BIOCHEMISTRY
including biophysical chemistry & molecular biology
Rapid Report

Biochemistry **2009,** *48,* 8771–8773 8771
DOI: 10.1021/bi901309c

Solution Structure of Psb27 from Cyanobacterial Photosystem II[†,‡]

Peter D. Mabbitt,[§] Gilles J. P. Rautureau,[∥] Catherine L. Day,[§] Sigurd M. Wilbanks,[§] Julian J. Eaton-Rye,*[,§] and Mark G. Hinds*[,∥]

[§]*Department of Biochemistry, University of Otago, Dunedin 9054, New Zealand, and* [∥]*Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia*

Received July 29, 2009; Revised Manuscript Received August 19, 2009

ABSTRACT: Psb27 is a highly conserved component of photosystem II. The three-dimensional structure has a well-defined helical core, composed of four helices arranged in a right-handed up−down−up−down fold, with a less ordered region of the structure located at the N-terminus. The position of conserved residues on the surface suggests conserved functional roles for distinct interconnected features encompassing a P−Φ−P loop, a polar patch spanning helices α3 and α4, and the N-terminal sequence.

Photosystem II (PSII)[1] of plants, algae, and cyanobacteria catalyzes the light-driven splitting of water and reduction of plastoquinone as the initial step of photosynthesis. High-resolution X-ray crystallographic structures have been obtained for this membrane−protein complex from the thermophilic cyanobacterium *Thermosynechococcus elongatus* (*1*, *2*). Twenty protein subunits have been assigned in the electron density maps; however, PSII from the mesophilic cyanobacterium *Synechocystis* sp. PCC 6803 contains additional hydrophilic proteins in the thylakoid lumen that are not present in the X-ray-derived structures (*3*). Two of these exhibit similarity to the plant PsbP and PsbQ subunits associated with the lumenal face of PSII (*4*, *5*). However, whereas cyanobacterial PsbQ is important for the stability of the complex and required for maximum activity (*6*, *7*), PsbP is present in substoichiometric amounts and its precise function remains to be clarified (*8*, *9*).

A third additional subunit is Psb27, an ∼11 kDa polypeptide that is targeted to the thylakoid lumen in both cyanobacteria (*10*) and *Arabidopsis thaliana* (*11*) by an N-terminal sequence. Δ*psb27* cells of *Synechocystis* sp. PCC 6803 are photoautotrophic under optimal laboratory conditions and exhibit a doubling time similar to that of the wild type, but growth following exposure to high light is impaired under nutrient-limiting conditions or in a strain lacking PsbT (*12*, *13*). In addition, *A. thaliana* plants lacking Psb27 recover more slowly than the wild type after exposure to photoinhibitory light (*11*).

In *Thermosynechococcus elongatus*, Psb27 possesses a post-translational lipid modification at an N-terminally located lipobox motif which is conserved in cyanobacteria but absent in *A. thaliana* (*10*). Isolation of PSII complexes from *T. elongatus* yielded a distinct population of monomeric PSII that had bound Psb27 lipoprotein but lacked the PsbO, PsbU, and PsbV proteins associated with the active enzyme. The Psb27-containing complexes do not contain the Mn_4Ca center of the water-splitting active site (*10*, *14*) and accumulate newly synthesized D1 reaction center protein, suggesting Psb27-containing PSII is an intermediate complex in PSII biogenesis (*10*). In addition, assembly of the Mn_4Ca complex by photoactivation is impaired in Δ*psb27* cells from *Synechocystis* sp. PCC 6803 (*12*).

An inherent susceptibility of PSII to light-induced damage is counteracted by a repair cycle that continually replaces photodamaged protein (reviewed in ref *15*). The available data suggest that Psb27 has a role in this photosystem repair cycle, although when and where Psb27 binds are not known. The structural details presented in this report provide essential information for the elucidation of the function of Psb27 in PSII biogenesis and repair.

Psb27 from *Synechocystis* sp. PCC 6803 [residues 1−110 of the processed protein (Figure 1)] was overexpressed in *Escherichia coli* as a fusion protein and purified as described in the Supporting Information. Analysis of the purified protein by multiple-angle light scattering and circular dichroism (CD) spectroscopy showed that Psb27 is monomeric and helical (Figures S1−S3 of the Supporting Information). To investigate if the pH change in its translocation from the cytosol to the lumen affected the conformation of Psb27, samples were analyzed by CD spectroscopy over a range of pH values from 3.5 to 9.0. No change in secondary structure was detected, and therefore, the pH of 6.7 was selected for NMR analysis. At this pH, the $^{1}H-^{15}N$ HSQC spectrum of 0.5 mM Psb27 gave a set of well-dispersed resonances that indicated the protein was folded. Sequential resonance assignment and structural constraints were determined from a series of heteronuclear three-dimensional NMR experiments on uniformly ^{15}N-labeled protein or ^{13}C- and ^{15}N-labeled protein. Initial structures were calculated using CYANA and refined using XPLOR-NIH. The final structures were calculated using a total of 2802 experimentally derived distance constraints, 168 dihedral angle constraints (ϕ, 84; ψ, 84), and 57 hydrogen bonds. The final set of 20 structures had no experimental distance violations of > 0.30 Å or dihedral angle violations of > 5.0°, and 99.3% of the residues occurred in the allowed regions of the Ramachandran plot (Table S1 of the Supporting Information; Protein Data Bank entry 2KMF).

Psb27 consists of a single domain with an all α-helical fold (Figures 1 and 2). The core of the protein is formed by four amphipathic α-helices (α1, residues 11−26; α2, 35−52; α3, 63−82; α4, 89−108) arranged in an up−down−up−down

[†]This work was supported by Marsden Grant 08-UOO-043 to J.J.E.-R. P.D.M. was supported by an Otago University postgraduate scholarship.

[‡]Atomic coordinates have been deposited in the Protein Data Bank as entry 2KMF.

*To whom correspondence should be addressed. J.J.E.-R.: telephone, +64 3 479 7865; fax, +64 3 479 7866; e-mail, julian.eaton-rye@otago.ac.nz. M.G.H.: telephone, +61 3 9345 2537; fax, +61 3 9347 0852; e-mail, mhinds@wehi.edu.au.

[1]Abbreviations: CD, circular dichroism; PCC, Pasteur Culture Collection; PSII, photosystem II.

Published on Web 08/21/2009

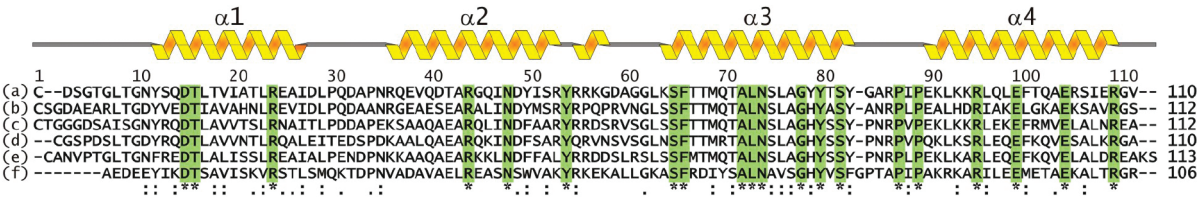

FIGURE 1: Alignment of representative Psb27 sequences from cyanobacteria with that of *A. thaliana*. The signal sequence is not shown. The α-helices of Psb27 from *Synechocystis* sp. PCC 6803 are depicted. Invariant residues are highlighted in green. Sequence conservation: asterisks, conserved; two dots, conservative substitutions; and one dot, limited substitutions. Psb27 sequences (accession codes in brackets): (a) *Synechocystis* sp. PCC 6803 (P74367), (b) *Synechococcus* sp. WH8102 (Q7U5D5), (c) *Synechococus elongatus* PCC 7942 (Q31RE4), (d) *Anabaena variabilis* PCC 7937 (Q3MFN4), (e) *T. elongatus* BP1 (Q8DG60), and (f) *A. thaliana* (Q9LR64), aligned using Clustal-W.

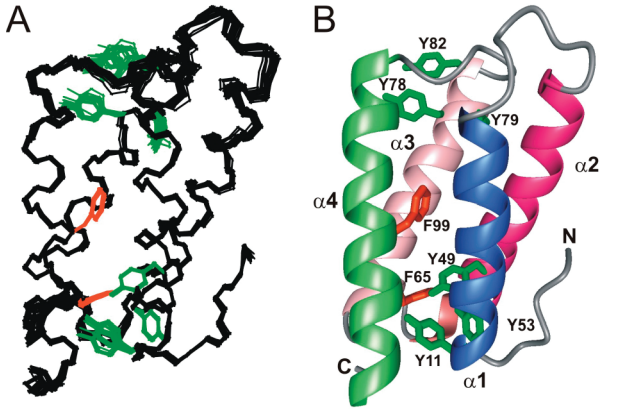

FIGURE 2: Solution structure of Psb27. (A) Backbone superposition (C^{α}, N, and C′) over the residues of regular secondary structure of the family of 20 NMR-derived structures representative of the Psb27 solution structure. (B) Ribbon diagram of the structure closest to the mean. The side chains of clustered aromatic residues are shown (Tyr in green and Phe in orange). Residues 5−110 are depicted and the helices labeled as in Figure 1.

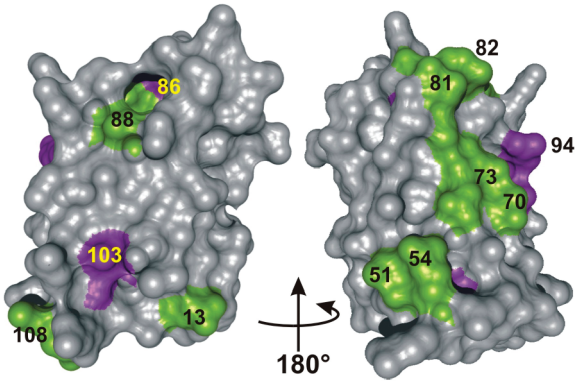

FIGURE 3: Molecular surface of Psb27. Residues conserved between cyanobacteria and plants are colored. Invariant surface-exposed residues are colored purple [invariant residues, T15, Y53, S64, F65, Y79, P86, R94 E98, and E103 (see Figure S4 for the alignment)], and highly conserved exposed residues are colored green (highly conserved residues, Y11, Q13, D14, V18, L22, I26, Y49, S51, R54, M68, T70, A71, L72, N73, S74, L75, G77, Y78, S81, Y82, I87, P88, K92, F99, A102, and R108). Only those residues with ≥25% accessible surface area are labeled. The left view is the same as Figure 2, and the right view is rotated 180° with respect to the vertical axis.

right-handed antiparallel topology. The hydrophobic core is composed of the inward-facing hydrophobic side chains of the amphipathic helices. Short loops connect the helices with α1 and α2 connected by a pair of linked *β*-turns (Asp29−Ala32 and Ala32−Arg35), and helices α2 and α3 joined by a more extended loop, which includes a turn of α-helix (Tyr53−Lys56) and a bend (Ala59−Gly61). The conserved P−Φ−P motif, where Φ is I, L, or V (Figures 1 and S4 of the Supporting Information), resides in the seven-residue α3−α4 loop. Only the four N-terminal residues of Psb27 together with the five vector-derived residues are disordered, as measured by their low backbone angular order parameters. Residues 5−11 are well-ordered and pack between helices α1 and α2, with hydrogen bonds from the completely conserved carboxylate of Asp14 to the amides of Thr8 and Gly9 appearing to stabilize this position. In addition, Asp14, Gln45, Asp48, and Arg52 form a cavity occupied by conserved Leu7, and this interaction also anchors residues 5−11 in this extended position.

The interfaces between the four helices are composed of well-conserved hydrophobic residues (Figure 2). A prominent and conserved aromatic cluster formed by Tyr11, Tyr49, Tyr53, and Phe65 is located at the N-terminal end of the bundle (at the amino terminus of α1 and the C-terminus of α4). At the opposite end of the bundle, a polar patch (formed by conserved residues Thr67, Thr70, Tyr78, Tyr82, and Lys92, adjacent to invariant charged residues Arg94 and Glu98) spreads along the surfaces of helices α3 and α4 from the P−Φ−P motif (Figure 3). This motif was identified as a potential site of protein interactions (see the Supporting Information). Preceding this motif in all cyanobacterial Psb27 sequences is the surface-exposed Arg85, and immediately following it is a well-conserved exposed region (Glu-Lys-Leu in cyanobacteria and Ala-Lys-Arg in plants). Strikingly, the most highly conserved regions of the hydrophobic core of Psb27 are those residues directly beneath the P−Φ−P loop.

Comparisons of the sequence of *Synechocystis* sp. PCC 6803 Psb27 with those of other proteins in a BLAST search did not reveal any significant sequence identity with proteins of known three-dimensional structure, suggesting that Psb27 represents a novel protein family. However, a search of structural databases using secondary structure matching showed that Psb27 shares its right-handed four-helical topology with cytochrome *c*′, but there is little discernible sequence conservation (10.3% identical and 18.3% similar). An alignment of the primary sequences across a family of representative plant and cyanobacterial Psb27 proteins revealed the presence of highly conserved residues between the plant and cyanobacterial sequences, some of which are invariant (Figures 1, 3, and S4 of the Supporting Information). This suggests that many of the structural features observed in Psb27 from cyanobacteria are present in the plant protein. For example, the majority of surface-exposed conserved residues reside on the α3−α4 face of Psb27, while the opposite face presented by α1 and α2 has fewer exposed conserved residues (Figure 3). The α3 residues present a contiguous surface, and interestingly, the conserved patch on α3 is encircled by charged residues (Figures 3 and S5 of the Supporting Information). These features are suggestive of a common interaction site for Psb27 on PSII in the plant and cyanobacterial photosystems. However, in the absence of biochemical constraints and without a structure of the

PSII monomer, docking simulations failed to locate a unique binding site for Psb27 (see the Supporting Information).

Psb27 is targeted to the thylakoid membrane by an N-terminal signal that is removed by proteolysis, and the resulting 110-residue protein is post-translationally modified by covalent attachment of lipid at the N-terminus (*10*). The role of this lipid modification has not been fully elucidated, but a likely function is to colocalize Psb27 with other PSII components in the membrane. While the structure presented here shows that Psb27 is a tightly packed four-helix bundle with a hydrophobic core, the N-terminal residues are disordered and could form a flexible linker connecting the globular protein through the lipid moiety to the membrane without significantly constraining its orientation. The presentation of a cluster of residues, many of which are conserved between plants and cyanobacteria, on the surface of Psb27 suggests potential functional roles in protein−protein interactions. The conservation of the P−Φ−P motif, the polar patch, and the buried residues connecting them suggests communication between these regions is important for function.

Psb27 is necessary for efficient repair of PSII after photodamage, and its presence may therefore confer a significant advantage to cells under adverse environmental conditions such as high light and low temperature. The solution structure presented here identifies three interconnected and highly conserved features. The P−Φ−P loop, the polar patch on α3 and α4, and the poorly defined N-terminus represent initial targets for functional studies directed at elucidating the mechanistic role of Psb27 in turnover and repair of PSII.

ACKNOWLEDGMENT

NMR data were acquired at the Bio21 Institute NMR facility, the University of Melbourne (Melbourne, Australia).

SUPPORTING INFORMATION AVAILABLE

Supplementary figures, detailed experimental procedures, NMR statistical data, and prediction of interaction sites on PSII. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Ferreira, K. N., Iverson, T. M., Maghlaoui, K., Barber, J., and Iwata, S. (2004) *Science 303*, 1831–1838.
2. Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A., and Saenger, W. (2009) *Nat. Struct. Mol. Biol. 16*, 334–342.
3. Roose, J. L., Wegener, K. M., and Pakrasi, H. B. (2007) *Photosynth. Res. 92*, 369–387.
4. Kashino, Y., Lauber, W. M., Carroll, J. A., Wang, Q., Whitmarsh, J., Satoh, K., and Pakrasi, H. B. (2002) *Biochemistry 41*, 8004–8012.
5. Thornton, L. E., Ohkawa, H., Roose, J. L., Kashino, Y., Keren, N., and Pakrasi, H. B. (2004) *Plant Cell 16*, 2164–2175.
6. Summerfield, T. C., Shand, J. A., Bentley, F. K., and Eaton-Rye, J. J. (2005) *Biochemistry 44*, 805–815.
7. Roose, J. L., Kashino, Y., and Pakrasi, H. B. (2007) *Proc. Natl. Acad. Sci. U.S.A. 194*, 2548–2553.
8. Summerfield, T. C., Winter, R. T., and Eaton-Rye, J. J. (2005) *Photosynth. Res. 84*, 263–268.
9. Sveshnikov, D., Funk, C., and Schröder, W. P. (2007) *Photosynth. Res. 93*, 101–109.
10. Nowaczyk, M. M., Hebeler, R., Schlodder, E., Meyer, H. E., Warscheid, B., and Rögner, M. (2006) *Plant Cell 18*, 3121–3131.
11. Chen, H., Zhang, D., Guo, J., Wu, H., Jin, M., Lu, Q., Lu, C., and Zhang, L. (2006) *Plant Mol. Biol. 61*, 567–575.
12. Roose, J. L., and Pakrasi, H. B. (2008) *J. Biol. Chem. 283*, 4044–4050.
13. Bentley, F. K., Luo, H., Dilbeck, P., Burnap, R. L., and Eaton-Rye, J. J. (2008) *Biochemistry 47*, 11637–11646.
14. Mamedov, F., Nowaczyk, M. M., Thapper, A., Rögner, M., and Styring, S. (2007) *Biochemistry 46*, 5542–5551.
15. Nixon, P. J., Barker, M., Boehm, M., de Vries, R., and Komenda, J. (2005) *J. Exp. Bot. 56*, 357–363.