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Crystal Structure of PsbQ from *Synechocystis* sp. PCC 6803 at 1.8 Å: Implications for Binding and Function in Cyanobacterial Photosystem II[†,‡]

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ABSTRACT: In *Synechocystis* sp. PCC 6803, PsbQ is associated with photosystem II (PSII) complexes with the highest activity and stability. However, this subunit is not found in PSII X-ray crystallographic structures from *Thermosynechococcus elongatus* or *Thermosynechococcus vulcanus*. We present the crystal structure of cyanobacterial PsbQ determined in the presence and absence of Zn^{2+}. The protein has a well-defined helical core, containing four helices arranged in an up−down−up−down fold. A conserved potential interaction site composed of a divalent metal binding site and adjacent hydrophobic pocket has been identified.

Water splitting in oxygenic photosynthesis is conducted by photosystem II (PSII)[1] in thylakoid membranes of chloroplasts and cyanobacteria (*1*, *2*). A group of extrinsic hydrophilic proteins (PsbO, PsbP, PsbQ, PsbU, PsbV, and Psb27) are associated with the lumenal face of the photosystem, but the composition of bound subunits varies between phyla. X-ray crystallographic studies from the cyanobacteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* have confirmed the structure and position of PsbO, PsbU, and PsbV (*1*−*3*). However, in green algae and plants, PsbU and PsbV are absent (*4*). Although not found in the currently available X-ray-derived structures of PSII, homologues of PsbP, PsbQ, and Psb27 are encoded in the genome of *T. elongatus*, and all three subunits were identified within PSII preparations from *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) (*4*, *5*).

In cyanobacteria, the PsbQ, PsbP, and Psb27 homologues contain a motif for an N-terminal lipid-modified cysteine and thus form a unique group of extrinsic PSII lipid-modified proteins: lipid modification of this cysteine has been confirmed for Psb27 and PsbQ (*6*−*8*). Psb27 appears to play a role in PSII assembly (*7*, *9*), and its solution structure has been determined (*10*, *11*). The structure and function of PsbP remain unclear, and it appears to be substoichiometric (*6*, *12*, *13*). PsbQ, however, is required for optimal PSII activity, appears to be stoichiometric, and is associated with the most stable and active PSII complexes isolated from *Synechocystis* (*6*, *14*, *15*). This indicates that PsbO, PsbU, PsbV, and PsbQ are all present in the mature form of PSII in this cyanobacterium.

PsbQ structures have been obtained from *Spinacia oleracea* (spinach), and these required Zn^{2+} for crystallization (*16*, *17*). We have obtained the X-ray-derived structure of PsbQ from *Synechocystis,* both in the presence and in the absence of bound Zn^{2+}. Comparison of this structure with its plant homologue identified several novel structural elements that may be required for both the function and binding of PsbQ to cyanobacterial PSII.

The homologue of PsbQ from *Synechocystis* (Sll1638) was overexpressed in *Escherichia coli* as a fusion protein with glutathione *S*-transferase (Figure S1 of the Supporting Information). The fusion protein was purified by affinity chromatography and proteolytic cleavage to yield a recombinant peptide including the entire predicted mature sequence of PsbQ (residues 22−149) with a Gly-Pro-Leu-Gly-Ser pentapeptide N-terminal extension. The protein was concentrated between 14 and 18 mg/mL, and crystals were obtained using the hanging-drop, vapor-diffusion method. The X-ray crystal structure in the absence of Zn^{2+} was determined by multiwavelength anomalous dispersion using both a selenomethionine derivative and a native form of the recombinant protein. The structure of PsbQ in the presence of Zn^{2+} was determined by molecular replacement using the structure obtained in the absence of Zn^{2+}. In the structure without Zn^{2+}, residues Thr32−Pro146 were ordered, whereas in the presence of Zn^{2+}, all residues and two zinc atoms could be fitted to the electron density.

A sequence alignment highlights the low level of conservation among cyanobacteria, algae, and higher-plant primary sequences and illustrates the secondary structural differences between PsbQ from *Synechocystis* and the spinach protein (Figure 1). Both in the presence and in the absence of Zn^{2+}, cyanobacterial PsbQ folds as a four-helix up−down−up−down bundle similar to PsbQ from spinach except that in *Synechocystis* the second α-helix (H2) is split by a small loop between residues Gly77 and Leu79 (Figure 2A). The four helices span residues 35−64 (H1), 68−92 (H2), 95−121 (H3), and 127−145 (H4). Other notable differences include longer H1 and H3 helices compared with those of the spinach structure and an apparent lack of any structural motifs within the cyanobacterial N-terminus. The N-terminus of spinach PsbQ has two parallel *β*-strands, the first of which is essential for binding of spinach PsbQ to PSII (*17*, *18*). The absence of these in cyanobacterial PsbQ suggests binding mechanisms differ between organisms.

There are six invariant residues in cyanobacteria: Leu59, Trp68, Gly77, Pro78, Leu93, and Asp116 (Figures 1 and 2A,B). In addition, 11 highly conserved residues are present: Ile38, Ile63, Asn67, Ile75, His76, Leu79, Leu89, Gln98, Leu112, Leu115, and Ala119. The majority of conserved residues are positioned near the apex of PsbQ away from both termini (Figure 2A). Helix 2 is disrupted by the occurrence in cyanobacteria of a dipeptide,

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[‡]Atomic coordinates have been deposited in the Protein Data Bank as entry 3LS0 (without Zn^{2+}) and 3LS1 (with Zn^{2+}).

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[1]Abbreviations: PCC, Pasteur Culture Collection; PSII, photosystem II; rmsd, root-mean-square deviation.

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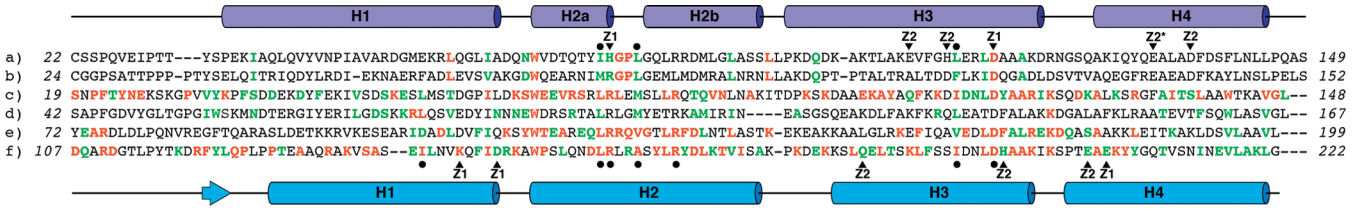

FIGURE 1: Alignment of representative PsbQ sequences from cyanobacteria (rows a and b), algae (row c, red algae; row d, brown algae; and row e, green algae), and higher plants (row f). Numbering is from the initial Met of the unprocessed polypeptides. The secondary structure of *Synechocystis* PsbQ (purple) is compared to that of spinach PsbQ (blue). α-Helices are depicted as cylinders. Only one of two β-strands (arrow) in the N-terminus of spinach PsbQ is included in this alignment. Residues that coordinate Zn^{2+} (▲) and conserved residues that form the H2H3 cavity (●) are indicated, and *Synechocystis* residues that coordinate Z1 are also part of the H2−H3 cavity. For each phylum, the invariant (orange) and highly conserved (green) residues are shown. PsbQ sequences (GenBank accession codes in parentheses): (a) *Synechocystis* sp. PCC 6803 (NP_440389), (b) *T. elongatus* BP-1 (NP_682847), (c) *Porphyra yezoensis* (AV434507), (d) *Guillardia theta* (CAH04626), (e) *Chlamydomonas reinhardtii* (P12852), and (f) *S. oleracea* (P12301). Sequences were aligned using Clustal-W.

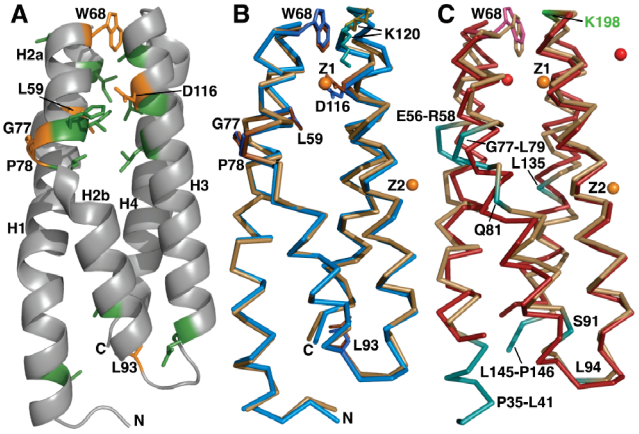

FIGURE 2: Helical structure of PsbQ. (A) Cyanobacterial PsbQ (without Zn^{2+}, residues 32−146) displaying invariant (orange) and highly conserved (green) residues (cf. Figure 1). (B) C_α trace of PsbQ (residues 32−146) determined in the presence (tan) and absence (blue) of Zn^{2+}. Z1 and Z2 atoms are orange. (C) C_α trace of PsbQ comparing the structures from *Synechocystis* (tan, residues 35−146) and the helical domain of spinach (red, residues 128−222). *Synechocystis* residues that are displaced by >2 Å compared with those of spinach are colored cyan, and K198 from spinach is colored green. Z1 and Z2 atoms are orange; spinach PsbQ Zn atoms are red. Images were displayed with PyMOL.

Gly-Pro, in a region otherwise well conserved across phyla. The residues involved in the loop that disrupt H2 include the invariant Gly77 and Pro78, flanked by a basic residue (His or Arg) and a hydrophobic residue (most commonly Leu or Met) forming a motif found in only cyanobacteria. In other phyla, the arginine equivalent to His76 is invariant and the closely positioned Asp116 is conserved with the exception of sequences from brown algae (Figures S2−S5 of the Supporting Information). The invariant Trp68, which is nestled between loop regions connecting H1 to H2a and H3 to H4, is present in all PsbQ sequences and may be an integral structural element at the conserved apex of the four-helix bundle. In this apex region, cyanobacteria lack a lysine that is otherwise conserved [Lys198 in spinach (Figure 1)].

No major structural differences are evident between the α-carbon backbones of the structures obtained in the presence or absence of Zn^{2+}, which is supported by a root-mean-square deviation (rmsd) of 0.695 Å between these structures (Figure 2B). Likewise, invariantly conserved residues occupy similar positions with the exception of the side chain of Asp116, which coordinates the Z1 Zn^{2+} ion and which has moved with respect to His76 (Figure S6 of the Supporting Information). Despite only 17% sequence identity between the helical domains of PsbQ from *Synechocystis* and spinach, the α-carbon backbones are clearly related (rmsd = 1.425 Å); positions that deviate by >2 Å include Leu41, Glu56−Arg58, Gly77−Leu79, Gln81, Ser91, Leu94, Leu135, Leu145, and Pro146 (Figure 2C). The similarity between the two structures supports the notion that a cyanobacterial PsbQ was the ancestral version of green algal and higher-plant PsbQ (*19*).

The molecular surface of *Synechocystis* PsbQ shows Trp68 is exposed and many of the conserved residues (Ile75, His76, Leu79, Leu112, and Asp116) are clustered in the vicinity of a cavity on the H2−H3 face close to the conserved Gly-Pro motif (Figures 1 and 3A−C). On the opposite side (Figure 3B), the H4−H1 face has a cavity near the bottom that involves three conserved residues (Ile38, Leu89, and Leu93) and the cyanobacterium-specific extension of H1 [residues Pro35−Gln42 (Figure 1)]: the cavity is positioned beneath a ridge formed by a potential hydrogen bond interaction between residues Gln42 and Gln147 (Figure S7 of the Supporting Information). The H4−H1 cavity is absent in the spinach structure; however, a ridge is formed by a potential hydrogen bond interaction between the conserved Lys136 and the carboxyl group of Gly222.

Zinc was required for crystallization of PsbQ from spinach (*16*, *17*), and Zn^{2+} occupies two well-ordered binding sites in the crystals of the *Synechocystis* protein. However, the Zn^{2+} binding sites are not conserved between species (Figures 1 and 2C). The Z2 binding site reported here appears to be an artifact because the ligands (Glu107, His111, and Asp137 from one monomer and Glu133 from the adjacent monomer) are not conserved within cyanobacteria (Figures S5 and S8 of the Supporting Information). Even though Zn^{2+} also mediated a dimer interface in crystals of spinach PsbQ, the interface differs in the two species, arguing against any requirement for dimer formation (Figure 2C and ref *17*).

In contrast, two of the ligands to the Z1 Zn^{2+} are well-conserved (His76 and Asp116), indicating this site may be an authentic site of interaction for divalent cations in vivo. The Z1 Zn^{2+} is also bound by a ligand from a second monomer (Gly17), although this is not an authentic PsbQ residue but a relic of the fusion protein. Exposure of Zn^{2+} on the surface of PsbQ may, however, reflect a specific cation-mediated interaction with another PSII subunit.

The molecular surfaces of the H2−H3 face in the presence and absence of Zn^{2+} (Figure 3A,C) are similar, the exceptions being that the cavity appears larger with Zn^{2+} bound and the position of the Lys120 side chain differs. The electrostatic surface of PsbQ (Figure S9 of the Supporting Information) shows the *Synechocystis* protein has a dipole with a positive H2−H3 face and a negative H4−H1 face, whereas the spinach homologue has

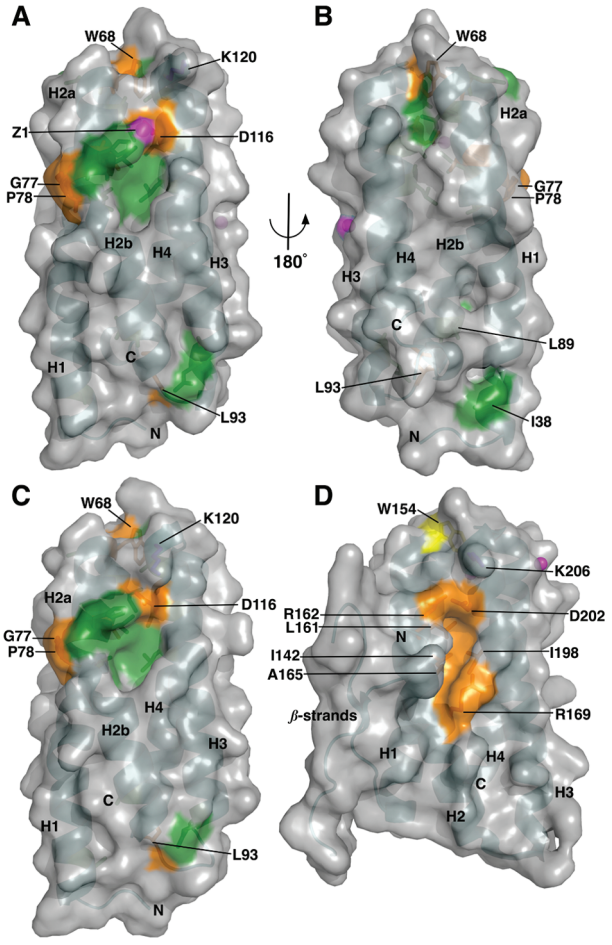

FIGURE 3: Molecular surface of PsbQ. (A) Cyanobacterial PsbQ with Zn^{2+} (residues 32−149) oriented showing the H2−H3 face. (B) Same as panel A but showing the H4−H1 face. (C) Cyanobacterial PsbQ without Zn^{2+} (residues 32−146) showing the H2−H3 face. (D) Spinach PsbQ (residues 84−222) showing the H2−H3 face. In panels A−D, invariant residues are colored orange, highly conserved residues green, and Zn^{2+} ions magenta. In panel D, invariant residues around the central cavity and the conserved W154 (yellow) are shown.

only a positive H2−H3 face. The addition of Zn^{2+} extends the positive charge to the top of the *Synechocystis* PsbQ H2−H3 face similar to the charge on the spinach protein which has been proposed to be the region that interacts with plant PsbO (*19*). Intriguingly, in the presence of Zn^{2+}, the H2−H3 cavity accommodates the hydrophobic side chain of a leucine from a symmetry-related molecule (Figure S6 of the Supporting Information): the ion binding site and hydrophobic pocket may together form a determinant for PSII binding. The spinach structure also contains an H2−H3 cavity that consists primarily of conserved residues (Ile142, Leu161, Arg162, Ala165, Arg169, Ile198, and Asp202) (Figure 3D). This cavity may therefore be a shared feature of PsbQ proteins across different phyla. The conformational change of Lys120 in the presence of Zn^{2+} (Figure 3A,C) results in the side chain being located away from the Zn^{2+} ion in a manner similar to that of the equivalent lysine in the spinach structure (Lys206 in Figure 3D). In the absence of Zn^{2+}, a H_2O molecule is present in place of Z1 and Lys120 forms H_2O-mediated interactions with His76 and Asp116 (Figure S6 of the Supporting Information). Conservation of His76 and Asp116 as well as the corresponding residues in other phyla, along with their proximity to the H2H3 cavity, supports our hypothesis that this region is important for the role of PsbQ. Furthermore, the similarity of the surface of *Synechocystis* PsbQ, in the presence of Zn^{2+}, to that of spinach PsbQ suggests divalent cation binding may induce a functional conformational change.

SUPPORTING INFORMATION AVAILABLE

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

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