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Structure of CyanoP at 2.8 Å: Implications for the Evolution and Function of the PsbP Subunit of Photosystem II[†,‡]

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ABSTRACT: We present here the crystal structure of CyanoP (Tlr2075) from *Thermosynechococcus elongatus* at 2.8 Å. CyanoP is a substoichiometric component of the isolated cyanobacterial Photosystem II (PSII) complex, distantly related to the PsbP extrinsic subunit of the oxygen-evolving PSII complex in higher plants and green algae. Despite the relatively low degree of sequence similarity, we have found that CyanoP adopts the same β-sandwich fold as higher-plant PsbP and contains a well-conserved metal (zinc)-binding site that is also present in plant PsbP. Our results support the idea that CyanoP represents the basal structural fold of the PsbP superfamily.

Photosystem II (PSII) functions as the oxygen-evolving enzyme of oxygenic photosynthesis and is a multisubunit pigment−protein complex found in the thylakoid membranes of chloroplasts and cyanobacteria (*1*). It uses four photons of light to oxidize water to oxygen, with the concomitant reduction of plastoquinone to plastoquinol. The water oxidation reaction occurs at the oxygen-evolving complex (OEC), a Mn_4Ca cluster ligated by side chains of the D1 and CP43 proteins. PSII contains a number of extrinsic proteins that bind to the lumenal side of the complex (*2*). The precise complement of these extrinsic proteins depends on the taxon. Cyanobacterial PSII contains the PsbO, PsbV, and PsbU subunits, which are present in the published crystal structures of PSII from the thermophilic cyanobacteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* (*3*−*5*), whereas higher-plant PSII contains the PsbP and PsbQ extrinsic proteins in addition to PsbO. PsbP is required for the stability and assembly of the PSII complex (*6*), and its absence leads to an increased requirement for Cl^- and Ca^{2+} ions for oxygen evolution in vitro (*7*). PsbP is needed for photoautotrophy (*6*, *7*) and plays a role in accumulating PSII−LHC II supercomplexes and forming the granal stacks (*8*). Structures of higher-plant PsbP have been determined from tobacco (*9*) and spinach (*10*) [Protein Data Bank (PDB) entry 2VU4], but the PsbP-binding site in PSII remains unclear.

Higher plants express multiple isoforms of PsbP and also a number of PsbP-like proteins (PPLs): for instance, there are two PsbP (PsbP1 and PsbP2) and eight PPL subunits encoded by *Arabidopsis thaliana* (*2*). The functions of the PPL proteins are largely unknown; however, PPL1 is involved in the repair of PSII following photodamage, and PPL2 is required for accumulation of the chloroplast NDH-1 complex in *Arabidopsis* (*11*).

The prokaryotic cyanobacteria also contain a PsbP-like protein (*12*), here termed CyanoP. PsbP and CyanoP are not well-conserved, with a level of sequence identity over the mature protein of only 24%. In contrast to higher-plant PsbP mutants, CyanoP null mutants are still able to grow photoautotrophically as well as the wild type (WT) (*12*) but have been reported to show reduced growth rates and fewer active PSII complexes when Ca^{2+} and Cl^- are removed from the medium (*12*). The absence of CyanoP in a PSII deletion mutant has provided strong evidence that CyanoP is a bona fide PSII subunit (*13*). However, only substoichiometric amounts of CyanoP are detected in isolated His-tagged PSII preparations (*12*, *13*), possibly because CyanoP is lost during purification (*13*). Overall, the current data suggest an accessory role for CyanoP in PSII, possibly in optimizing the function of the Mn_4Ca cluster (*14*) or in assembly and repair of the complex.

To gain structural insights into CyanoP, we overexpressed the *T. elongatus* subunit as a His-tagged protein in *Escherichia coli*. CyanoP has a predicted "lipobox" (*15*) sequence in its N-terminal region suggestive of a lipoprotein. Consistent with this, CyanoP is more strongly attached to the membrane than PsbO in both *T. elongatus* (Figure S1 of the Supporting Information) and *Synechocystis* 6803 (*13*).

Thus, the soluble domain starting from the lipid-binding Cys residue was chosen for expression. The protein was purified by affinity chromatography, and the His tag was removed by thrombin cleavage (Figure S2 of the Supporting Information) to yield a polypeptide corresponding to the predicted mature protein with an additional N-terminal glycine residue; the lipid-binding Cys20 residue was changed to Ser. The protein was concentrated to 24 mg/mL and crystallized using sitting-drop vapor diffusion. The crystal structure was determined using Zn-SAD and refined using data to 2.8 Å (Figure 1 and Table S1 of the Supporting Information). Electron density was visible corresponding to residues Ser24 to Tyr183, the C-terminal residue of the protein. A loop consisting of residues 133−137 was not visible in the electron density and so is unmodeled. This loop is also missing in the deposited PsbP structures from higher plants but is not highly conserved among PsbP sequences. There were no Ramachandran plot outliers. Overall, the structure of CyanoP is very similar to that of PsbP with a 1.6 Å root-mean-square deviation for both deposited plant PsbP structures over the aligned Cα atoms.

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[‡]The coordinates and structure factors of CyanoP have been deposited in the Protein Data Bank as entry 2XB3.

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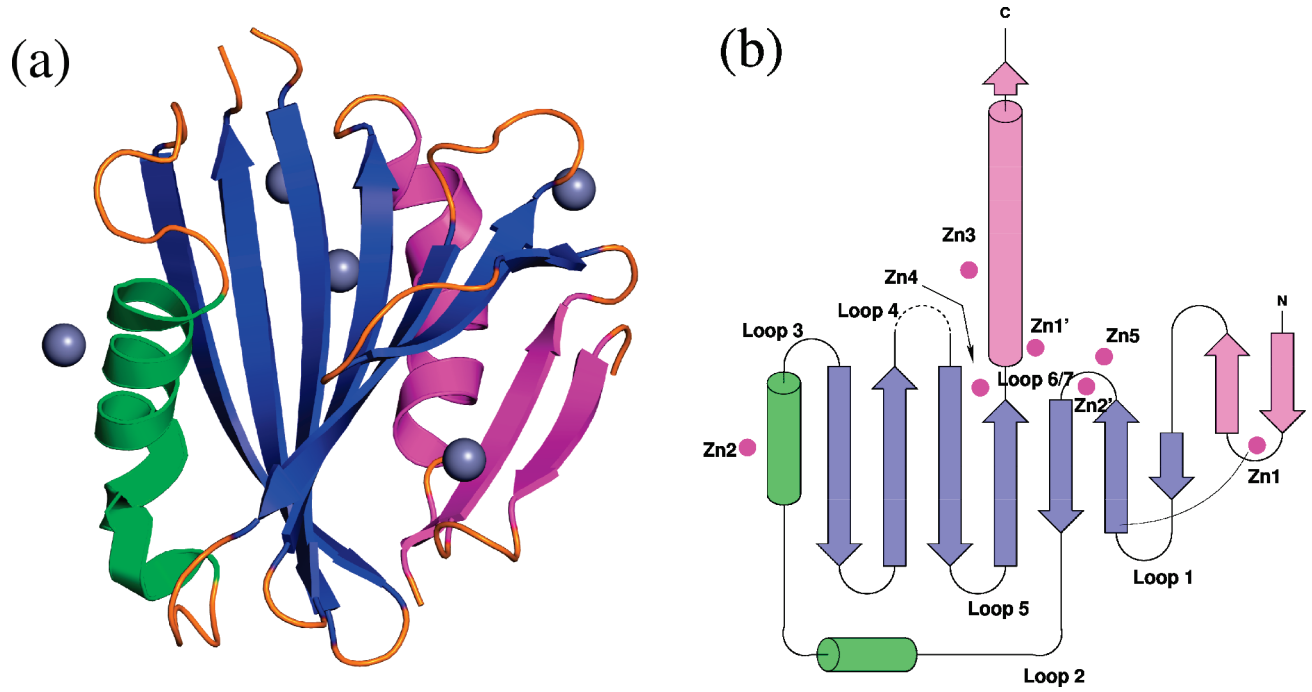

FIGURE 1: (a) Cartoon ribbon diagram of CyanoP from *T. elongatus*, with zinc atoms shown as spheres. (b) Cartoon topology diagram of CyanoP. The N-terminal extension and C-terminal helix (magenta) form the back of the β-sandwich. The main central sheet is colored blue, and the "front" helices are colored green. The approximate binding sites of the five zinc sites are shown. Primes indicate a zinc from a symmetry-related molecule, as in Table 1. Residues 133−137 are not visible in the electron density map and are represented by a dotted line.

Table 1: Zinc Sites Found in CyanoP[a]

Zn atom	ligating residues	Zn atom	ligating residues
1	Asp31, Asp34, Asp54, Glu164′	4	His142, Glu163
2	Glu87, Asp91, His58′, Thr63′	5	Asp59
3	Glu170, Glu170′		

[a]A prime indicates that the residue comes from a symmetry-related molecule in the crystal lattice. Thr63 is bound to the zinc via its main chain carbonyl oxygen atom. These sites are shown in Figure S3 of the Supporting Information.

The main difference between the CyanoP and PsbP structures is that PsbP contains the insertion of short sequences into the loop regions and an extension at the N-terminus, which is implicated in regulating Cl^- and Ca^{2+} binding in PSII (*16*, *17*). From an evolutionary perspective, the structural data support the recent suggestion by Sato (*18*) that CyanoP represents the basal form of the PsbP family of proteins that has diversified into eight distinct families after the divergence of cyanobacteria and plastids. Family A contains the PsbP subunits involved in the OEC of higher plants, whereas families B−G, H1, and H2 contain PsbP-like proteins. Family H2 corresponds to cyanobacterial PsbP-like proteins (CyanoP). An alignment of selected PsbP-like proteins is shown in Figure S3 of the Supporting Information, with associated sequence conservation mapped onto the structure in Figure S4 of the Supporting Information.

There are five zinc atoms in the CyanoP structure (positions verified by peaks in the phased anomalous difference map and enumerated in Table 1 and Figure S5 of the Supporting Information), which because of their positions at crystal contacts leads to seven distinct zinc-binding sites on the surface of the protein. Zinc site 4 is equivalent to the single zinc site in the deposited structure of PsbP from spinach (PDB entry 2VU4). Plant PsbP has been reported to bind manganese tightly at one site (*19*). Several possible manganese-binding residues have been suggested on the basis of the effect on tryptophan fluorescence (*20*). Of these proposed residues, His144 and Asp165 (PDB entry 1V2B numbering) correspond to our zinc 4 site, and Asp51 corresponds to the zinc 5 site. Only the binding sites for zinc sites 4 and 5 are highly conserved, although no zinc site is absolutely conserved in the PsbP and CyanoP sequences. Many of the zinc-binding residues are also conserved in the PsbP-like proteins (Table S2 of the Supporting Information), which would suggest that binding of metal ions might be a common feature of members of the PsbP superfamily.

A recent structure of CyanoQ, a PsbQ-like protein, from *Synechocystis* (*21*) has also shown it to be a zinc-binding protein, with a high degree of structural similarity to plant PsbQ (*22*). The presence of zinc in the crystal structures of CyanoQ, PsbQ, PsbP, and now CyanoP is suggestive of a physiological function for this metal in PSII. It is known that zinc inhibits the activity of plant PSII and causes dissociation of PsbP and PsbQ from the core complex (*23*, *24*). It is, however, possible that some of the zinc sites in our crystal structure are physiological manganese or calcium sites, and the zinc is present as an artifact of the crystallization conditions required to crystallize this particular form of CyanoP.

Like plant PsbP, CyanoP shares a fold with the Mog1 protein with a 2.7 Å root-mean-square deviation (*25*). A DALI (*26*) search showed that CyanoP (and PsbP) also share a fold with the DUF1795 family of proteins (Domain of Unknown Function 1795), also pfam08786 (*27*), of which there are two examples in the PDB, from *Pseudomonas aeruginosa* (PDB entry 1TU1, Ordered locus PA0094, Uniprot entry Q9I738) and *Jonesia denitrificans* (PDB entry 3LYD, Uniprot entry C7QYG7). None of the CyanoP zinc-binding residues are conserved in these proteins. These two DUF1795 proteins are of unknown function but are dimeric, unlike CyanoP, which size exclusion chromatography shows to be monomeric (data not shown). Superpositions of

CyanoP and related proteins are shown in Figure S6 of the Supporting Information. It has been proposed that plant PsbP was recruited from a Mog1p-like GTP binding protein to aid the GTP-regulated degradation of D1 (*9*). However, this eukaryotic mechanism is unlikely given the evidence that PsbP-like proteins were probably present in the earliest PSII complexes, and that the more similar DUF1795 family is so far only prokaryotic.

How PsbP binds to the PSII core complex is unknown, although preliminary placements of PsbP and PsbQ have been proposed on the basis of low-resolution cryo-electron microscopy particle reconstructions (*28*). Cross-linking data have led to the identification of a site of interaction between plant PsbP and PsbO (*29*, *30*). Recently, an interaction with PsbQ has been proposed for *Chlamydomonas reinhardtii* PsbP (*31*), although one residue, Lys174, is proposed to bind to both PsbQ and PsbO. This residue is a glutamine in *T. elongatus* CyanoP. The same *Chlamydomonas* study also found an interaction between the α-subunit of cytochrome b_{559} (PsbE) and PsbP. Given the positions of PsbO and PsbE at opposite sides of the PSII complex, it seems implausible for CyanoP to bind both of these, although green alga and higher-plant PsbP may bind in different ways.

Electrostatic potentials were calculated for PsbP and CyanoP (Figure S7 of the Supporting Information). The rear surface, on the right-hand side of the figure, loosely comparable to the magenta parts of the protein in Figure 1, shows a broad pattern of positive charge along the "top" and negative charge along the "bottom" that is conserved between PsbP and CyanoP, indicating a possible conserved functional surface involved in binding to PSII. This region must be close to the N-terminus, due to the predicted lipid anchor at this point. The overall level of sequence conservation of PsbP-like proteins is low (Figure S3 of the Supporting Information), making it difficult to predict functional surfaces of CyanoP as there are no totally conserved surface residues. Overall, our results show that the fold of CyanoP and PsbP is highly conserved despite a low level of sequence identity. A common zinc-binding site that might be physiologically significant has been identified in CyanoP and higher-plant PsbP. The determination of a crystal structure of isolated CyanoP is an important step toward determining its role in the function of PSII and the evolution of the PsbP superfamily.

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SUPPORTING INFORMATION AVAILABLE

Supplementary figures, detailed experimental procedures, and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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