Biochimica et Biophysica Acta 1837 (2014) 1435–1446

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

# The importance of the hydrophilic region of PsbL for the plastoquinone electron acceptor complex of Photosystem II☆

Hao Luo [a,1], Simon A. Jackson [a], Robert D. Fagerlund [a,2], Tina C. Summerfield [b], Julian J. Eaton-Rye [a,*]

[a] Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
[b] Department of Botany, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

ARTICLE INFO

*Article history:*
Received 7 January 2014
Received in revised form 12 February 2014
Accepted 15 February 2014
Available online 24 February 2014

*Keywords:*
Electron transfer
Herbicide binding
Photosystem II
PsbL
$Q_B$
*Synechocystis* sp. PCC 6803

ABSTRACT

The PsbL protein is a 4.5 kDa subunit at the monomer–monomer interface of Photosystem II (PS II) consisting of a single membrane-spanning domain and a hydrophilic stretch of ~15 residues facing the cytosolic (or stromal) side of the photosystem. Deletion of conserved residues in the N-terminal region has been used to investigate the importance of this hydrophilic extension. Using *Synechocystis* sp. PCC 6803, three deletion strains: Δ(N6–N8), Δ(P11–V12) and Δ(E13–N15), have been created. The Δ(N6–N8) and Δ(P11–V12) strains remained photoautotrophic but were more susceptible to photodamage than the wild type; however, the Δ(E13–N15) cells had the most severe phenotype. The Δ(E13–N15) mutant showed decreased photoautotrophic growth, a reduced number of PS II centers, impaired oxygen evolution in the presence of PS II-specific electron acceptors, and was highly susceptible to photodamage. The decay kinetics of chlorophyll *a* variable fluorescence after a single turnover saturating flash and the sensitivity to low concentrations of PS II-directed herbicides in the Δ(E13–N15) strain indicate that the binding of plastoquinone to the $Q_B$-binding site had been altered such that the affinity of $Q_B$ is reduced. In addition, the PS II-specific electron acceptor 2,5-dimethyl-*p*-benzoquinone was found to inhibit electron transfer through the quinone-acceptor complex of the Δ(E13–N15) strain. The PsbL Y20A mutant was also investigated and it exhibited increased susceptibility to photodamage and increased herbicide sensitivity. Our data suggest that the N-terminal hydrophilic region of PsbL influences forward electron transfer from $Q_A$ through indirect interactions with the D–E loop of the D1 reaction center protein. Our results further indicate that disruption of interactions between the N-terminal region of PsbL and other PS II subunits or lipids destabilizes PS II dimer formation. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.



## 1. Introduction

Photosystem II (PS II) is a multi-subunit complex embedded in the thylakoid membrane of cyanobacteria, algae and plants where it catalyzes the oxidation of water and photoreduction of plastoquinone [1,2]. The structure of PS II from the thermophilic cyanobacteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* has been obtained by X-ray crystallography [3–5]. These studies have shown PS II to be dimeric, with each monomer containing the chlorophyll *a*-binding core antenna proteins CP43 and CP47 together with the D1/D2 reaction center subunits and at least 16 additional polypeptides and 70 cofactors [3–6]. The complexity of PS II is in contrast to the purple bacterial reaction center, which contains 4 subunits and 14 cofactors [7], despite structural and functional similarities in the core reaction centers of these related photosystems [8–10].

The structural complexity of the PS II supercomplex is in part attributed to the presence of low-molecular-weight (LMW) transmembrane polypeptides [11–13]. Thirteen LMW subunits have been identified on the periphery of each PS II monomer [3–6]. Gene inactivation studies suggest that the absence of these subunits frequently results in mutants

*Abbreviations:* BN-PAGE, Blue-native polyacrylamide gel electrophoresis; DCMU, 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; DM, n-Dodecyl β-D-maltoside; DCBQ, 2,6-Dichlorobenzoquinone; DCPIP, 2,6-Dichlorophenolindophenol; DMBQ, 2,5-Dimethyl-*p*-benzoquinone; HEPES, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid; $I_{50}$, Concentration of inhibitor to reduce activity by 50%; Kb, Kilobase; $K_D$, Apparent dissociation constant; kDa, Kilodalton; OD, Optical density; OEC, Oxygen-evolving complex; PCC, Pasteur Culture Collection; PCR, Polymerase chain reaction; PQ, Plastoquinone; PS II, Photosystem II; $Q_A$, Primary quinone electron acceptor of Photosystem II; $Q_B$, Secondary quinone electron acceptor of Photosystem II; S states, Oxidation states of the manganese–calcium cluster of the oxygen-evolving complex of Photosystem II; TES, 2-[Tris(hydroxymethyl)methyl]amino-1-ethanesulfonic acid; Tris, Tris(hydroxymethyl) aminomethane

☆ This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

* Corresponding author. Tel.: +64 3 479 7865; fax: +64 3 479 7866.
*E-mail address:* julian.eaton-rye@otago.ac.nz (J.J. Eaton-Rye).
[1] Current address: Novo Nordisk Foundation for Biosustainability, Technical University of Denmark, Kogle Alle 4, Hørsholm 2970, Denmark.
[2] Current address: Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA.

http://dx.doi.org/10.1016/j.bbabio.2014.02.015
0005-2728/

with impaired photoautotrophic growth or PS II assembly [14–17]. In addition, although LMW subunits do not appear to directly participate in linear photosynthetic electron transport, several subunits have been shown to regulate electron flow within PS II [18–21].

The PsbL protein is one of three LMW polypeptides (the others are PsbM and PsbT) located at the monomer–monomer interface of the cyanobacterial PS II crystal structure [3–6]. This highly conserved ~4.5 kDa subunit consists of a single transmembrane helix and a cytosol-exposed N-terminal region. Inactivation of *psbL* in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803) severely retarded photoautotrophic growth [21,22].

It has been suggested that the C-terminus of PsbL is required for the oxidation of the redox active tyrosine $Y_Z$ on the D1 protein by the oxidized primary donor of PS II, $P680^+$, as determined by electron paramagnetic resonance spectroscopy using PsbL-reconstituted PS II complexes from spinach [23]. However, in the cyanobacterial PS II structure PsbL is not in the vicinity of $Y_Z$ or the adjacent oxygen-evolving complex (OEC). We investigated the role of the PsbL C-terminus in *Synechocystis* 6803 and found that the last four C-terminal residues were required for attachment of PsbL to the CP43-less PS II assembly intermediate complex. The failure of PsbL to dock to the CP43-less PS II complex in our mutant blocked the assembly of functional oxygen-evolving photosystems [24]. We also demonstrated that mutations targeting other conserved residues of the PsbL transmembrane domain resulted in increased susceptibility to photodamage.

Among the LMW subunits of PS II, PsbL is unique in having its N-terminal region exposed to the cytosolic side of the membrane. To extend our understanding of the role of PsbL in PS II, this hydrophilic stretch of residues has been investigated by the introduction of short deletions using site-directed mutagenesis in *Synechocystis* 6803.

## 2. Materials and methods

### 2.1. Synechocystis 6803 growth conditions

The glucose tolerant strain of *Synechocystis* 6803 [25] was grown in BG-11 media under constant illumination at ~30 μmol photons $m^{-2}$ $s^{-1}$. Cultures were maintained on solid BG-11 plates in the presence of 5 mM glucose, 20 μM atrazine, 10 mM TES-NaOH (pH 8.2), 0.3% sodium thiosulfate and appropriate antibiotics [26]. The liquid cultures were grown mixotrophically in the presence of 5 mM glucose and appropriate antibiotics. In both solid and liquid media, the antibiotics used were chloramphenicol at 15 μg $mL^{-1}$ and kanamycin at 25 μg $mL^{-1}$.

### 2.2. Generation of the PsbL mutant strains

The mutant strains were created using an oligonucleotide-directed PsbL mutagenesis system [24]. The Δ(N6–N8), Δ(P11–V12) and Δ(E13–N15) strains were made using oligonucleotides: 5′-ATGG ACAGAAATTCA//CGCCAACCGGTGGAA-3′, 5′-AACCCAAACCGCCAA// GAATTGAACCGCACT-3′ and 5′-AACCGCCAACCGGTG//CGCACTTCTTTA TAC-3′, respectively, where the two backslashes indicate the position of the deletion.

### 2.3. Measurement of photosynthetic activity

Photosynthetic activity was measured either by oxygen evolution in whole cells or by monitoring the reduction of 2,6-dichlorophenolindophenol (DCPIP) in isolated thylakoid membranes. Oxygen evolution in whole cells was performed according to [24]. For whole chain oxygen evolution measurements 15 mM sodium bicarbonate was used.

Isolation of thylakoid membranes from *Synechocystis* 6803 cells was performed according to [24]. Thylakoid membranes corresponding to 10 μg $mL^{-1}$ of chlorophyll *a* were resuspended in the assay buffer (50 mM Tricine–NaOH (pH 7.5), 600 mM sucrose, 30 mM $CaCl_2$ and 1 M betaine) containing 100 μM DCPIP. The light-induced reduction of DCPIP was monitored spectrophotometrically at 590 nm using a Cary 118 UV–vis spectrophotometer (Varian, Palo Alto, CA, USA) and the sample was illuminated at room temperature with red light provided by a FLS2 light source (Hansatech, King's Lynn, UK) passed through a Melles Griot RG 665 optical filter. The photomultiplier was protected with a Melles Griot BG 38 filter.

### 2.4. PS II assembly

The relative level of assembled PS II reaction centers was estimated on a chlorophyll basis by employing a [$^{14}$C]-atrazine herbicide-binding assay [27,28]. Assembly of the PS II native complexes was visualized using blue native-polyacrylamide gel electrophoresis (BN-PAGE) followed by western blot analysis as described in [24]. The estimation of the level of PsbL expressed in the specific mutants was also determined with a PsbL-specific antibody as described in [24].

### 2.5. Variable chlorophyll a fluorescence measurements

Cells in liquid culture were harvested at an $OD_{730\ nm}$ of 1.0–1.2 (Jasco V-550 UV/vis spectrophotometer; Jasco International) by centrifugation at 2760 *g* for 10 min at room temperature and washed twice in buffered BG-11 containing 25 mM HEPES–NaOH (pH 7.5). Cells were resuspended at a chlorophyll *a* concentration of 10 μg $mL^{-1}$ and incubated for 60 min under the standard growth conditions described above with constant shaking at 120 rpm. Prior to each measurement, cells were diluted 1:3 with fresh BG-11 (pH 7.5) media and incubated in the dark for 4 min at room temperature.

Variable chlorophyll *a* fluorescence measurements were performed using a double modulation fluorometer (PS I instruments, Brno, Czech Republic). Chlorophyll *a* fluorescence induction was measured over a period of 5 s under a continuous blue actinic light (455 nm) at 2800 μmol photons $m^{-2}$ $s^{-1}$. Decay of the variable chlorophyll *a* fluorescence was measured in the 50 μs to 60 s time range after a 30 μs saturating single turnover flash (455 nm). Component analysis of the fluorescence relaxation kinetics attributed to forward and back electron transfer was performed according to [29]. When added, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or diuron was at a final concentration of 40 μM and 2,5-dimethyl-*p*-benzoquinone (DMBQ) was at 200 μM.

### 2.6. Photodamage and recovery measurements

To assess the sensitivity of different mutants to photodamage, cells at 10 μg $mL^{-1}$ were subjected to high intensity white light at 2000 μmol photons $m^{-2}$ $s^{-1}$ provided by a Kodak Ektalite 1000 slide projector for up to 45 min, followed by recovery under 30 μmol photons $m^{-2}$ $s^{-1}$ light provided by 250 W metal halide lamps [24]. When utilized, kanamycin (50 μg $mL^{-1}$) was added 10 min prior to starting the assay to prevent protein synthesis.

## 3. Results

### 3.1. Conserved residues in the N-terminus of PsbL

The PsbL N-termini of 34 cyanobacterial strains varied in length from 13 to 17 amino acids; the shortest were from *Gloeobacter violaceus* and *T. elongatus* and the longest from *Trichodesmium erythraeum* (Supplementary Fig. S1). In the majority of the cyanobacteria, including *Synechocystis* 6803, the hydrophilic region at the N-terminus contained 15 amino acids; however, even among these strains the first five amino acids were not conserved. Only four residues were conserved in all the cyanobacterial strains examined, and these were: Pro-7, Glu-13, Leu-14 and Asn-15 (numbers are based on the *Synechocystis* 6803 sequence). The Asn-6 and Asn-8 residues differed only in the PsbL