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Solution NMR structure of photosystem II reaction center protein Psb28 from *Synechocystis* sp. strain PCC 6803

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INTRODUCTION

Oxygenic photosynthesis is initiated by photosystem II (PSII) in the thylakoid membranes of plants, algae and cyanobacteria. PSII is a multi-subunit pigment-protein complex responsible for splitting water into oxygen gas, hydrogen ions and electrons transferred to electron acceptors during photosynthesis.¹ Two homologous membrane-spanning proteins D1 (PsbA) and D2 (PsbD) form the PSII complex core.¹ Peripherally, two chlorophyll (Chl)-binding inner antenna proteins CP47 (PsbB) and CP43 (PsbC) are bound to the D1-D2 PSII complex core.¹ These four large proteins are surrounded by a large number of smaller membrane proteins.² Most of these small proteins have been observed in the crystal structures of the PSII complex from cyanobacteria.^{3,4} However, one small protein, Psb28, previously detected as a nonstoichiometric component of PSII,⁵ was not observed in the crystal structures indicating that Psb28 might not be a true PSII subunit. Recent studies revealed that Psb28 was preferentially bound to PSII core complex lacking CP43 (RC47) and involved in the biogenesis of CP47.⁶ Understanding the association of Psb28 with the PSII core complex should provide additional insight into its role in PSII-mediated function. However, the structure of Psb28 has remained unknown up until now.

In this note, we report the solution NMR structure of Psb28 protein encoded by gene *slh1398* [gi952386] of *Synechocystis* sp. strain PCC 6803 (SWISS-PROT ID: PSB28_SYNY3,

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NESG target ID: SgR171).⁷ This protein, also named Psb13 or ycf79, belongs to the Psb28 protein family (Pfam ID: PF03912), which is currently made up of ~48 protein sequences (E score less than 0.001 using PSI-BLAST, Table S1). Both PSI-BLAST sequence similarity and Dali⁸ structure similarity searches indicate that this is the first atomic resolution structure available for the Psb28 family. ConSurf⁹ was used to identify conserved surface residues potentially involved in binding to the PSII core complex.¹⁰

MATERIALS AND METHODS

The *sll1398* gene from *Synechocystis* sp. strain PCC 6803 was cloned into the pET21 expression vector (Novagen) containing a C-terminal affinity tag (LEHHHHHH) yielding the plasmid SgR171-21.1. Plasmids were transformed into codon enhanced BL21 (DE3) pMGK *E. coli* cells, cultured at 37°C in MJ minimal medium¹¹ containing (¹⁵NH₄)₂SO₄ and uniformly *U*-¹³C-glucose (NC) as the sole nitrogen and carbon sources. Initial cell growth was carried out at 37°C until OD₆₀₀ up to 0.5-1.0, then induced with isopropyl-β-D-thiogalactopyranoside (IPTG). The temperature was then shifted to 17 °C, and the cultures were grown overnight with vigorous shaking. Expressed proteins were purified using an AKTAexpress (GE Healthcare) two-step protocol consisting of HisTrap HP affinity chromatography followed directly by HiLoad 26/60 Superdex 75 gel filtration chromatography. Typical yields for expression in MJ minimal media were 70.0 mg/L. *U*-¹⁵N and 5% ¹³C-enriched (NC5) Psb28 was expressed and purified for stereospecific isopropyl methyl assignments of all Leu and Val residues.¹² Final NC and NC5 NMR samples were concentrated to 0.58 and 0.44 mM respectively, in 95% H₂O/5% D₂O solution buffer containing 20 mM MES, 100 mM NaCl, 10 mM DTT and 5 mM CaCl₂ at pH 6.5. The monomeric state of Psb281D was confirmed by determination of $\tau_c = 8.4 \pm 0.1$ ns at 20°C from 1D ¹⁵N *T*₁/*T*₂ measurements¹³ (using peak integration between 10.5ppm-8.5ppm to minimize contributions from disordered regions of the protein) and gel-filtration chromatography with mass detection by static light scattering.

All NMR data were recorded at 20°C on Varian INOVA 600 MHz and Bruker AVANCE 850 MHz spectrometers, processed with NMRPipe¹⁴ and analyzed with Sparky.¹⁵ Proton chemical shifts were referenced to external DSS. Backbone and side-chain resonance assignments were made using conventional triple resonance experiments recorded on NC sample including 2D ¹⁵N-HSQC and ¹³C-HSQC, 3D HNCACB, CBCA(CO)NH, HNCA, HN(CO)CA, HNCB, HBHA(CO)NH, H(CC)(CO)NH-TOCSY, (H)CC(CO)NH-TOCSY, H(C)CH-TOCSY and (H)CCH-TOCSY. Backbone assignments were made following auto-assignments using AutoAssign 2.30^{16,17} from Rutgers University and the PINE server from NMRFAM (<http://pine.nmr.fam.wisc.edu/>).¹⁸ All Leu and Val methyl groups resonances were stereospecifically assigned. Final ¹H, ¹³C and ¹⁵N resonance assignments (94.2%) were deposited in the BioMagResDB (BMRB accession number 16782).

NOE data were obtained using 3D ¹⁵N-edited NOESY-HSQC ($\tau_m = 70$ ms), ¹³C-edited NOESY-HSQC ($\tau_m = 70$ ms) and 4D ¹³C-¹³C-HMQC-NOESY-HMQC ($\tau_m = 70$ ms) experiments. Initial structure calculations were performed based on manually picked ¹⁵N and ¹³C NOESY peak lists with AutoStructure 2.1.1¹⁹ and Cyana 2.1.^{20,21} NOE-based distance constraints were initially derived from NOE cross peaks automatically assigned by these two programs, followed by manual editing to eliminate some incorrect ambiguous NOE assignments, to produce a final list of NOESY distance constraints used in structure calculations. Dihedral angle constraints were computed by Talos+²² based on chemical shifts. There were 80 backbone-backbone hydrogen bond constraints automatically identified by the Autostructure¹⁹ calculation and 64 of these, in regions with secondary structure, were kept for structure calculations using XPLOR-NIH. Final structures were calculated by XPLOR-NIH-2.20 based on these constraints.²³ The final ensemble of 20

structures with lowest energy out of 150 was further refined with an improved simulated annealing protocol that uses many of the updated features of Xplor-NIH²⁴ including the IVM module for torsion angle and rigid body dynamics, a radius of gyration term to represent the weak packing potential, and database potentials of mean force to refine against Ca/Cb chemical shifts, multidimensional torsion angles, and a backbone hydrogen-bonding term, and deposited into the Protein Data Bank (PDB ID: 2KVO). Structure statistics and global structure quality factors are shown in Table I. Structure statistics and global structure quality factors were computed using PSVS version 1.4²⁵ which includes analysis by Verify3D, ProsaII, PROCHECK, and MolProbity raw and statistical Z-scores. The RPF analysis program²⁶ was used to determine the global goodness-of-fit of the final structure ensembles with the final refined NOESY peak list data.

RESULTS AND DISCUSSION

A high-quality NMR structure of Psb28 protein (120 amino acids including C-terminal 6xHis-tag) was determined [Fig. 1(A)] (Table I). The structure features seven β -strands (β 1: 3-5; β 2: 17-22; β 3: 29-36; β 4: 52-55; β 5: 60-63; β 6: 66-71; β 7: 74-83;), two short 3_{10} helices (h1:39-41; h2:46-48), one long α -helix (α 1:87-103) and nine loop regions (L1: 6-16; L2: 23-28; L3: 37-38; L4: 42-45; L5: 49-52; L6: 56-59; L7: 64-65; L8: 72-73; L9: 84-86) [Fig. 1(B)]. Three β -strands (ordered 1-4-5) form an anti-parallel β -sheet, while the other four β -strands (ordered 2-3-7-6) form a second anti-parallel β -sheet. The α 1 flanks β 2 of the second sheet, and is nearly perpendicular to the first β -sheet plane. 20 hydrophobic residues (I4, V18, L20, A30, F33, F35, M52, L54, I61, T63, V66, I78, A80, V82, W90, F94, M94, F96, M97 and Y100) from these two β -sheets and α 1 constitute the Psb28 protein hydrophobic core. Two short 3_{10} helices are found between β 3 and β 4. The secondary structure elements are locally and globally well-defined [Fig. 1(A)].

Unusual chemical shifts observed in Psb28 (Fig.S1) can be understood upon examination of the Psb28 fold. For example, one of the H^β protons of L54 is located in the ring current field perpendicular to the aromatic ring of F96 and one of the H^β protons of P16 is located in the ring current field perpendicular to the aromatic ring of F35 (Fig.S1, blue arrow) causing significant shielding of these protons: L54 H^β (-0.85 ppm) and P16 H^β (-0.22 ppm). The chemical shift for these protons was calculated using the ShiftX server (<http://redpoll.pharmacy.ualberta.ca/shiftx/>).²⁷ The most shielded H^β protons for these residues had average calculated chemical shifts of 1.12 ± 0.54 ppm (lower range of 0.49 ppm) and 1.22 ± 0.54 ppm (lower range of 0.84 ppm), respectively. The calculated chemical shifts of these protons reproduced the shielding trends experimentally observed in this structure relative to the average chemical shifts of 2.05 and 1.57 ppm, respectively, reported for these protons in the BMRB. On the other hand, both R95 H^β protons located in the plane of the aromatic ring of F96 experienced a ring current field that caused significant deshielding of these resonances, as expected (3.44 ppm and 3.32 ppm, respectively). The calculated chemical shift for the R95 H^β was 2.11 ± 0.13 with an upper range of 2.34 ppm, a deshielding trend relative to the expected of 1.79 ppm consistent with the experimental observation.

The structure with the lowest energy in pdb ensemble (Table S2) was subjected to SCREEN analysis using MarkUS²⁸, which resulted in the identification of nine solvent accessible cavities. The biggest cavity (C1) consisted of 28 residues with a surface area of $418.1 \text{ \AA}^2$, while the smallest (C9) consisted of six residues with a surface area of $35.5 \text{ \AA}^2$. Based on the Consurf analysis (Table S3), four cavities C1, C3, C4 and C9 had 7, 3, 5 and 4 highly conserved residues respectively, whereas the other five cavities had none or only one conserved residue. Cavities C4 and C9 had the highest conserved residues percentages (5/7 and 4/6, respectively), compared to C1 (7/28) and C3 (3/7). Conserved residues in C4 (I4, E12, P16, F35 and Y100) and C9 (A2, D56, R95 and F96), which are mostly found in the

loop regions L1, L2, L6, N-terminus (1-2) and the C-terminus (104-120), are shown [Fig. 1(C)]. The electrostatic surface potential of Psb28 around C4 and C9, which had average electrostatic potentials of -2.61 V and -9.64 V, respectively (Table S2), is shown in Figure 1(D). ConSurf analysis further supported the evolutionary significance of cavities C4 and C9 identifying 23 highly conserved Psb28 residues (listed in Table S3) with 4 conserved residues in L1 (between β 1 and β 2), 3 in L6 (between β 4 and β 5) and 6 in α 1, all found in C4 and C9. ConSurf plots indicated that C4 and C9 occur on the same side of the protein surface [Fig. 1(E)], whereas the other side had few highly conserved residues [Fig. 1(F)]. These analyses indicate that C4 and C9 might play an important functional role in PSII complex formation or function.

Dobakova *et al.* found Psb28 at substantially reduced levels in pulled-down complexes in mutants lacking PsbH protein, which led to their conclusion that the Psb28 binding site on CP47 was located in the vicinity of the PsbH binding site.⁶ Yao *et al.* proposed an alternative model where Psb28 may either bind directly to CP47 or in the vicinity of ScpC and ScpD, both which also bind to PSII and are located close to CP47.²⁹ Therefore, it was suggested that Psb28 might stabilize binding between CP47 and ScpC/ScpD.²⁹ It has also been reported that Psb28 can be completely washed out from membranes by 1M CaCl₂ or 0.1M Na₂CO₃, or 0.1M NaOH.⁶ Therefore, association of Psb28 with the PSII complex core may be driven by ionic interactions. Considering that C9 contains four charged residues D56, D57, R95 and R99 (Table S3), it is plausible that C9 might be a site of ionic associations to the PSII complex core, though supporting experimental evidence is not yet available. The work reported here should enable future chemical shift perturbation studies to help identify binding interactions with Psb28.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Rainbow-colored superposition of C α trace of 20 lowest energy conformers of Psb28 (1-105). The disordered C-terminal tails are not shown for clarity. (B) Rainbow-colored ribbon drawing of Psb28 (1-105) with lowest overall energy. Seven β -strands and three α -helices are labeled. N- and C- termini are labeled as "N" and "C". (C) Rainbow-colored ribbon drawing of Psb28(1-110). Eight highly conserved residues (I4 behind helix3 is not labeled) in C4 and C9 were labeled. (D) Electrostatic potential surface diagram for identical size and orientation as in (C), C4 and C9 are labeled. (E) ConSurf plot of Psb28 for identical size and orientation as in (C). ConSurf analysis was conducted for the Psb28 protein family (47 in total using PSI-BLAST), standard ConSurf residue coloring reflecting the degree of residue conservation over the entire family was used (color scheme: magentas, highly conserved; cyan, variable). Eight conserved surface residues in C4 and C9 were labeled using the single letter code for each residue. (F) ConSurf plot of (E) rotated by 180° about the vertical axis.

Table 1

NMR Structure Statistics for Photosystem II Reaction Center Psb28 Protein from *Synechocystis* sp. strain PCC 6803^a

Conformationally-restricting constraints ^b	
Distance constraints	
Intra-residue (i=j)	395
Sequential (i-j =1)	399
Medium-range (1< i-j <5)	174
Long-range (i-j ≥5)	487
Total	1455
Distance constraints per residue	12.5
Hydrogen bond constraints	
Long-range (i-j ≥5)/total	40/64
Dihedral angle constraints	92
Total number of restricting constraints	1611
Number of constraints per residue (long range / total)	4.5/13.9
Residue constraint violations ^b	
Average number of distance violations per structure	
0.1-0.2 Å	6.30
0.2-0.5 Å	1.4
>0.5 Å	0
Average RMS distance violation/constraint (Å)	0.01
Maximum distance violation (Å)	0.36
Average number of dihedral angle violations per structure	
1-10°	0.2
>10°	0
Average RMS dihedral angle violation/constraint (degree)	0.09
Maximum dihedral angle violation (degree)	1.70
RMSD from average coordinates (Å) ^{b,c}	
Backbone /Heavy atoms	0.6/1.1
Ramachandran plot statistics ^{b,c}	
Most favored regions (%)	96
Additional allowed regions (%)	3.5
Generously allowed (%)	0.5
Disallowed regions (%)	0
Global quality scores (raw/Z-score) ^b	
Verify3D	0.35/-1.77
ProsaII	0.35/-1.24
Procheck (phi-psi) ^c	-0.08/0.00
Procheck (all) ^c	0.01/0.06

Molprobability clash	23.81/-2.56
RPF Scores ^d	
Recall	0.997
Precision	0.904
F-measure	0.948
DP-score	0.785

^a Structure statistics were computed for the ensemble of 20 deposited structures.

^b Calculated using PSVS version 1.4 program. Average distance violations were calculated using the sum over r^{-6} . Residues 1-120 were analyzed.

^c Ordered residues ranges (with sum of phi and psi > 1.8): 3-6, 16-22, 29-37, 40-48, 52-55, 60-64, 66-71, 75-103.

^d RPF scores reflected the goodness-of-fit of the final ensemble of structures including disordered residues to the final NOESY peak list and resonance assignment data.