Biochimica et Biophysica Acta 1837 (2014) 1427–1434

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

# Some Photosystem II properties depending on the D1 protein variants in *Thermosynechococcus elongatus*☆

Miwa Sugiura [a,b,*], Alain Boussac [c]

[a] Proteo-science Research Center, Ehime University, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[b] PRESTO, Japan Science and Technology Agency (JST), 4-1-8, Honcho, Kawauchi, Saitama 332-0012, Japan
[c] iBiTec-S, CNRS UMR 8221, CEA Saclay, 91191 Gif-sur-Yvette, France

ARTICLE INFO

*Article history:*
Received 3 October 2013
Received in revised form 17 December 2013
Accepted 25 December 2013
Available online 2 January 2014

*Keywords:*
Photosystem II
PsbA
D1
*Thermosynechococcus elongatus*
Cyanobacterium

ABSTRACT

Cyanobacteria have multiple *psbA* genes encoding PsbA, the D1 reaction center protein of the Photosystem II complex which bears together with PsbD, the D2 protein, most of the cofactors involved in electron transfer reactions. The thermophilic cyanobacterium *Thermosynechococcus elongatus* has three *psbA* genes differently expressed depending on the environmental conditions. Among the 344 residues constituting each of the 3 possible PsbA variants there are 21 substitutions between PsbA1 and PsbA3, 31 between PsbA1 and PsbA2 and 27 between PsbA2 and PsbA3. In this review, we summarize the changes already identified in the properties of the redox cofactors depending on the D1 variant constituting Photosystem II in *T. elongatus*. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.



## 1. Introduction

The light-driven oxidation of water in Photosystem II (PSII) is the first step in the photosynthetic production of most of biomass, fossil fuels and $O_2$ on Earth. PSII in cyanobacteria is made up of 17 membrane protein subunits and 3 extrinsic proteins (PsbY was not detected in [1] but seen in [2]). Altogether these bear 35 chlorophylls (Chl), 2 pheophytins (Phe), 2 hemes, 1 non-heme iron, 2 plastoquinones ($Q_A$ and $Q_B$), a $Mn_4CaO_5$ cluster, at least 2 $Cl^-$, 12 carotenoids and 25 lipids [1]. The excitation resulting from the absorption of a photon is transferred to the photochemical trap that undergoes charge separation. The positive charge is then stabilized on $P_{680}$ which is composed of four Chl*a* molecules, $P_{D1}/P_{D2}$ and $Chl_{D1}/Chl_{D2}$, and two pheophytin *a* molecules, $Phe_{D1}/Phe_{D2}$. Then, $P_{680}^{+\cdot}$ oxidizes $Tyr_Z$, the Tyr161 of the D1 polypeptide, which in turn oxidizes the $Mn_4CaO_5$ cluster. On the electron acceptor side the electron is transferred to the primary quinone electron acceptor, $Q_A$, and then to $Q_B$, a two-electron and two-proton acceptor, *e.g.* [3–5]. The $Mn_4CaO_5$ cluster both accumulates oxidizing equivalents and acts as the catalytic site for water oxidation. The enzyme cycles sequentially through five redox states denoted $S_n$ where *n* stands for the number of stored oxidizing equivalents. Upon formation of the $S_4$ state two molecules of water are rapidly oxidized; the $S_0$ state is regenerated and $O_2$ is released [4–8].

Cyanobacterial species have multiple *psbA* variants coding for the D1 protein, *e.g.* [9–17]. These different genes are known to be differentially expressed depending on the environmental conditions, *e.g.* [9–15]. In particular, specific up/down-regulation of one of these genes under high light conditions is indicative of a photo-protection mechanism. For example [12], the mesophilic cyanobacterium, *Synechocystis* PCC 6803, has three *psbA* genes. Two of these (*psbAII* and *psbAIII*) produce an identical D1. Nevertheless, while *psbAII* is expressed under the "normal" cultivation conditions, transcription of *psbAIII* is induced by high light or UV light [12]. The expression of *psbAI* seems triggered by micro-aerobic conditions [15]. Other cryptic *psbA* genes were found to be induced in micro-aerobic conditions [18,19]. A class of "rogue" D1 protein has also been recently described [20]. Recent reviews on the

*Abbreviations:* PSII, Photosystem II; $Q_A$, primary quinone acceptor; $Q_B$, secondary quinone acceptor; Chl, chlorophyll; $P_{680}$, chlorophyll dimer acting as the second electron donor; $P_{D1}$ and $P_{D2}$, Chl monomer of $P_{680}$ on the D1 or D2 side, respectively; $Chl_{D1}$ and $Chl_{D2}$, accessory Chl on the D1 or D2 side, respectively; $Phe_{D1}$ and $Phe_{D2}$, pheophytin on the D1 or D2 side, respectively; $Tyr_Z$, the Tyr161 of the D1 polypeptide; EPR, Electron Paramagnetic Resonance; SQDG, sulfoquinovosyldiacylglycerol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PPBQ, phenyl-*p*-benzoquinone; SDS, sodium dodecyl sulfate; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; WT*1, WT*2, WT*3, cells containing only the $psbA_1$, $psbA_2$, and $psbA_3$ genes, respectively; 43H, *T. elongatus* strain with a His-tag on the C terminus of CP43

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

* Corresponding author at: Cell-Free Science and Technology Research Center, Ehime University, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan. Tel./fax: +81 89 927 9616.
*E-mail addresses:* miwa.sugiura@ehime-u.ac.jp (M. Sugiura), alain.boussac@cea.fr (A. Boussac).

0005-2728/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbabio.2013.12.011

cyanobacterial *psbA* gene family [14] and strategies for the *psbA* gene expression are available in the literature [21].

The thermophilic cyanobacterium *Thermosynechococcus elongatus* has three different *psbA* genes in its genome [22]. From the translated sequences and among the 344 residues of the PsbA proteins, 21 differ between PsbA1 and PsbA3, 31 between PsbA1 and PsbA2 and 27 between PsbA2 and PsbA3 (Fig. 1). The $psbA_1$ gene is constitutively expressed under "normal" laboratory conditions, while the transcription of $psbA_3$ occurred under high-light or UV light conditions [13,23,24]. The transcription of the $psbA_2$ gen has been reported to be, at least partially, induced under microaerobic conditions [15]. In contrast, with the case of the other cyanobacteria mentioned above, the differences in the D1 sequences in *T. elongatus* raise the possibility that the regulation at the transcription level is not a mere adjustment of the protein synthesis but rather an acclimation at the functional level whereby the functional properties of PSII are adjusted to cope with the increased photon flux. In this review, we summarize the changes in the properties of the redox cofactors depending on the D1 variant constituting PSII in *T. elongatus* that have been already identified.

## 2. The pheophytin, $Phe_{D1}$

Among the amino acids which differ between PsbA1, PsbA2 and PsbA3, the residue at position 130 has caught much attention as shown in Figs. 1 and 2. Raman spectroscopy [25], EPR [26] and FTIR studies [27] have shown that it is H-bonded to the $13^1$-keto of $Phe_{D1}$. In *T. elongatus* [27], the FTIR difference spectra of PsbA1-PSII exhibited the $13^1$-keto C=O bands at 1682 and 1605 $cm^{-1}$ in $Phe_{D1}$ and $Phe_{D1}^{-\cdot}$, respectively, while the corresponding bands in PsbA3-PSII were observed at frequencies lower by 1–3 and 18–19 $cm^{-1}$, respectively. This larger frequency shift in $Phe_{D1}^{-\cdot}$ than $Phe_{D1}$ by the change of the H-bond donor was well reproduced by density functional theory calculations for the Phe models H-bonded. Thus, the substitution of a glutamine in PsbA1-PSII for a glutamate in PsbA2-PSII and PsbA3-PSII results in a stronger H-bond with, as a consequence, an expected less negative $E_m(Phe_{D1}/Phe_{D1}^{-\cdot})$ in PsbA3-PSII than in PsbA1-PSII [28,29]. Such a change is therefore expected to modulate the energy level of the $P_{680}^{+\cdot}Phe_{D1}^{-\cdot}$ radical pair, the free energy change associated with charge separation being larger with Glutamate than with Glutamine.

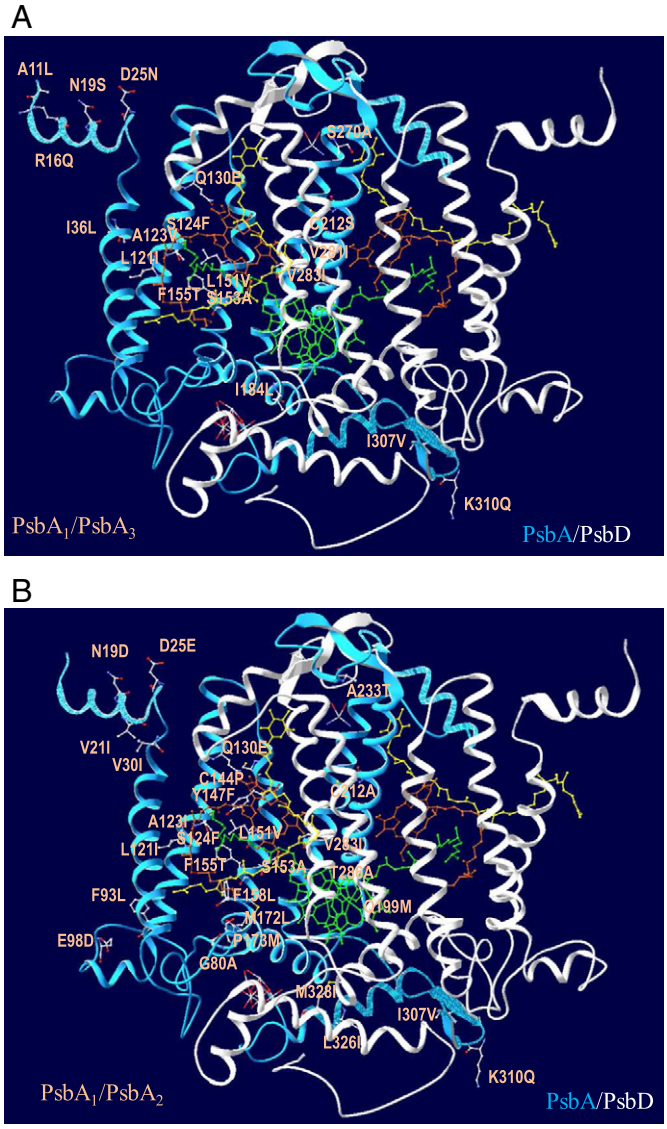


**Fig. 1.** Amino acid differences between either PsbA1 and PsbA3 (A) or PsbA1 and PsbA2 (B). Blue helices and white helices belong to D1 and D2, respectively. Numbers correspond to the amino acid sequence of D1. Letters are amino acids of PsbA1, PsbA3 and PsbA2.

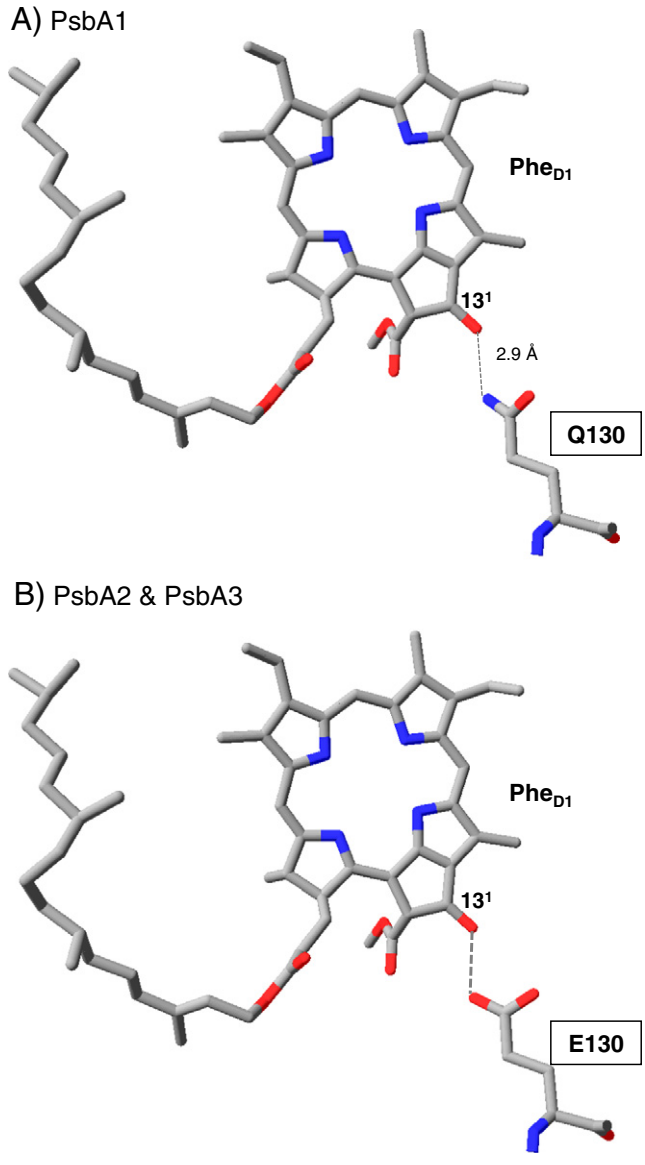


**Fig. 2.** Structures around $Phe_{D1}$ in PsbA1 (A) and PsbA2 and PsbA3 (B). The $13^1$-keto C=O of $Phe_{D1}$ gives weakly H-bond to Gln130 of PsbA1 with the distance of 2.9 Å. This residue is substituted by Glu in both PsbA2 and PsbA3. In those PSII, the hydrogen bond between the $13^1$-keto C=O of $Phe_{D1}$ and Glu130 was stronger than that in PsbA1-PSII [27].