Biomol NMR Assign (2009) 3:247–249
DOI 10.1007/s12104-009-9186-0

ARTICLE

Sequence-specific ^{1}H, ^{13}C, and ^{15}N backbone assignment of Psb27 from *Synechocystis* PCC 6803

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Received: 9 July 2009 / Accepted: 5 September 2009 / Published online: 30 September 2009

Abstract Photosystem II (PSII) is a large membrane protein complex that uses light to split water into molecular oxygen, protons, and electrons. Here we report the ^{1}H, ^{15}N and ^{13}C backbone chemical shift assignments for the Psb27 protein of Photosystem II from *Synechocystis* PCC 6803. These assignments will now provide the basis for the structural analysis of the Psb27 protein.

Keywords NMR resonance assignment · Photosystem II · Psb27 · *Synechocystis* PCC 6803

Biological context

Photosystem II (PSII) catalyzes the first step of the photosynthetic light reaction of cyanobacteria, algae, and vascular plants: the light induced oxidation of water, which is accompanied by the release of oxygen as a side product. All animal life depends on this important reaction because it sustains the oxygenic atmosphere of the planet. However, both light and the presence of oxygen inherently cause a constant damage of the PSII complex. Especially under high light conditions, PSII is susceptible to oxidative damage. One strategy of the cell to avoid persistent PSII inactivation is the continuous exchange of damaged proteins—especially of the D1 core subunit. This unique repair cycle (Mulo et al. 2008) includes (1) at least partial disassembly of the complex and replacement of damaged D1, (2) integration of newly synthesized D1 with a C-terminal extension (pD1), (3) N-terminal processing of pD1 by the CtpA protease, (4) assembly of the oxygen evolving complex and (5) binding of the extrinsic proteins on the lumenal side of PSII. Whereas detailed structural information is available for the assembled and fully active PSII complex (Guskov et al. 2009), only little is known about the transient complexes involved in the repair cycle. One of these complexes contains the transiently bound subunit Psb27 (Ikeuchi et al. 1995; Kashino et al. 2002) which was shown to prevent the binding of the extrinsic proteins (Nowaczyk et al. 2006; Mamedov et al. 2007) in order to facilitate the assembly of the manganese cluster (Roose and Pakrasi 2008). In cyanobacteria, Psb27 exhibits a specific lipid modification (Nowaczyk et al. 2006) which seems to support the binding to the complex. Here we report the assignment of the ^{1}H, ^{15}N and ^{13}C backbone resonances for the mature Psb27 subunit of *Synechocystis* PCC 6803 which was heterologously overexpressed in *E. coli*.

This paper is dedicated to Prof. Dr. Achim Trebst on the occasion of his 80th birthday.

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Methods and experiments

Protein expression and purification

The sequence coding for the mature Psb27 protein comprising 110 amino acids without the N-terminal signal

sequence from *Synechocystis* PCC 6803 was amplified by PCR with primers, 5′-GGAATTCCATATGTGCGACAGC GGCACAGGA-3′ and 5′-CGGGATCCCCAAGAAATTC CAG-3′, digested with *Nde*I and *Bam*HI and cloned into the plasmid pET3xb (Novagen).

For protein expression, *E. coli* BL21(DE3)pLysS cells (Novagen) were transformed with the pET3xb-psb27 plasmid and plated onto LB media supplemented with 50 μg/ml chloramphenicol and 120 μg/ml ampicillin. After 14 h of growth at 37°C, precultures of 50 ml LB were inoculated with single colonies and incubated for approximately 8 h at 37°C. After centrifugation (10 min, 10,000 rpm, 4°C) the cells were resuspended in M9 media and used for inoculation of 1 l M9 media enriched with ^{15}N ammonium chloride and ^{13}C glucose. Cells were grown at 37°C, agitated at 220 rpm to an OD_{600} of approximately 0.6 and protein expression was induced by the addition of 0.5 mM isopropyl 1-thio-β-D-galactopyranoside (IPTG). The cells were harvested after 5 h of incubation at 37°C and stored at −20°C. Before disruption by French press at 1,000 psi, the cells were resuspended in 20 mM MES pH 6.5, 10 mM EDTA, 1 mM PMSF. Cell debris was removed by centrifugation (20 min, 13,000 rpm, 4°C, SS34) and the supernatant was diluted to a protein concentration of approximately 5 mg/ml. After addition of ammonium sulfate up to 50% saturation and centrifugation (20 min, 13,000 rpm, 4°C, SS34), the ammonium sulfate concentration in the supernatant was increased to 80% saturation, followed by centrifugation (20 min, 13,000 rpm, 4°C, SS34). The precipitated proteins were resuspended in 10 ml 20 mM Tris pH 8.0 and dialyzed twice against 1 l 20 mM Tris pH 8.0. The prepurified extract was loaded on a Resource S column (1 ml, GE Healthcare), which was previously equilibrated with 20 mM Tris pH 8.0 and connected to a FPLC system (Äktapurifier 100, GE Healthcare). After a short wash step, the Psb27 protein was eluted by a linear gradient of 0–250 mM NaCl and fractions containing the Psb27 protein were pooled and dialyzed twice against 1 l 20 mM phosphate buffer adjusted to a pH of 6.5. Psb27 was then concentrated by ultrafiltration (cut off 5 kDa) to a final concentration of approximately 1 mM. Psb27 was shown to be monomeric in solution as judged by blue native PAGE analysis (data not shown).

NMR spectroscopy

Uniformly labelled ^{15}N and ^{13}C samples of 1 mM were prepared in 20 mM phosphate buffer adjusted to a pH of

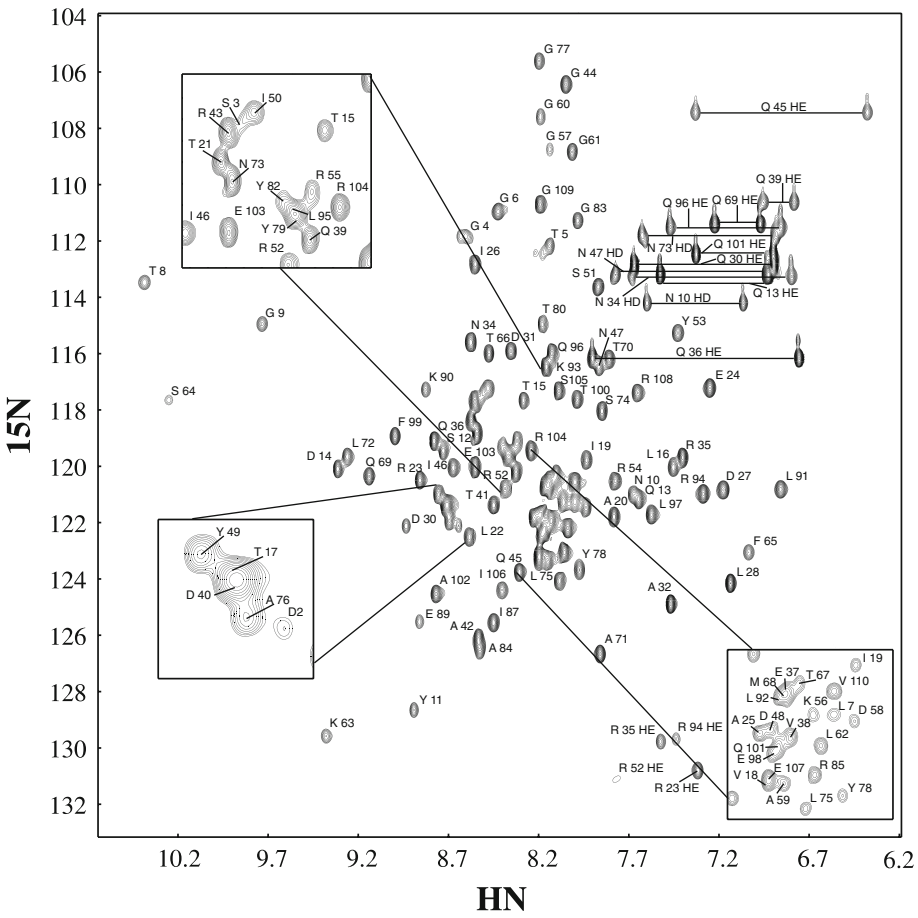

Fig. 1 ^{1}H–^{15}N HSQC spectrum of rPsb27 at 298 K and 600 MHz. Sequence specific assignment of backbone ^{1}H and ^{15}N frequencies of mature Psb27 is indicated. Signals connected by *horizontal lines* correspond to side chain amide groups of asparagines and glutamine residues

6.5 supplemented with 10% (v/v) D_2O. All spectra were recorded at 298 K on a Bruker DRX600 spectrometer equipped with a z-axis gradient unit and a triple resonance probehead. Backbone assignments were obtained from three dimensional HNCA, CBCA(CO)NH, and HNCO. Side chain assignments were obtained from three dimensional HCCH-TOCSY, HCCH-COSY, HNHA, HNHB, HBHA(CO)NH, ^{15}N-NOESY-HSQC, and ^{13}C-NOESY-HSQC (Marley et al. 2001). All spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed with NMRView (Johnson and Blevins 1994) as previously published (Stoll et al. 2001).

Assignments and data deposition

The ^{1}H–^{15}N HSQC of recombinant Psb27 (rPsb27) is shown in Fig. 1. The assignment of the backbone resonances of Psb27 from *Synechocystis* PCC 6803 has been completed to 100%. Overall, 75% of all detectable backbone and side chain frequencies have been assigned. In detail, we have obtained a degree of assignment of 86% for all atoms and 91% for all protons of amino acids which are part of the hydrophobic core stabilizing the Psb27 fold, i.e. I, L, V, M, and A. The assignment of side chains that are solvent exposed is lower which can be attributed to chemical exchange and spectral overlap. This holds true for solvent exposed amino acids such as K and R residues, for example. In addition, preliminary analysis of the secondary structure revealed that the extent of assignment in helical regions is higher than in the (flexible) loop regions. A list of ^{1}H, ^{13}C and ^{15}N chemical shift assignments has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-16398. These assignments now provide a basis for the detailed investigation of Psb27 from *Synechocystis* PCC 6803.

Acknowledgements We thank the German Research Council (Sonderforschungsbereich 480, project C1 to M.R. and Sonderforschungsbereich 642, project A6 to R.S.), SolarH2 and the Protein Research Department of the Ruhr-University Bochum for infrastructural and financial support.

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