acido-philic organisms are thus unique in that they have a strong activity in the low pH region. Introduction of the gene encoding the plasma membrane ATPase from *Cyanidium* or *Cyanidioschyzon* into ordinary organisms may yield novel acido-tolerant organisms that are resistant to acid rain or can survive under acidic environments. This may provide a useful approach to overcoming the environmental acidification caused by increasing pollutions.

3. Characteristics of photosystems in *Cyanidium caldarium*

The photosynthetic apparatus of *C. caldarium* is essentially the same as that of other red algae (*Rhodophyta*), which appears to represent a transitional state between cyanobacteria and photosynthetic eukaryotes. The chloroplast of red algae, one of the most primitive groups of eukaryotic algae, is considered to have arisen from a single endosymbiotic event of a primitive cyanobacterial cell with a eukaryotic host. The ultrastructure of red algal chloroplasts is similar to that of cyanobacteria, where thylakoid membranes are not differentiated into stacked and unstacked membrane regions as found in chloroplasts of higher plants and green algae. Both, cyanobacteria and red algae do not synthesize chlorophyll (Chl) *b* and contain phycobilisomes as the light-harvesting antenna for the PSII complex instead of Chl *a*/*b* (or Chl *a*/*c*)-binding proteins found in higher plants and other eukaryotic algae. On the other hand, PSI of red algae has an intrinsic light-harvesting complex (LHCI) (Wolfe et al., 1994) similar to that found in all other major groups of photosynthetic eukaryotes. Thus, the red algal PSII is closely related to prokaryotic cyanobacteria, whereas its PSI is more similar to that of eukaryotic photosynthetic organisms. In the following, we focus on the characteristics of the two photosystems, PSI and PSII, of *C. caldarium*, and compare them with those of cyanobacteria and other eukaryotic algae and higher plants.

3.1. PHOTOSYSTEM II

PSII catalyzes the light-induced electron transfer from water to plastoquinone, leading to the evolution of molecular oxygen which is essential for oxygenic life on earth. The core part of protein subunits - which exists predominantly in a dimeric form - and the function of PSII are essentially the same among cyanobacteria, red algae and higher plants. The red algal PSII resembles cyanobacterial PSII in that both contain phycobilisomes as the light-harvesting antenna and a similar set of extrinsic proteins required for oxygen evolution. The crystal structure of PSII (Figure 3) has been solved for two species of thermophilic cyanobacteria (Kamiya and Shen 2003; Ferreira et al., 2004; Loll et al., 2005). Based on the crystal structure, two subunits, D1 and D2, which constitute the reaction center of PSII, are located in the center of PSII and have five trans-membrane helices each. These two subunits are surrounded by two chlorophyll *a*-binding proteins CP47 and CP43, which have six trans-membrane helices each. In addition, a large number of small, membrane-spanning subunits are located in conjunction with these four large subunits. In cyanobacteria, the number of these small subunits amounts to 13, namely, PsbE, F, H, I, J, K, L, M, Tc, X, Y, Z, and Ycf12 (Loll et al., 2005; Kashino et al., 2007; Shen et al., 2008). Genes corresponding to all of these subunits have been found in the genome of the red alga *Cyanidioschyzon merolae* (Matsuzaki et al., 2004), a close relative of *C.*

caldarium. At the lumenal side, there are three hydrophilic, peripheral subunits that function to maintain the oxygen-evolving activity. In the case of cyanobacteria, these three extrinsic proteins are the 33 kDa protein (PsbO), cytochrome c_{550} (PsbV), and 12 kDa protein (PsbU) (Shen and Inoue, 1993). These proteins were also found in *C. caldarium* PSII (Enami et al., 1998) (see below).

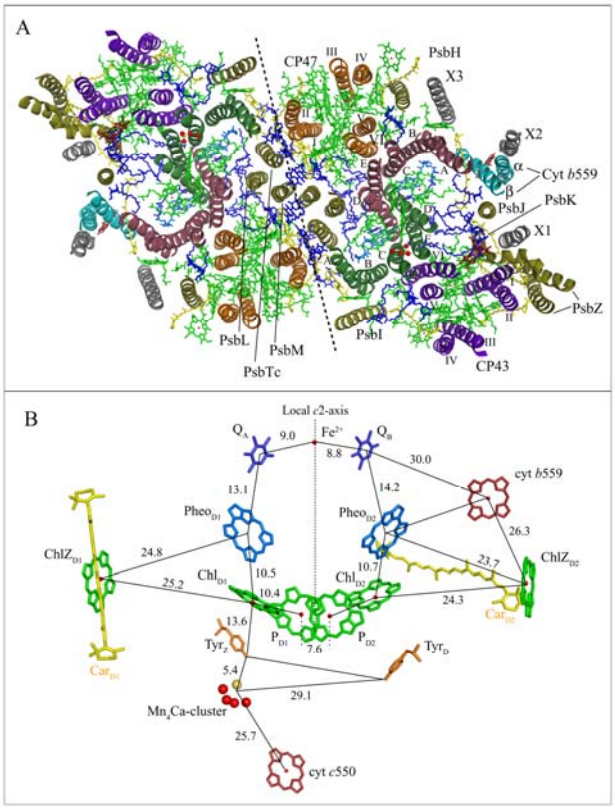

Figure 3. **A**. Crystal structure of PSII dimer at 3.0 Å resolution (Loll et al., 2005, PDB code: 2axt). Top view from the stromal side. Color code: deep green, D1; dark purple, D2, orange, CP47; purple, CP43.; light green, chlorophylls; blue, lipids; dark yellow, carotenoids. Other subunits are indicated in the figure. For clarity, the extrinsic proteins as well as the hydrophilic loops of intrinsic subunits at the lumenal side were omitted. **B**. The arrangement of electron transfer chain of PSII. Side view along the membrane plane. The numbers represent distances in Å.

The PSII core complex contains 36 chlorophylls (Kamiya and Shen 2003; Ferreira et al., 2004; Loll et al., 2005) (Figure 3A), among which 6 are associated with D1 and D2 subunits, and the remaining chlorophylls are associated with CP47 and CP43 which are thus designated as intrinsic antenna subunits of PSII. The initial charge separation takes place in the reaction

center chlorophyll(s) of PSII (designated as P680) upon capture of light, which subsequently transfers an electron to a pheophytin molecule bound to D1 (Figure 3B). The electron is then transferred to the first bound plastoquinone acceptor Q_A on D2, and then to the second quinone acceptor Q_B on D1. Q_B acts as a two-electron gate which remains bound to D1 after one-electron reduction, but is released from its binding-site upon reduction by two electrons, which is then replaced by a free plastoquinone present in the plastoquinone pool of the thylakoid membrane. On the other hand, the oxidized reaction center chlorophyll abstracts an electron from a nearby tyrosine residue, namely, D1-Tyr 161 (designated as Tyr_Z), which in turn abstracts an electron from a Mn_4Ca-cluster containing four manganese atoms and one calcium atom at the donor side. Upon subsequent abstraction of four electrons, the Mn_4Ca-cluster is able to oxidize two molecules of water, leading to the release of one molecule of oxygen. This reaction thus proceeds stepwise, the intermediates of which have been designated as Si-states with i = 0-4 (Kok et al., 1970; Joliot, 2003). The initial, dark-stable state is S_1, and S_4 is a transient state immediately prior to the release of oxygen. In the crystal structure, a similar arrangement of electron transfer cofactors is found in both D1 and D2 subunits, but the active electron transfer pathway is located on the D1 side due to the presence of the Mn_4Ca-cluster on the D1 side only.

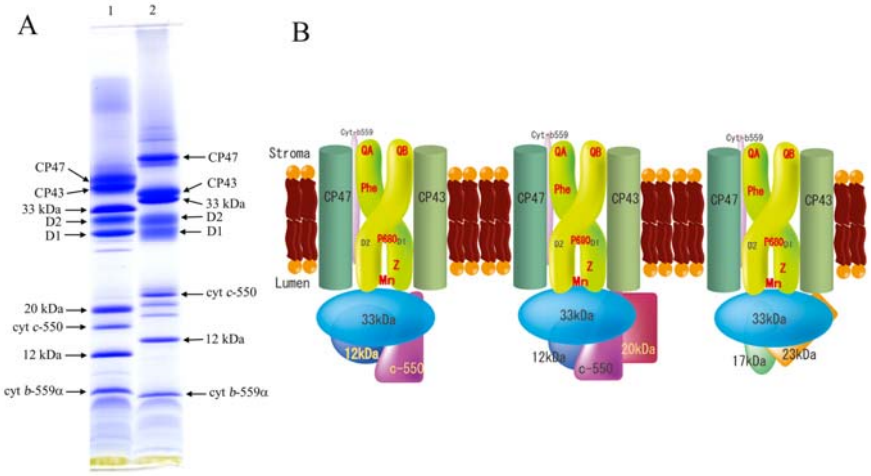

Figure 4. A. Protein composition of purified PSII from *Cyanidium caldarium* (lane 1) and the thermophilic cyanobacterium *Thermosynechococcus vulcanus* (lane 2). **B**. Schematic representation of PSII from cyanobacteria, red algae and higher plants, showing the differences of extrinsic proteins associated at the lumenal side and involved in oxygen evolution.

None of the PSII structures from eukaryotic organisms has been solved. Nevertheless, PSII of *C. caldarium* has been isolated to a high purity (Enami et al., 1995), and characterized in detail (Enami et al., 1998; 2000; 2003). In Figure 4A, the protein composition of *C. caldarium* PSII is shown in comparison with that of the thermophilic cyanobacterium

Thermosynechococcus vulcanus. As mentioned above, most of the protein subunits of PSII in cyanobacteria and red algae (and also higher plants) are similar, although the electrophoretic mobility of some subunits slightly differs for the cyanobacterial PSII and *C. caldarium* PSII. However, there is an important difference in the composition of extrinsic proteins: the *C. caldarium* PSII contains an extrinsic protein of 20 kDa (PsbQ') in addition to the three subunits homologous to cyanobacterial PsbO, PsbV, and PsbU (Enami et al., 1995; 1998; 2000; 2003; Ohta et al., 2003) (Figure 4A and B). This 20 kDa protein has been shown to be required for effective binding of the other two proteins, PsbV and PsbU, in the red algal PSII (Enami et al., 1998). In contrast, PsbV (cytochrome c_{550}) of cyanobacterial PSII is able to bind and function independent of other extrinsic proteins (Shen and Inoue 1993; Shen et al., 1995). Interestingly, PsbQ' from *C. caldarium* has a low homology with PsbQ (17 kDa protein) from green algae (Ohta et al., 2003). Like higher plants, green algae contain PsbP (23 kDa protein) and PsbQ as extrinsic proteins instead of PsbV and PsbU found in cyanobacteria. Thus, the extrinsic proteins of the red algal PSII appear to represent an intermediate state between cyanobacteria and higher plants (Figure 4B). In relation to this, it should be pointed out that PsbP-like and PsbQ-like proteins have been found in cyanobacterial thylakoids and PSII complexes (Kashino et al., 2002). The specific binding of PsbQ-like protein to cyanobacterial PSII was suggested after co-purification of PSII with a His-tagged PsbQ-like protein (Roose et al., 2007). However, cyanobacterial PSII used for crystallization, did not contain PsbQ- and PsbP-like proteins. The association of these two proteins with cyanobacterial PSII is thus not as tight as that in red algae or higher plants. Moreover, both proteins have been suggested to be lipo-proteins and not required for the binding of PsbV and PsbU in the cyanobacterial PSII. Thus, the binding characteristics and function of these extrinsic proteins in the cyanobacterial PSII are apparently different from that of PsbQ' in the red algal PSII.

3.2. PHOTOSYSTEM I

PSI mediates light-induced electron transfer from plastocyanin or cytochrome c_{553} to ferredoxin, thereby generating electrons required for the reduction of $NADP^+$ which in turn is utilized for the reduction of CO_2 to organic carbon. The core part of PSI contains 11-14 subunits denoted from PsaA to PsaO. They exhibit a high overall similarity in cyanobacteria, algae and higher plants, although PsaG and PsaH are found only in eukaryotic PSI but not in cyanobacterial PSI (Xu et al., 2001; Scheller et al., 2001). PSI of eukaryotic algae and higher plants contains a cluster of light-harvesting proteins (LHCI, encoded by Lhca genes) that are not present in cyanobacterial PSI. Red algal PSI also contains an intrinsic chlorophyll *a*-binding antenna (Wolfe et al., 1994) encoded by Lhcr genes homologous to the Lhca genes found in green algae and higher plants (Jansson et al., 1999). Moreover, like green algae and higher plants, *C. caldarium* PSI exists in

a monomeric form (Gardian et al., 2007), whereas the cyanobacterial PSI exists predominately in a trimeric form (Jordan et al., 2001). Thus, red algal PSI is more similar to that of green algae and higher plants than to that of cyanobacteria. In Figure 5A, the protein composition of PSI from *C. caldarium* is compared with that of the cyanobacterial PSI from *T. vulcanus*.

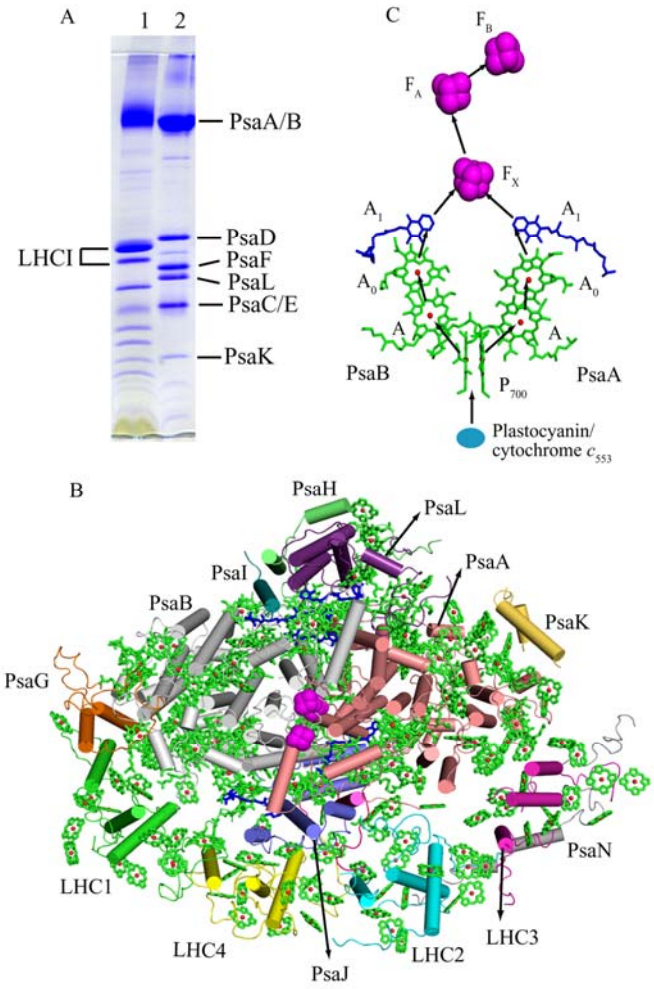

Figure 5. A. Protein composition of PSI purified from *Cyanidium caldarium* (lane 1), in comparison with cyanobacterial PSI from *Thermosynechococcus vulcanus* (lane 2). **B**. The structure of PSI monomer from pea at 3.4 Å resolution (Amunts et al., 2007, PDB code: 2oO1). Top view from the stromal side of the thylakoid membrane. For clarity, the PsaC, D, E subunits at the stromal side were omitted, and PsaF is scarcely visible due to its position at the lumenal side. Molecules in green represent chlorophylls, those in blue represent carotenoids and phylloquinones, and those in magenta represent iron-sulfur centers. **C**. The electron transfer chain of PSI based on the crystal structure shown in **B**.

The crystal structure of PSI from both cyanobacteria and higher plants has been reported (Jordan et al., 2001; Amunts et al., 2007). Figure 5B shows the crystal structure of PSI from pea at a 3.4 Å resolution (Amunts et al., 2007). The core part of pea PSI contains 13 subunits, namely, PsaA, B, C, D, E, F, G, H, I, J, K, L, and N. In addition, 4 LHCI subunits designated LHC1-4, are associated in the periphery of the complex. This gives rise to an asymmetric conformation of the complex, as all 4 LHCI subunits are located in one side of the complex. The opposite side is occupied by PsaL and PsaH, of which, PsaH has been suggested to serve as a docking site for mobile LHCII possibly migrated from PSII upon state-transitions (Takahashi et al., 2006; Amunts et al., 2007). This subunit is not present in cyanobacterial as well as red algal PSI, since the antennae of PSII in both organisms are membrane-peripheral phycobilisomes instead of LHCII. Another subunit, PsaG, has been suggested to be important for the association of LHCI with PSI core, and is also not present in the cyanobacterial PSI. The gene of this subunit was also not found in the genome of *C. merolae* (Matsuzaki et al., 2004), suggesting that the association of LHCI with PSI core in red algae may be somewhat different from that in higher plants. In relation to this it might be interesting to note that PSI from higher plants contains 4 Lhca proteins, whereas 5 Lhcr genes have been identified in the thermoacidophilic red alga (*Cyanidiaceae*) *Galdieria sulphuraria* (Marquardt et al., 2001), and the genome of *C. merolae* contains only 3 homologous Lhcr genes (Matsuzaki et al., 2004). The exact number of LHCI subunits of *C. caldarium* still needs to be clarified in the future.

A significant difference between PSI and PSII is the number of chlorophylls associated: PSI core binds approximately 100 chlorophylls, most of which are associated with the PSI reaction center subunits PsaA, PasB, and each LHC1 subunit binds 14 chlorophylls (Amunts et al., 2007). In total, PSI-LHCI complex of higher plants has around 160 chlorophylls; this number is likely to be similar in red algal PSI. This is in sharp contrast to the 36 chlorophylls found in the PSII core from cyanobacteria to red algae and higher plants (Kamiya and Shen 2003; Ferreira et al., 2004; Loll et al., 2005). This low number of chlorophylls in the PSII core is compensated by the large number of chlorophylls associated with LHCII in green algae and higher plants (typically 200-250 chlorophylls), or phycobili-proteins in cyanobacteria and red algae.

The central electron transfer chain of PSI is very similar from cyanobacteria to red algae and higher plants. The reaction center of PSI, designated P700, is composed of a chlorophyll dimer bound to PsaA and PsaB separated by a center-to-center distance of 3.6 Å (Jordan et al., 2001; Amunts et al., 2007) (Figure 5C). This distance is significantly shorter than the distance found in the purple bacterial reaction center (7.4-7.6 Å) (Deisenhofer et al., 1995) or PSII (7.6 Å) (Loll et al., 2005), suggesting a stronger interaction between the two chlorophylls in the PSI reaction center. P700 accepts an electron from plastocyanin or cytochrome c_{553} at the lumenal side in green algae and higher plants, whereas only cytochrome c_{553} is present and denotes electrons to

P700 in cyanobacteria and red alga. The electron from P700 is transferred to a chlorophyll monomer A_0, and subsequently to a phylloquinone acceptor A_1, both of which are bound to PsaA/B. The electron from A_1 is transferred to Fx, an interpolypeptide 4Fe-4S cluster located at the stromal surface of the membrane, and, subsequently to F_A, F_B, two 4Fe-4S clusters bound to the extrinsic subunit PsaC at the stromal side. The components of PSI electron transfer chain are arranged in two quasisymmetrical branches (Figure 5C) formed by the polypeptides PsaA and B, which are homologous to each other. This suggested that both branches may be active in light-induced electron transfer (Nelson and Yocum, 2006). In the case of cyanobacteria, however, site-directed mutagenesis combined with spectroscopic studies has indicated that most of the electron transfer takes place on the PsaA-branch. In contrast, studies on algal and higher plant PSI have yielded evidence for a significant activity on the PsaB-branch. Whether both branches are active or only one branch is active in the red algal PSI needs to be clarified in future studies.

4. References

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