Structure of the far-red light utilizing photosystem I of *Acaryochloris*

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Abstract

Acaryochloris marina is a cyanobacterium that can, uniquely, use far-red light for oxygenic photosynthesis. Here, we report the structure of the photosystem I reaction center of *A. marina* determined by cryo-electron microscopy at 2.5 Å resolution. The structure reveals a unique arrangement of electron carriers and light harvesting pigments. The primary electron donor P740 is a dimer of chlorophyll *d/d'* and the primary electron acceptor pheophytin *a*, a metal-less chlorin different from the chlorophyll *a* common to all other oxygenic type I reaction centers. The architecture of the 11 subunits and identity of key components help explain how the low energy yield from far-red light is efficiently utilized for driving oxygenic photosynthesis.

Main Text

Photosynthesis is performed by two types of photoreaction systems, namely type I and type II reaction centers. Photosystems I¹ and II² (PSI and PSII, belonging to type I and type II, respectively) work sequentially in oxygenic photosynthesis in plants and cyanobacteria³. They typically use chlorophyll (Chl) *a* (Extended Data Fig. 1) as the major pigment that absorbs visible light (670–700 nm) (Extended Data Fig. 2). Anoxygenic photosynthetic bacteria use bacteriochlorophylls (BChl) that absorb 800–900 nm light with either a type I or type II reaction center. *Acaryochloris marina* (*A. marina*) is a cyanobacterial species that uses the novel pigment, Chl *d* (Extended Data Fig. 1), which absorbs 700–750-nm light *in vivo*⁴ (Extended Data Fig. 2). *A. marina* was isolated from colonial ascidians, which harbor mainly Chl *a*-type cyanobacteria, resulting in an environment with low visible light and high far-red light. *A. marina* has exploited this with the niche-filling introduction of Chl *d*^{5,6}. The discovery of *A. marina* prompted intensive research into the mechanism of this low-energy- driven system. Spectroscopic and pigment analyses revealed that Chl *d* indeed plays a central role in the photoreaction, although the organism also contains Chl *a* at around 5% relative abundance^{4,5,7}. With the acquisition of Chl *d*, *A. marina*, uniquely harnesses far-red light, which is the lowest energy light that can be used in all oxygenic photosynthetic organisms. The cyanobacterium can be seen as a missing-link between organisms possessing visible light/Chl *a*-based oxygenic photosynthesis and 800–900 nm light/BChl-based anoxygenic photosynthesis. Chl *d* has a peak wavelength of absorption at around 697 nm in methanol (Extended Data Fig. 3) (so-called Qy band; 700–750 nm *in vivo*, Extended Data Fig. 2), which is longer than the 665.2 nm of Chl *a* (670–700 nm *in vivo*, Extended Data Fig. 2 and 3) by ~30 nm, which means that the photon energy absorbed is ~80 mV (10%) lower. How PSI and PSII of *A. marina* drive the similar photochemical reactions that occur in the Chl *a*- dependent systems of other oxygenic photosynthetic organisms has been a long-standing puzzle.

PSI generates reducing power for NADPH production by accepting electrons originating from PSII³. PSI of *A. marina* has a similar subunit composition to that of the PSIs of other oxygenic photosynthetic organisms^{8,9} (Extended Data Table 1). However, the peak wavelength of the light-induced redox difference absorption spectrum of *A. marina* PSI showing that of reaction center is longer (740 nm) than

that of Chl *a* (700 nm) in the PSI of plants and typical cyanobacteria¹⁰ (Fig. 1A). Reaction center P740 thus named has been assumed to be a heterodimer of Chl *d* and *d'*^{11,12} by analogy to P700, which is the reaction center composed of a heterodimer of Chl *a*/Chl *a'* in all other PSIs (Fig. 1A)^{1,13}. Cofactors of the PSI electron transfer chain in *A. marina* (Fig. 1B) and the reduction potential of P740 are also similar to those of P700 in other organisms^{8,9,14}.

To date, several structures of type I reaction centers have been determined from higher plants^{15,16}, green algae^{17,18}, red algae¹⁹, diatoms²⁰, cyanobacteria^{1,21,22} and an anoxygenic bacterium²³. All structures have Chl *a* in the reaction center with the exception of the one from the anoxygenic bacterium which has BChl. The PSI of *A. marina* with Chl-*d* in its reaction center and its ability to utilize such low energy light is thus unique. Here, we present the first structure of the PSI trimer isolated from *A. marina* revealed by cryo-electron microscopy (cryo-EM) at 2.48 Å resolution to understand the mechanism for utilization of far-red light (Fig. 1B).

Overall structure, protein subunits, and cofactors of *A. marina* PSI

The PSI trimer complex was isolated from *A. marina*. Detailed methods of sample preparation, data processing, and structural refinement by cryo-EM, and biochemical data, are presented in Supplementary Materials (Extended Data Figs. 4–11, Extended Data Tables 1–4). The model of the PSI trimer was refined to give a correlation coefficient and Q-score, which are indices for validating the correctness of a model, of 0.80 and 0.678, respectively (Extended Data Table 4). These index values can be considered reasonable at 2.48 Å resolution (Extended Data Fig. 10).

The PSI trimer had dimensions of 100 Å depth, 200 Å length, and 200 Å width, surrounded by detergent micelles (Fig. 2). The overall structure resembles those of PSI trimers reported for other cyanobacteria^{1,22,24}. The root-mean-square deviations using secondary structure matching between the monomer model (chains A–M) and Protein Data Bank (PDB) structures 1JB0 of *Thermosynechococcus elongatus*¹, 5OY0 of *Synechocystis* sp. PCC 6803²², and 6KMX of *Halomicronema hongdechloris*²⁴ are 0.90 Å, 0.90 Å, and 0.87 Å, respectively.

The *A. marina* PSI monomer contains 11 subunits [PsaA, PsaB, PsaC, PsaD, PsaE, PsaF, PsaJ, PsaK, PsaL, PsaM, and Psa27], all of which could be assigned to the cryo-EM density map (Fig. 2, Extended Data Figs. 5 and 12, and Extended Data Table 1). PsaX (a peripheral subunit in other cyanobacteria such as *T. elongatus*), PsaG and PsaH (subunits in higher plants) were missing in the density map, consistent with the absence of their genes from the *A. marina* genome⁶. The unique name Psa27 was given to a novel subunit protein in *A. marina* that has low sequence similarity (29.4%) with PsaI of *T. elongatus* (Extended Data Table 5)⁸. Here, we found that Psa27 is in the same location as PsaI in *T. elongatus* and has a similar fold. Psa27 also contributes to the structural stabilization of the PSI trimer just as PsaI does in *T. elongatus*. Thus, we concluded that Psa27 and PsaI are in effect the same subunit.

The loop regions of PsaB (residues 290–320 and 465–510) were not identified due to disorder because of the absence of PsaX, suggesting that PsaX stabilizes these regions in other cyanobacteria. Most of subunits PsaE, PsaF, and PsaK were modeled using polyalanine because of poor regional map quality (Extended Data Figs. 12, 13, and Extended Data Table 1), possibly suggesting that some part of those subunits dissociated during sample preparation. The cofactors assigned (Extended Data Fig. 14) are 71 Chl *d* (Extended Data Table 6), one Chl *d'* (an epimer of Chl *d*), 11 α -carotenes (α -Car) but no β -Car (Extended Data Table 7)^{6,10}, two pheophytins (Pheo) (a derivative of Chl; however, unlike Chl, no Mg²⁺ ion is coordinated by the tetrapyrrole ring), two phylloquinones (PhyQs), three iron–sulfur clusters, two phosphatidylglycerols, one monogalactosyl diacylglycerol, and 84 water molecules. The Chls and α -Car pigments are mainly involved in the electron transfer reactions and light-harvesting.

Electron transfer components

The important cofactors involved in electron transfer (Fig. 1B) are four Chls (three Chl *d* and one Chl *d'*), two Pheos, two PhyQs (A_1), and three iron–sulfur clusters (F_X , F_A , and F_B) (Fig. 3A). The Chls and PhyQs are arranged in two branches, the A-branch and the B-branch, which are related by a pseudo- C_2 axis as in other type I reaction centers, and are stabilized by amino acid residues of subunits PsaA and PsaB. The reaction center, P740, is composed of Chl *d'* (P_A) and Chl *d* (P_B) (Fig. 4A), which are coordinated by residues His678/A and His657/B, respectively (capitalized letters following the slash (/) indicate the subunit names such as PsaA or PsaB) (Fig. 3B and C). The distance between the ring planes (the π – π interaction distance) of Chl *d'* and Chl *d* is 3.5 Å (Fig. 3D) (3.6 Å in *T. elongatus*). Two Tyr residues, Tyr601/A and Tyr733/A, are positioned within hydrogen bonding distance of P_A . These configurations are similar to those in the PSI from *T. elongatus* (Fig.1), although cofactor compositions are different.

In *A. marina* PSI, we uniquely identified three water molecules (W1–W3) around P_A , forming hydrogen bonds with surrounding amino acid residues (Tyr601/A, Ser605/A, Asn608/A, Ser741/A, and Try745/A) and one Chl *d* (Chl *d*32) (Fig. 3B and C). For the *T. elongatus* P_A , only one water molecule forms hydrogen bonds with surrounding amino acid residues (Tyr603/A, Ser607/A, Ile610/A, Thr743/A, and Phe747/A) and Chl *a'* (P_A). There are no water molecules around P_B in *A. marina*, but some water molecules surround P_B in *T. elongatus* without forming hydrogen bonds. This difference around P_B may come from the difference in two amino acid residues: Val594/B in *A. marina* vs. Thr597/B in *T. elongatus*, and Asn598/B in *A. marina* vs. His601/B in *T. elongatus*. The hydrogen bonding pattern around P_A probably contributes to the charge distribution ratio ($P_A^{\cdot+}/P_B^{\cdot+}$)^{1,25}, and therefore, is likely different in the two organisms. The midpoint potential values, $E_m(P_A)$ and $E_m(P_B)$, are influenced by the protein environment, in particular, by the presence of charged residues, and the E_m difference, $E_m(P_A) - E_m(P_B)$ ($= \Delta E_m$), is an important factor in determining the $P_A^{\cdot+}/P_B^{\cdot+}$ ratio²⁶.

Previous studies have suggested that the primary electron acceptor A_0 in *A. marina* PSI (Fig. 1A) is Chl *a*^{9,27}, in line with other species^{1,28}. However, we found that there was no mass corresponding to Mg²⁺ at

the center of the tetrapyrrole rings of A_{0A} and A_{0B} , but rather a hole in the cryo-EM density map (Fig. 4A). This indicates that A_0 is actually Pheo, a derivative of Chl, which is consistent with pigment analysis identifying the presence of Pheo *a* (Extended Data Table 7 and Extended Data Fig. 15). Furthermore, the position of Leu665/B (Extended Data Fig. 16B) ²⁹, pointing to A_{0B} and in the same place as conserved Met688/B that ligates A_{0B} in *T. elongatus* PSI ^{1,29}, supports Pheo *a* as A_{0B} in *A. marina*, because Leu cannot serve as a ligand to Mg^{2+} in Chl. Thus, surprisingly, A_{0A} and A_{0B} are assigned as Pheo *a* in *A. marina* PSI (Fig. 1B; Fig. 3A; Fig. 4A–C).

The amino acid residue nearest to A_{0A} in *A. marina* is Met (Met686/A) (Extended Data Fig. 16A) ²⁹ and the identity of close amino acid residues may modify the reduction potentials of the two A_0 and their absorption peak wavelength(s). Two mechanisms have been proposed for electron transfer in PSI reaction centers, one using both A- and B-branches

30,31, and the other using the A-branch preferentially ^{32,33}. Only the A-branch may be active in *A. marina* PSI ²⁹. Details of the electron transfer mechanism in relation to the $P_A^{\cdot+}/P_B^{\cdot+}$ ratio can now be filled in using the precise structure of *A. marina* PSI.

Why is Pheo *a* the A_0 in *A. marina* PSI? Pheo *a* has not been identified in any PSI before, although it is the primary electron acceptor in all type II reaction centers ³. While the reaction center P740, which is composed of Chl *d/d'*, uses low-energy far-red light of 740 nm (1.68 eV), it generates reducing power almost equivalent to that of the P700 (Chl *a/a'*, 1.77 eV) in plants and most cyanobacteria ⁸. Then, the excited state of P740 (P740*) produces 0.09 eV-weaker reducing power than P700* (i.e., 77 – 1.68 eV). This could result in a slower electron transfer rate to A_0 and an increase in reverse reaction, due to the smaller driving force. However, the rates of electron transfer from P740* to A_0 , and to PhyQ are actually comparable to those from P700* to A_0 and to PhyQ ²⁷, therefore, Pheo *a* as A_0 must achieve the same electron transfer efficiency as the Chl *a*-type PSI.

The midpoint potential value (E_m) vs. the normal hydrogen electrode (NHE) of P740 is 439 mV ^{8,9,14}, whereas that of P700 is 470 mV ³⁴. However, most of the amino acid residues around A_0 in *A. marina* PSI are similar to those in *T. elongatus*, so it is unlikely that the protein structure around A_0 influences the E_m value. We obtained E_m (vs. NHE) values of purified Chl *a*, Chl *d*, and Pheo *a* in acetonitrile of –1100, –910, and –750 mV, respectively (Extended Data Fig. 17) ^{34,35}. Therefore, according to the E_m value, it seems reasonable for the Chl *d*-driven PSI to use Pheo *a* as A_0 , as the energy gap is sufficient for the primary electron transfer step. However, a possible effect on the rate of the following A_0 to PhyQ step should be investigated as the rate is also known to be comparable to that in Chl *a*-type PSI.

A. marina contains a limited but distinct amount of Chl *a* ^{8,10,29}, and we found a small amount in the PSI (1–2 Chl *a* per PSI monomer) (Extended Data Table 7). One possible locality is as the accessory Chls, Acc_A and Acc_B (Figs. 1 and 4). However, unfortunately, Acc could not be conclusively identified as Chl *d* or

Chl *a*, because the functional group containing C3¹ of Acc Chl could not be precisely defined from the cryo-EM density map. While quantum mechanical/molecular mechanical calculations show that the formyl group of Chl *d* adopts two orientations (Extended Data Fig. 1C, D)³⁶, the oxygen atom of the formyl group on the Chl *d* molecule in a vacuum is more stable when oriented towards the C5 H atom, suggesting the conformer in Extended Data Fig. 1C. In contrast, the vinyl group of Chl *a* can adopt either orientation. The assignment of Chl *d* to the Accs in this study is actually due to the difference spectrum of P740, which suggests that the large electrochromic shift of Acc at 710 nm arises from Chl *d*⁷.

The Mg²⁺ in Acc_A and Acc_B are coordinated by water molecules forming hydrogen bonds with Asn588/B and Asn602/A, respectively (Fig. 4B and C). In *T. elongatus* PSI, the methyl ester groups of the Chl *a* in Acc_A and Acc_B affect the charge and spin distributions on P700^{26,37–39}. The distance between the carbonyl O atom in Acc_A and the Mg²⁺ in P_A, an epimer of Chl *a*, is 5.4 Å, whereas in Acc_B it is 7.1 Å. In contrast, in *A. marina*, these distances were 6.2 Å (Acc_A–P_A) and 6.6 Å (Acc_B–P_B), respectively (Fig. 4D), suggesting a different charge distribution with that in *T. elongatus*.

The amino acid residues surrounding PhyQ (A_{1A} and A_{1B}) are switched from those in *T. elongatus* PSI - Met720/A and Leu665/B in *A. marina* vs. Leu722/A and Met668/B in *T. elongatus* (Extended Data Fig. 18A, B). The arrangement of the phytol chain of A_{1B} is also different from that in other cyanobacteria (Extended Data Fig. 18B). These structural differences may suggest that the protein environment within *A. marina* PSI is modified to optimize electron transfer involving Chl *d*. However, cofactors F_X, F_A, and F_B and their surrounding structures in *A. marina* PSI are nearly identical to those in other cyanobacteria (Fig. 3A and Extended Data Fig. 18), indicating that the reduction potentials are likely the same. We suggest that F_X, F_A, F_B, and their surrounding structures must be invariant for the reduction of NADP⁺.

Pigment environments: antennas and red chlorophylls

In the PSI, a large number of Chl *d* and α-Car harvest low energy far-red light and finally transfer the energy to P740. The arrangements of Chl *d* and α-Car in *A. marina* are similar to those of Chl *a* and β-Car, respectively, in *T. elongatus*, as shown in Extended Data Fig. 19, in which, the Chl *d*s are numbered according to the nomenclature of Chl *a* in *T. elongatus*⁴⁰. However, there are differences in some amino acid residues surrounding the cofactors, slight gaps in the arrangement of the cofactors, and lack of some Chls, which, we assume, reflects optimization for efficient use of far-red light.

According to the theoretical study of Adolphs *et al.*⁴⁰, the changes in site-energy of the three Chl *a* molecules whose band positions are most significantly shifted in *T. elongatus* PSI are produced by the following factors: (1) interaction with the backbone of α-helices, in particular with that of residues

Gly316/B–Met320/B, causing Chl *a* 65 to be the third-lowest site-energy site of all the pigments; (2) the amide dipole in the side-chain of Gln115/A causing a decrease of the site-energy of Chl *a* 12; and (3) the positive charge of Arg24/M that induces a significant increase in the site-energy of Chl *a* 94.

The first factor is related to what are known as “red Chls”^{1,41}. Red Chls are a dimer or trimer of Chls, whereby the peak wavelengths of their Q_y bands are shifted to wavelengths longer than the 700-nm peak of P700. In *T. elongatus* PSI, 9–11 Chl *a* per PSI monomer are considered to be red Chls. In particular, the trimer of Chl *a* 77–79 positioned in the region of Gly316/B–Met320/B is proposed to be a red Chl (Extended Data Fig. 19A)¹. In *A. marina*, this latter region seems highly flexible, and Chl *d* 55, 56, 63–67, and 77–79 could not be assigned as counterparts of Chl *a* molecules. The high flexibility of this region likely comes from the absence of PsaX. The seeming absence of Chl *d* 77–79 may be the reason why the *A. marina* PSI intrinsically does not exhibit features of a long-wavelength Chl *d* (Extended Data Figs. 2, 6, and 7)^{7,42}.

Regarding the second factor, in *A. marina* PSI, the Mg²⁺ ions of two Chls, namely Chl *d* 13 and Chl *d* 32, are within 10 Å of those of Chl *d* 12 (Extended Data Fig. 19B). The amino acid residue that is an axial ligand of Chl *d* 12, corresponding to Gln115/A of *T. elongatus*, is His (His116/A) in *A. marina*. This difference may affect the site-energy of Chl *d* 12 and surrounding Chls (Chl *d* 13 and 32).

Regarding the third factor above, Arg24/M in *T. elongatus* PSI corresponds to Asn (Asn24/M) in *A. marina*, which has a polar uncharged side-chain and no Chl *d* that corresponds to Chl *a* 94 of *T. elongatus* PSI could be identified in the vicinity of Asn24/M (Extended Data Fig. 19C). The *A. marina* PSI appropriately lacks the Chl with most increased site-energy that captures high-energy, short-wavelength light in *T. elongatus*.

Differences in some of the amino acid residues in the *A. marina* PSI compared with those of *T. elongatus* (Extended Data Table 5), result in different arrangements of some cofactors. For example, Phe49/J in *A. marina*, the counterpart of His39/J that is an axial ligand of Chl *a* 88 in *T. elongatus* PSI, explains the absence of the corresponding Chl *d* (Extended Data Fig. 19D). Owing to the absence of PsaX in *A. marina*, the Chl *d* corresponding to Chl *a* 95 in *T. elongatus* whose axial ligand is Asn23/X is also missing. These differences help explain the unique features of light harvesting in *A. marina*.

The arrangements of pigments adjacent to Psa27 (PsaI) and PsaL, whose sequence identity with other cyanobacterial PsaLs is low, are specific to *A. marina* (Extended Data Fig. 19A, black dashed line region, and Extended Data Fig. 20, and 21). Chl *d* 38, 52, 53, and the ring (α or ϵ) of Car4007 near Chl *d* 53 are set within the surrounding structure differently from those in *T. elongatus* (Extended Data Fig. 20A). This impacts on the distances between Car4010 and Chl 92 (8.1 Å in *A. marina* vs. 4.9 Å in *T. elongatus*), and Car4010 and Chl 47 (7.9 Å in *A. marina* vs. 6.9 Å in *T. elongatus*) (Extended Data Fig 20B and C). In the *A. marina* PSI, Chls 37 and 91–93 are in close proximity to PsaL, and Gln92/L (Ala94/L of *T. elongatus*) and Trp27/L (Phe30/L of *T. elongatus*) are positioned within hydrogen-bonding distance of Chl *d* 91 and 92, respectively (Extended Data Fig. 20D and E). Moreover, the formyl group of Chl *d* 37 and 93 and Ser62/L

may form hydrogen bonds via one water molecule (Extended Data Fig. 21). Again, these divergences must affect energy transfer efficiencies in the two organisms.

A. marina PSI is a trimer as is the case for other cyanobacterial PSIs, but the amino acid sequence identity of each protein subunit is low, and, not unexpectedly, the divergence is most pronounced around the unique pigments, for example Chl *d*, α -Car, and Pheo *a*-A₀ (Extended Data Table 5). The structure exhibits unexpectedly high flexibility of photosynthetic machinery. Again, the alterations must reflect optimization to drive efficient photochemistry. The structure would also bridge the evolutionary gap between the Chl *a*-type oxygenic reaction centers (adapted to visible light) and the BChl-type reaction centers (adapted to 800–900-nm light).

In conclusion, the structure and identification of electron carriers and key light-harvesting pigments provide the architecture for understanding how low energy far-red light is utilized by *A. marina* PSI. However, the full picture must wait for the structure of PSII, which is responsible for oxygen evolution through water-splitting, to be elucidated³.

Materials And Methods

1. Cell culture and thylakoid membrane preparation

Acaryochloris marina was cultivated in artificial sea water Marine Art SF-1 (Osaka Yakken, Osaka, Japan) enriched with Daigo IMK (Nihon Pharmaceutical, Tokyo, Japan), using 7.5-L culture bottles (Rectangular Polycarbonate Clearboy, Nalgene, Rochester, NY) under continuous white light at 5–15 $\mu\text{mol photons/m}^2\text{s}$ and 23°C with continuous air-bubbling for 25–28 days. Cells were harvested by centrifugation at $7,800 \times g$ for 10 min, suspended in buffer A containing 50 mM MES–NaOH (pH 6.5), 5 mM CaCl_2 , 10 mM MgCl_2 ,

1.0 M betaine, and 5% (w/v) glycerol, and stored frozen until use. The cells were broken at 1.5 mg Chl *d*/mL with the same volume of glass beads (0.2 mm diameter) at 0°C by 24 cycles of 10-s breaking, and 2-min cooling in the presence of DNase I (0.5 $\mu\text{g/mL}$; Sigma, St. Louis, MO) and a protease inhibitor mixture (280 $\mu\text{L}/100\text{ mL}$ cell suspension; Sigma). Thylakoid membranes were collected by centrifuging the broken cells at $31,000 \times g$ for 10 min. The resulting pellets of thylakoid membrane were resuspended in buffer A.

2. Preparation of PSI trimer from *marina* membrane

Prior to the purification of PSI complex, solubilization of thylakoids was tested using 13 detergents that are frequently used to solubilize membrane protein complexes: *n*-decyl- β -D-maltopyranoside (DM), *n*-undecyl- β -D-maltopyranoside (UDM), *n*-dodecyl- β -D-maltopyranoside, *n*-tetradecyl- β -D-maltopyranoside, *n*-dodecyl- α -D-maltopyranoside, *n*-octyl- β -D-glucopyranoside, 6-cyclohexyl-1- β -D-maltopyranoside, *N*-lauroylsarcosine, *n*-decyl- β -D-maltopyranose neopentyl glycol (LMNG), decyl maltose neopentyl glycol, sodium cholate, and digitonin. Because the first three detergents had almost the same solubilization

efficiency, UDM was selected in this work to solubilize thylakoids. DM was used in sucrose density gradient ultracentrifugation because it was the best among those three detergents for maintaining the stability of the purified PSI trimer.

Thylakoid membranes were solubilized with 1.0% (w/v) UDM at 1 mg Chl *d*/mL on ice for 10 min. The extracts were separated from insoluble membranes by centrifugation at $75,600 \times g$ for 10 min, and layered onto a stepwise sucrose gradient [1.4, 1.2, 1.1, 1.05, 1.0, 0.9, 0.8, and 0.7 M sucrose in buffer A supplemented with 0.2% (w/v) DM] for ultracentrifugation at $100,000 \times g$ for 20 h. After centrifugation, fractionation was performed from bottom to top, and the protein content of each fraction was investigated by blue native- polyacrylamide gel electrophoresis (BN-PAGE) using linear gradient gels of 3%–12% polyacrylamide (Extended Data Fig. 2). Fractions exhibiting a band of PSI trimer of ~720 kDa, corresponding to sucrose concentrations of 1.05–1.0 M, were collected.

For electron microscopic analysis, the PSI trimer in the above fractions was washed using Amicon centrifugal filters (pore-size 100,000 Da) with buffer A supplemented with 0.002% LMNG, and applied to a second stepwise sucrose gradient ultracentrifugation consisting of 1.4, 1.2, 1.1, 1.05, 1.0, 0.9, 0.8, and 0.7 M sucrose in buffer A supplemented with 0.002% LMNG. LMNG was used for electron microscopic analysis because of its low critical micellar concentration.

For biochemical analyses, the fractions obtained after the first sucrose density gradient ultracentrifugation were applied to an anion exchange column (HiLoad 16/10 Q Sepharose HP) and eluted with a linear gradient of 0–600 mM KCl.

3. Polypeptide analysis

Denatured polypeptides were separated on a gel containing sodium dodecyl sulfate (SDS), 6 M urea, and 18% or 16%–22% (w/v) acrylamide as described previously ⁴³ with or without dithiothreitol, and then stained with Coomassie Blue (Extended Data Fig. 5). Separated polypeptides were identified by mass spectrometry (MS) analysis after in-gel digestion by trypsin (Extended Data Table 2).

4. Spectroscopic analysis

Chl *a* and Chl *d* concentrations were determined as described by Porra et al. ⁴⁴ and Li et al. ⁴⁵, respectively, using methanol as the solvent and a UV-2700 spectrophotometer (Shimadzu; Kyoto, Japan) with a slit-width of 1 nm at 25°C. Absorption spectra of the PSI trimers were

measured at 25°C using an UV-2700 spectrophotometer (Extended Data Fig. 6). For cell suspensions, an integrating sphere, model ISR-2600, was used with the UV-2700 spectrophotometer (Extended Data Figs.

2 and 6). Absorption spectra at 77 K were measured using an MPS 2000 spectrophotometer equipped with a low-temperature measurement unit, LTS-2000 (Shimadzu) (Extended Data Fig. 7).

5. Pigment analysis

Photosynthetic pigments and quinones were extracted in methanol and quantified using reversed-phase high-performance liquid chromatography (HPLC) carried out using an LC-20AT with a CBM-20A system controller (Shimadzu), equipped with a Kinetex C18 column (5 μ m, 250 $\times$ 4.60 mm, 100 Å) (Phenomenex, Torrance, CA), as described previously ⁴⁶. The elution pattern of the pigments was detected using a Shimadzu photodiode array detector, SPD-M20A, with Shimadzu LabSolutions (ver. 5.82) analysis software at 430 nm for photosynthetic pigments (Extended Data Fig. 15) and 270 nm for PhyQ. Standard curves for Chl *a* and Chl *d* were obtained after quantification of Chl *a* from a cyanobacterium, *Synechocystis* sp. PCC 6803, and Chl *d* from *A. marina* as described above. The standard curve for Chl *d* was also used for Chl *d*. A Standard curve for Pheo *a* was made using Pheo *a* obtained by acidification of quantified Chl *a*. PhyQ (Vitamin K₁) was obtained from Wako Pure Chemicals (Osaka, Japan). Zeaxanthin and α - and β -carotene were purchased from the VKI Water Quality Institute (Hørsholm, Denmark). PhyQ bound to PSI trimer was quantified after the removal of Chl *a* contribution at almost the same retention time by acidification ⁴⁷.

6. DNA sequencing

Genes *psaA* and *psaB* were partially sequenced as follows (Extended Data Fig. 16). Pairs of primers for *psaA* (GTACAACTGCATCTCAATTG and CTATCCTAATGCGAGAATTC) and *psaB* (CCTTGCCCTTCTTCTGGATGC and TTAGCCGAGAGGAGCTGTTG) were used to amplify part of the genes by PCR using genomic DNA as the template. Then, the DNA sequence of the amplified, purified DNA fragment was analyzed using BigDye Terminator ver. 3.1 (Applied Biosystems; Foster City, CA) using the same primers.

7. Cryo-EM sample preparation and data collection

Three microliters of purified PSI [0.4 mgChl *d*/mL in a buffer containing 50 mM MES– NaOH (pH 6.5), 5 mM CaCl₂, 10 mM MgCl₂, and 0.002% LMNG] were applied to a holey carbon grid (Quantifoil R1.2/1.3 200 mesh grids, Microtools GmbH, Berlin, Germany) that had been pretreated by gold-sputtering ^{48,49} and glow-discharging (JEC-3000FC, JEOL, Japan). The grid was blotted with filter paper for 4 s, then immediately plunge-frozen in condensed ethane using an FEI Vitrobot Mark IV (ThermoFisher Scientific, Waltham, MA, USA) under 100% humidity at 4°C. The frozen grids were then introduced into a CRYO ARM 300 electron microscope (JEOL, Tokyo, Japan) equipped with a cold-field emission gun, after which inelastic scattered electrons were removed using an in-column type energy filter with an energy slit of 20

eV. Dose fractionated images were recorded on a K2 summit camera in counting mode with a nominal magnification of 60,000 $\times$, which corresponded to a physical pixel size of 0.823 Å. All image data were collected with a JEOL Automatic Data Acquisition System (JADAS)⁵⁰ with a dose rate of 11e⁻/Å²/s, 10 s exposures, 0.2 s/frame, and a defocus range of -0.8 to -1.8 µm. In total, we collected 3,225 (data 1) and 4,346 movie stacks (data 2) from two independent sample preparations.

8. Cryo-EM image processing

Cryo-EM movie stacks were grouped into 10 separate optics groups based on time of data collection. Drift corrected and dose-weighted summation of movie frames was performed using MotionCor2⁵¹, and contrast transfer function (CTF) parameters were estimated from the correct images with CTFFIND4⁵². Based on Thon-ring patterns, 4,237 images from data 1 and 2 were selected for particle picking. Good PSI particles were manually selected and subjected to reference-free two-dimensional (2D) classification with RELION-3.1beta. Next, homogeneous 2D class averages were used as templates for reference-based auto-picking, and a total of 774,416 particles were extracted with a pixel size of 1.646 Å and a box size of 220 pixels for further 2D classification. Good 2D class averages contained 242,550 particles, which were passed to three-dimensional (3D) classification with a *de novo* initial model constructed using cryoSPARCv2, and a well-aligned and symmetrical 3D class was reconstructed from 86,509 particles. The particles in the best 3D class were rescaled and re-extracted with a pixel size of 1.097 Å and a box size of 330 pixels, and 3D refinement with three-fold symmetry enforcement and post-processing yielded a 3.3-Å resolution map based on the gold standard Fourier shell correlation criterion. Particles in the cryo-EM density map were then subjected to Bayesian polishing for correction of particle-based beam-induced motion and CTF refinement of particle-based defoci and optics-group-based high-order astigmatism. Finally, 86,419 particles were selected by excluding those showing unrealistic defocus values and then subjected to 3D refinement and post-processing again, which yielded a 2.59-Å resolution map with C3 symmetrization. After beam tilt estimation and correction for every micrograph, 3D refinement and post-processing reached 2.48-Å and 2.97-Å resolution structures with and without C3 symmetrization, respectively. B-factor values for map sharpening were estimated as -83.67 Å² and -77.95 Å², respectively. For further details, see Extended Data Figs. 8 to 10 and Extended Data Tables 3 and 4.

9. Model building and refinement

An initial model of the PSI monomer from *A. marina* was built using homology modeling in MODELLER (version 9.23)⁵³ by referring to the structure of the PSI monomer from *T. elongatus* (PDB code: 1JB0). The identity of each amino acid in subunit proteins in *A. marina* PSI was determined using amino acid sequences obtained from UniProt (<https://www.uniprot.org/>). The resulting homology model of the PSI monomer was fitted to the whole cryo-EM density map (C3 map) using a “fit in map” program in UCSF Chimera (version 1.13)⁵⁴, and an initial model of the trimeric form of PSI from *A. marina* was created.

Next, the created PSI trimer model was fitted to the cryo-EM density map using a molecular dynamics simulation program (CryoFit) and simulated annealing in Phenix^{55,56}. Each model of the three monomers in the PSI trimer was refined separately, and the refined model was modified using COOT⁵⁷ manually to fit the cryo-EM density map. Subsequently, the obtained structure model was finally refined using *refmac5*⁵⁸ (Extended Data Fig. 11). Refinement statistics of the refined model was obtained using a comprehensive validation program in Phenix.

The cofactors such as Chl *d*, Chl *d'*, Chl *a*, Pheo, phyloquinone (PhyQ), iron–sulfur clusters, α -carotene (α -Car), monogalactosyldiacylglycerol (MGDG), and phosphatidylglycerol (PG) were identified and refined using geometry restraint information as described in the following section. For A_0 (A_{0A} and A_{0B}) in *A. marina* PSI, a cryo-EM density map of Mg^{2+} of Chl *d* could not be identified unambiguously, and thus the two A_0 were identified as Pheo. Validation of the refined structure model was assessed using Phenix, ModelZ⁵⁹, and MapQ⁶⁰. The refinement statistics of the models are summarized in Extended Data Table 1. Structure figures were generated and rendered with PyMOL and Chimera^{54,61}.

10. Identification of cofactors and their geometry restraint information

The restraints needed for the pigments in PSI from *A. marina* were generated by Electronic Ligand Bond Builder and Optimization Workbench (eLBOW)⁶² because the information was not registered in the restraints library in Phenix. The restraint information for Chl *d* was created from the model of Chl *d* identified in a high-resolution structure (PDB code: 2X1Z)⁶³, and information for Chl *d'*, an epimer of Chl *d*, was created with reference to the structures of Chl *d* and Chl *a'*, an epimer of Chl *a* (PDB codes: 1JB0 and 2X1Z). The orientation of the C3-formyl group of Chl *d'* was determined based on that of Chl *d*⁶³. The PSI trimer from *A. marina* contained α -Car instead of β -carotene (β -Car). The α -Car molecule possesses two rings—known as α - and ϵ -rings—at each end, while the β -Car molecule possesses two β -rings. Although the restraint information for α -Car was created using eLBOW, as for the other cofactors, α -Car was registered as an unknown ligand (three-letter code: UNL) in this study because it is hard to distinguish between α - and ϵ -rings from the present cryo-EM density map. Restraint information for Pheo, iron–sulfur clusters, MGDG (three-letter code: LMG), and PG (three-letter code: LHG) was created with reference to high-resolution structures (PDB codes: 1JB0 and 3WU2).

11. Identification of water molecules

Water molecules were identified manually from the cryo-EM density map at >2.0 root-mean-square level using COOT. Eighty-four water molecules were found in the refined structure model. Most of the water molecules were identified as ligands to Chl *d*.

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Declarations

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Author contributions

K.S-I., N.I-K., E.Y. and K.M. optimized purification conditions. K.S-I. purified the PSI trimer used in cryo-EM experiments. K.S-I., N.I-K., S.I., K.I. and Y.K. performed biochemical analyses. T.H. and K.Y. collected and analyzed cryo-EM data. K.K. built and refined the structural model. T.H., K.K., K.S-I., K.Y. and Y.K. wrote the manuscript with input from all other authors.

Competing interests

The authors declare no competing interests.

Additional Information

Data deposition is currently being conducted for PDB.

Extended Data is available for this paper.

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Figures

Figure 1

Comparison of electron transfer chain of cyanobacteria and higher plant PSI (A) and *A. marina* PSI identified in this work (B). In cyanobacterial and higher plant PSI, electrons released from reaction center P700, a heterodimer of Chl a and a', are transferred to ferredoxin (Fd) to reduce NADP⁺ via Acc (Chl a), A₀ (Chl a), A₁ (PhyQ), F_x (ion-sulfur center) and F_A/F_B (ion-sulfur center). The components are arranged in a pseudo-C₂ axis on a heterodimer reaction center protein complex (PsaA/PsaB) and the two routes (A- and B- branch located on PsaA and PsaB, respectively) are thought to be equivalent. Acc, accessory chlorophyll; A₀, primary electron acceptor; A₁, secondary electron acceptor; F_x, F_A and F_B, iron-sulfur centers; PhyQ, phylloquinone. In *A. marina* PSI, the electron transfer chain is the same, but some

cofactors differ from those in the PSIs of other organisms; the reaction center is P740 composed of Chl d/d', while Acc could be Chl d and A0 is Pheo.

Figure 2

Overall structure of the photosystem I (PSI) trimer from *Acaryochloris marina*. (A) Cryo-electron microscopy (cryo-EM) density map of PSI trimer viewed from the stromal side perpendicular to the membrane plane. Monomer-1, multicolored; monomer-2, orange; monomer-3, gray; detergent micelle, cloudy gray. (B) Cryo-EM density map of PSI trimer viewed from the side of the membrane plane. (C) Structural model of PSI monomer with view in the same direction as in (A). PsaA, green; PsaB, cyan; PsaC, orange; PsaD, yellow; PsaE, red; PsaF, gray; PsaJ, light blue; PsaK, magenta; PsaL, dark green; PsaM, teal blue; Psa27, blue. (D) Structural model of PSI monomer with view in the same direction as in (B).

Figure 3

Cofactors involved in the electron transfer reaction in *A. marina* PSI and their surrounding amino acid residues. (A) Arrangement of cofactors involved in the electron transfer reaction. (B) Arrangement of P740 and its surrounding structure. View from the side of the membrane plane. (C) As in (B), but view from the pseudo-C2 axis. (D) Arrangement of the amino acid residues influencing Em (P_A) and Em (P_B). Superposition of the structural model of PSI from *A. marina* with that from *Thermosynechococcus elongatus* (PDB code 1JB0). Transparent green and blue, PSI from *A. marina*; transparent grey, PSI from *T. elongatus*. Chlorophyll (Chl) d, green; Chl d', blue; water molecule, red.

Figure 4

Arrangement of A0, Acc, and the surrounding protein environment. The structural model of PSI from *A. marina* is superposed with that from *T. elongatus* (PDB code 1JB0). (A) Cryo-EM density map of PA, PB, A0A, and A0B. Each map is shown in a mesh representation at 1.0 sigma contour level. (B) Arrangement of A0, Acc, and the surrounding protein environment. (C) As in (B), but view from the pseudo-C2 axis. (D) Geometry of PA/PB and Acc (AccA and AccB). Chl d, green; Pheo, orange; water molecule, red.

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