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.

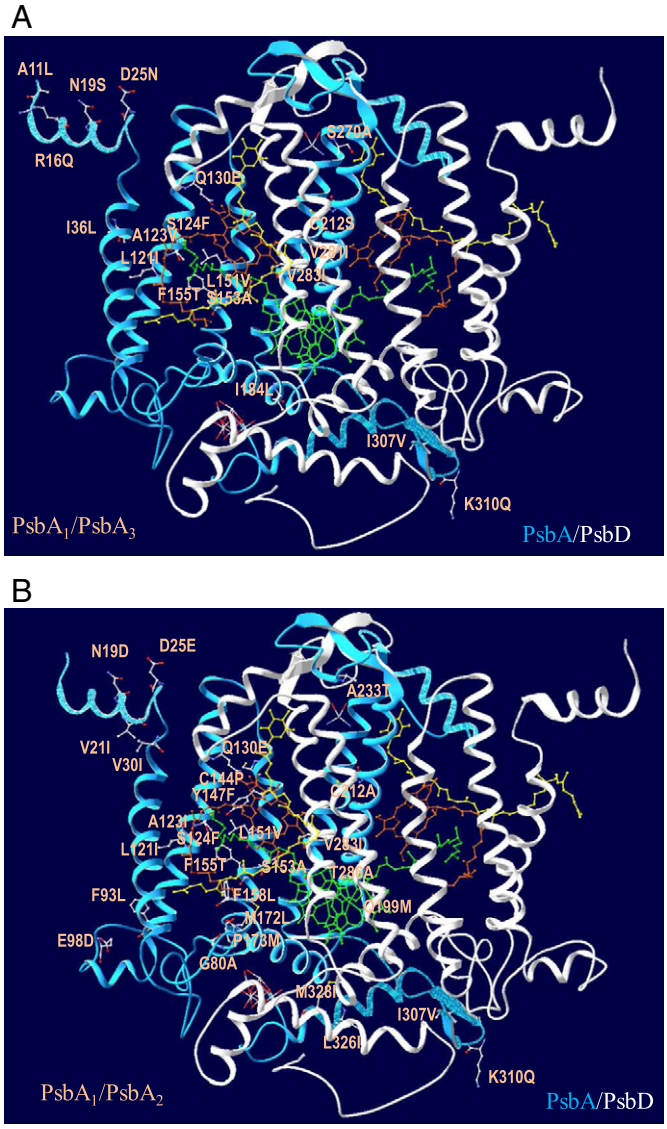

Fig. 1. Amino acid differences between either PsbA1 and PsbA3 (A) or PsbA1 and PsbA2 (B). Blue helices and white helices belong to D1 and D2, respectively. Numbers correspond to the amino acid sequence of D1. Letters are amino acids of PsbA1, PsbA3 and PsbA2.

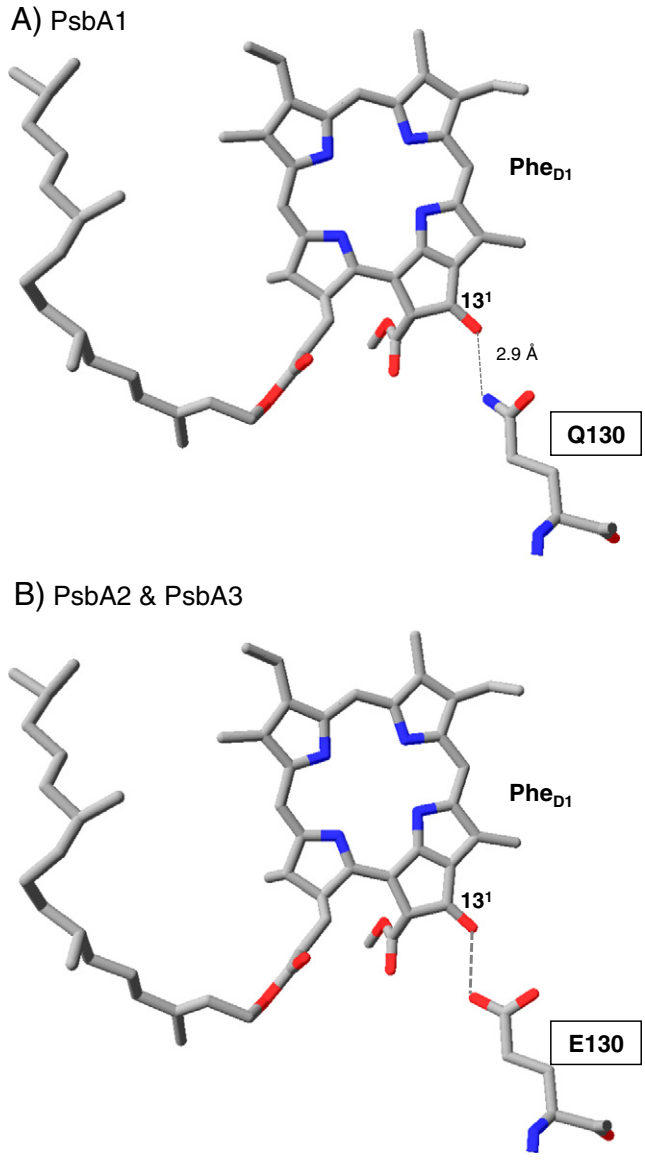

Fig. 2. Structures around Phe_{D1} in PsbA1 (A) and PsbA2 and PsbA3 (B). The 13^1-keto C=O of Phe_{D1} gives weakly H-bond to Gln130 of PsbA1 with the distance of 2.9 Å. This residue is substituted by Glu in both PsbA2 and PsbA3. In those PSII, the hydrogen bond between the 13^1-keto C=O of Phe_{D1}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

A clear and consistent view has emerged on the energetic consequences of the D1-Q130E substitution in *Synechocystis* PCC 6803 [30–32] or the D1-E130Q substitution in *Chlamydomonas reinhardtii* [33,34]. However, the increase of the redox potential of Phe_{D1} in *T. elongatus* was found to be only 17 mV from −522 mV in PsbA1-PSII [28] to −505 mV in PsbA3-PSII [29]. This increase was half the one observed upon single site directed mutagenesis in *Synechocystis* PCC 6803 [30–32]. This led us to propose that the effects of the D1-Q130E substitution could be, at least partly, compensated for by some of the additional amino-acid changes associated with the PsbA3 for PsbA1 substitution [16,29]. Thermoluminescence and fluorescence studies of PsbA1- and PsbA3-containing PSII from *T. elongatus* have shown that, although qualitatively consistent with the effect of the D1/Q130E point mutation, the consequences of the PsbA1 for PsbA3 substitution on the kinetics and thermodynamic characteristic of the $S_2Q_A^{-\cdot}$ charge recombination were also significantly less pronounced than in *Synechocystis* PCC 6803 [13,29,35]. This suggested that the physiologically relevant shift from PsbA1 to PsbA3 does not sum-up to the D1-Q130E change and that some (or all) of the 20 additional amino-acid substitutions contribute to determine the overall functional properties of the PsbA3 containing PSII. This suggestion found recently a strong support. Indeed, by studying the $S_2Q_A^{-\cdot}$ charge recombination in the presence of DCMU by thermoluminescence and fluorescence in a PsbA3/E130Q single site directed mutant [36] we found that the $E_m(Phe_{D1}/Phe_{D1}^{-\cdot})$ decreased by ≈30–35 mV, a shift with a similar amplitude, but of course in an opposite direction, to that observed in *Synechocystis* PCC 6803 [13,30–32] and similar both in terms of direction and amplitude to that observed in *C. reinhardtii* [33,34]. The E130Q mutation in PsbA3 also hardly affected the quantum efficiency as characterized by the yield of $S_2Q_A^{-\cdot}$ formation and the period four oscillations [36]. This is in agreement with previous reports [30,37] in which the Q130E change in *Synechocystis* PCC 6803 barely affected the nanosecond quantum yield of radical pair formation.

The $E_m(Phe_{D1}/Phe_{D1}^{-\cdot})$ has not yet been determined in PsbA2-PSII. In PsbA2-PSII, this amino acid of position 130 is also a glutamine like PsbA3-PSII. However, due to the compensatory effects evoked above, only a direct measurement by spectro-electrochemitry like that one done in PsbA1-PSII and PsbA3-PSII is expected to give us an accurate value in this variant. However, indirect observations like the electrochromic blue shift of the Phe_{D1} Q_x absorption around 545 nm undergone by Phe_{D1} upon reduction of Q_A (and known as the C-550 bandshift) are already available. The C-550 bandshift was found to be red shifted by ≈3.0 nm for the PsbA3-PSII sample, relative to the PsbA1-PSII sample, as expected. This is due to the stronger H-bond to the ^{1}13-keto of the Phe_{D1} from the carboxylate group of Q130 in PsbA3-PSII than from Q130 in PsbA1-PSII. In the PsbA2-PSII sample the electrochromic bandshift was found to be similar to that in the PsbA3-PSII sample which suggests that the electrostatic interaction which triggers this electrochromic bandshift is similar in PsbA2-PSII and PsbA3-PSII [36].

3. The primary quinone, Q_A

Measuring the fluorescence intensity against the electrode potential resulted in a value for $E_m(Q_A/Q_A^{-\cdot})$ of ≈−140 mV in PsbA1-PSII [38] and ≈−102 mV in PsbA3-PSII [29]. At first sight, this shift in $E_m(Q_A/Q_A^{-\cdot})$ may seem unexpected because Q_A is bound to PsbD (D2) rather than PsbA (D1). However, Q_A is linked to the Q_B binding site, made by the D1 protein, through the Q_A–H214(D2)–Fe–H215(D1)–Q_B molecular bridge. A FTIR study together with docking calculations [39] (see also [40]) suggested that the H-bond strength between D1/H215 and Q_B influences the H-bond strength between D2–H214 and Q_A through this molecular bridge, so that any change in the Q_B site may propagate through this H-bond wire to Q_A and possibly may lead to a shift of $E_m(Q_A/Q_A^{-\cdot})$.

Such a study has not yet been performed in PsbA2-PSII. However, by following the charge recombination in the light-induced radical pair $S_n Tyr_Z^{\bullet} Q_A^{-\cdot}$ at cryogenic temperatures by time-resolved EPR for different configurations of PSII that are expected to affect the driving force of the reaction (oxidation states S_0, S_1 or S_2 of the Mn_4CaO_5 cluster; PsbA1, PsbA2 or PsbA3 as D1 protein) similar $S_1 Tyr_Z^{\bullet} Q_A^{-\cdot}$ charge recombinations were observed in PsbA2-PSII and PsbA3-PSII [41]. Since, as it will be discussed below, the $E_m(Tyr_Z^{\bullet}/Tyr_Z)$ value seems not affected by the PsbA exchange in the S_1-state, this predicts that the $E_m(Q_A/Q_A^{-\cdot})$ value in PsbA2-PSII is likely similar to that in PsbA3-PSII.

4. The secondary quinone, Q_B, and the non-heme iron

Little is known on the properties of the secondary quinone Q_B that could vary depending on the D1 variant. Switching from PsbA1 to PsbA3 results in an amino acid substitution at position 270 (a serine in PsbA1 and an alanine in PsbA3 and PsbA2) as shown in Fig. 3 and it has been proposed that this might modify the structure of the Q_B site by changing the H-bond strength with D1–H215, and hence this might shift the $E_m(Q_A/Q_A^{-\cdot})$ value [42]. As mentioned in [43], the D1–S270A substitution may also contribute to loosen the H-bond with the head group of a lipid sulfoquinovosyldiacylglycerol (SQDG) located in the Q_B site and may be the rationale behind the difference of the binding characteristics of herbicides such as DCMU or bromoxynil to the Q_B site [16,43].

It has been observed that the addition of DCMU to PSII in the $Q_A Fe^{II} Q_B^{-\cdot}$ state induces the formation of the $Q_A Fe^{III} Q_B$ state in a fraction of centers, this fraction being larger in PsbA3-PSII than in PsbA1-PSII [43]. This observation could be explained in the framework of a square thermodynamic diagram in which the E_m, of the Fe^{II}/Fe^{III} couple in the presence and in the absence of the herbicide differs if indeed the binding constant of DCMU depends on the redox state of the non-heme iron. In this framework, the E_m of the non-heme iron was proposed to be slightly more positive in PsbA1-PSII than in PsbA3-PSII [43].

The limiting step of the overall oxygen evolution *in vitro* is the exchange of the doubly reduced Q_B molecule by an oxidized one. From an activity of ≈6000 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, in PsbA3-PSII (*i.e.* ≈50 O_2 molecules per second), one can infer for the limiting step a $t_{1/2}$ of 20 ms, which corresponds to 2 Q_B/Q_BH_2 exchanges. In both PsbA1-PSII and PsbA2-PSII the O_2 activity is generally found close to ≈3000–4000 μmol O_2 (mg Chl)$^{-1}$ h^{-1} [29,44]. This could suggest a slower Q_B/Q_BH_2 exchange in PsbA1-PSII and PsbA2-PSII than in PsbA3-PSII. In PsbA2-PSII, the oxygen evolving rate is about 75% of that of PsbA1-PSII. Since the amino-acid sequence around Q_B of PsbA2-PSII is similar to that of PsbA3-PSII further experiments will be required to understand the reasons of this apparent contradiction.

5. Electron transfer from Tyr_Z to $P_{680}^{+\cdot}$

The amino acid sequences around the Mn_4CaO_5 cluster are similar for PsbA1-PSII, PsbA2-PSII and PsbA3-PSII. Expectedly, the rate of the S_3 to S_0 transition ($t_{1/2}$ ≈ 1–2 ms) is similar in the 3 variants [44–46]. In contrast, in Mn-depleted PSII and at pH 9.2, the electron transfer rate from Tyr_Z to $P_{680}^{+\cdot}$ was found two fold faster in PsbA3 than in PsbA1 [16]. The faster reduction rate of $P_{680}^{+\cdot}$ in PsbA3-PSII likely stems from a faster intrinsic electron transfer rate constant between the tyrosinate and $P_{680}^{+\cdot}$ and therefore may reflect a difference in the free energy change associated with the electron transfer from Tyr_Z to $P_{680}^{+\cdot}$ and thus a different redox potential of either of these two players. We previously proposed that a hint in favor of a change in the redox potential of the $P_{680}^{+\cdot}/P_{680}$ couple was the observation that the slow component in the reduction of $P_{680}^{+\cdot}$, which develops in hundreds of microsecond time range and is usually assigned to the charge recombination between $P_{680}^{+\cdot}$ and $Q_A^{-\cdot}$ [47,48] was also faster in PsbA3-PSII than in PsbA1-PSII. However, considering the above results that both the $E_m(Q_A/Q_A^{-\cdot})$ and $E_m(Phe/Phe^{-\cdot})$ values also differ between the two types of PSII, this observation cannot be unequivocally interpreted

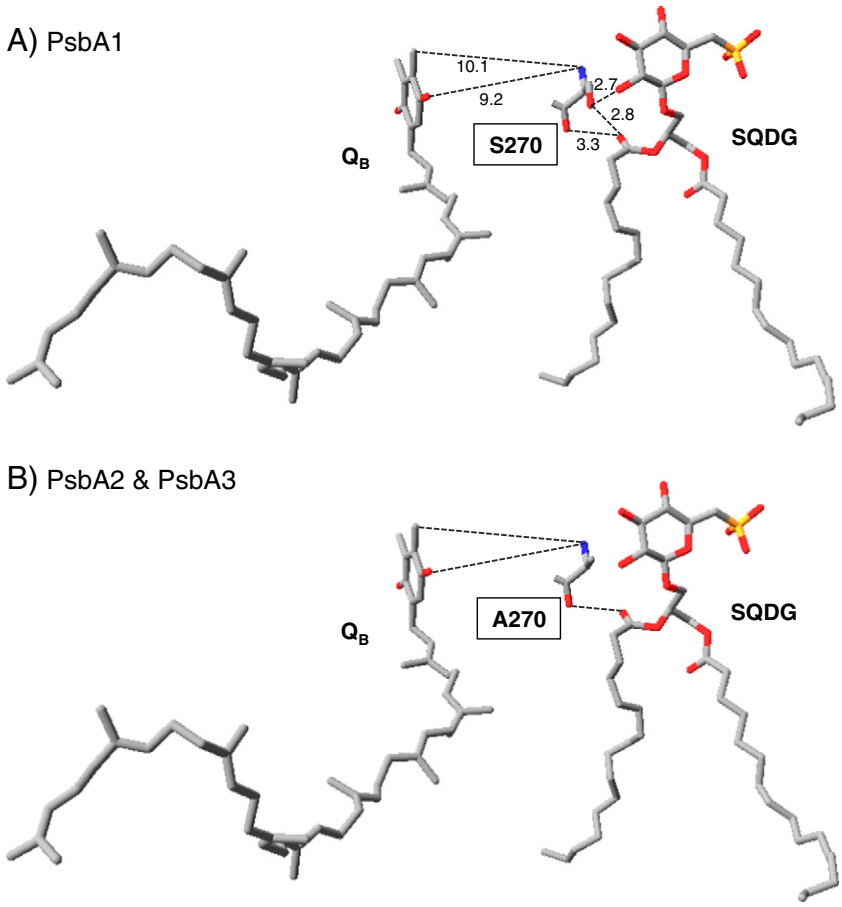

Fig. 3. Structure around SQDG, Ser270 and Q_B in PsbA1 (A). In PsbA2 and PsbA3, the Ser270 is substituted by Ala (B).

as reflecting a modification of the redox properties of P_{680} in Mn-depleted PSII.

In O_2 evolving PSII, the electron transfer rate from Tyr_Z to $P_{680}^{+\bullet}$ was found similar in PsbA3-PSII, *e.g.* [44], and in PsbA1-PSII, *e.g.* [49], whatever the S-state transition. In PsbA2-PSII, in the S_1-state, both the tens of ns and the tens of μs phases were found comparable to those in PsbA1/A3-PSII in terms of amplitude and $t_{1/2}$ [44]. However, in the S_2- and S_3-states the reduction of $P_{680}^{+\bullet}$ by Tyr_Z was much slower in PsbA2-PSII, particularly in hundreds of μs time domain. The finding that the reduction kinetics of $P_{680}^{+\bullet}$ is hardly affected in the S_1-state shows that possible changes of the properties of the electron acceptor side originating from the PsbA(1/3) to PsbA2 substitution did not significantly increase the percentage of centers in which the $P_{680}^{+\bullet}Q_A^{-\bullet}$ charge recombination occurred, at least with Q_B in the oxidized state (the experiment was performed in the presence of PPBQ). Therefore, it seems very unlikely that the slower $P_{680}^{+\bullet}$ reduction kinetics in hundreds of μs time domain and detected in the S_2 and S_3 states originate from a charge recombination that would be more efficient in PsbA2-PSII. According to the current understanding of the multiphasicity of the reduction of $P_{680}^{+\bullet}$ the ns components are kinetically limited by the electron transfer process, whereas the μs phases involve proton-coupled transfer reactions, *e.g.* [50–52]. In this framework, the results mentioned above would thus point to a slower proton transfer process in PsbA2-PSII. As shown in Fig. 4, two amino acid substitutions on the electron donor side of PsbA2-PSII may affect the orientation of the helices which respectively bear H190 and Tyr_Z: the C144P and P173M exchanges. These two substitutions may impact the H-bond between Tyr_Z and H190 and/or the H-bond network in which these two residues are involved. If such is indeed the case, this would be expected to affect the rates of the proton transfer steps associated with the oxidation of Tyr_Z. EPR spectroscopy, which has been shown to probe the geometry and the environment of the Tyr_Z phenol ring, *e.g.* [53,54], indeed revealed a change, in PsbA2-PSII, in the split EPR signal attributed to the $(S_2Tyr_Z^{\bullet})'$ state formed by the NIR-induced conversion of the manganese cluster, in the S_3-state, into an "activated" state able to oxidize Tyr_Z and thus leading to the formation of $(S_2Tyr_Z^{\bullet})'$ at the expense of the S_3Tyr_Z state [55].

6. Electron transfer from the Mn_4CaO_5 cluster to $Tyr_Z^{\bullet}$

The electron transfer rates between the Mn_4CaO_5 cluster and $Tyr_Z^{\bullet}$ have been essentially probed by following the absorption changes at 292 nm. Absorption changes at this wavelength reflect the Mn_4CaO_5 cluster valence changes and the Tyr_Z redox state changes occurring in the $S_1Tyr_Z^{\bullet}$ to S_2Tyr_Z, $S_2Tyr_Z^{\bullet}$ to S_3Tyr_Z, $S_3Tyr_Z^{\bullet}$ to S_0Tyr_Z, and $S_0Tyr_Z^{\bullet}$ to S_1Tyr_Z transitions [56]. No significant differences were found between the 3 PSII variants [44–46]. It was pointed out that the observation that the PsbA1/3 to PsbA2 exchange affects the μs components in the oxidation of Tyr_Z by $P_{680}^{+\bullet}$ while keeping unaffected the proton release associated with the $S_3Tyr_Z^{\bullet}$ to S_0Tyr_Z transition suggests that this particular proton release does not originate from the same H-bond network as the one involved in the proton transfer triggered by the formation of $Tyr_Z^{\bullet}$ [44]. The latter has been described as a sequence of push–pull steps that would be initiated by the transfer of the phenolic proton from Tyr_Z to Nε of H190. The identity of the "proton releaser" during the $S_3Tyr_Z^{\bullet}$ to S_0Tyr_Z is not known, and several candidates have been considered. A substrate water molecule is an obvious one. Alternatively, it could be a protonated base, proposed to be CP43-R357 [57], that would undergo a pK_a shift upon the formation of the $S_3Tyr_Z^{\bullet}$…$HN\varepsilon(H190)^+$ state and would, by acting as a proton acceptor from water, promote water splitting. These different proton transfer events thus have essentially different mechanistic implications. Although one mainly reflects electrostatic relaxation, the other sets the stage for all

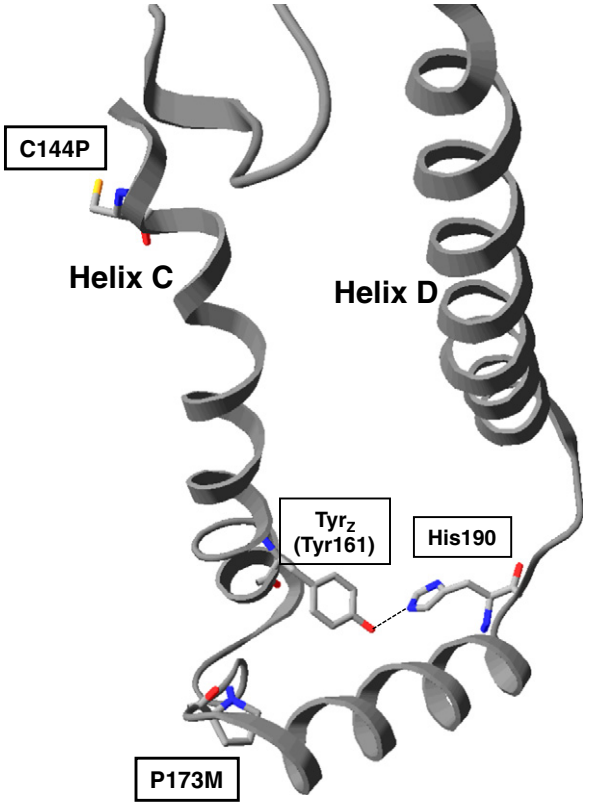

Fig. 4. Substitutions of Cys144Pro and Pro173Met on helix C in PsbA2. In both PsbA1 and PsbA3, these residues are Cys144 and Pro173. Hydrogen-bonded Tyr_Z and His190 are from the helix C and helix D. In PsbA2-PSII, distance of this hydrogen bond was longer than those in PsbA1- and PsbA3-PSII [44].

the players in the water-splitting process. In such a framework, it is not surprising that they involve different molecular actors, and these results support this expectation. Notably, they also point to a necessary conformational change to account for the fact that a new proton releaser that had stayed inactive until the formation of S_3 would come into play when $S_3Tyr_Z^{\cdot}\ldots HN\varepsilon(H190)^+$ is formed.

7. Differences in the interactions between the subunits of Photosystem II dependant on D1 protein variants

The structural stability of PSII upon a PsbA1/PsbA3 exchange has been investigated by studying the effects of PsbJ deletion in strains expressing either PsbA1 or PsbA3 [42]. The PsbJ subunit is a 4-kDa transmembrane polypeptide that is surrounded by PsbA, PsbK, and cytochrome b_{559} (Cyt b_{559} = PsbE + PsbF + heme). The PsbA3/ΔPsbJ-PSII was not significantly affected. Indeed, the polypeptide contents as revealed by SDS polyacrylamide gel electrophoresis and MALDI-TOF mass spectroscopy, the Cyt b_{559} properties, and the proportion of PSII dimer were similar to those found for PsbA3-PSII. In contrast, in 43H/ΔPsbJ-PSII (a strain having the His6-tag on CP43 and expressing PsbA1) the stability of the dimer was greatly diminished, the EPR properties of the Cyt b_{559} likely indicated a decrease in its redox potential, and many other PSII subunits were lacking. These results showed that most of the amino acid substitutions between PsbA1 and PsbA3, which could appear to be mainly conservative, must include side chains that are involved in a network of interactions between PsbA and the other PSII subunits. The 3D structure of PsbA3-PSII and PsbA2-PSII with a resolution comparable to that obtained for PsbA1-PSII will help us to identify these interactions.

Interestingly, it was recently reported that the deletion of PsbJ in a strain similar to our 43H/ΔPsbJ-PSII, *i.e.* a strain which possesses the 3 *psbA* genes, induced the expression of PsbA3 instead of PsbA1 [58]. In our conditions, we have found that the C-550 band-shift in 43H/ΔPsbJ-PSII was similar to that one in PsbA1-PSII (Sugiura, Rappaport, Boussac, unpublished). This result shows clearly that under our conditions the PsbA1/PsbA3 exchange did not occur upon the deletion of PsbJ. It seems likely that different culture conditions could be at the origin of such differences. They point out the importance of the culture conditions in the interpretation of the data.

8. PsbA1-PSII and PsbA3-PSII under photoinhibitory conditions

The sensitivity to high light illuminations of PSII with either PsbA1 (WT*1 cell strain) or PsbA3 (WT*3 cell strain) as the D1 protein have been found to be different [24,59]. It should be noted that in our work [59] the WT*1 strain had only the $psbA_1$ gene whereas in [24] the strain expressing PsbA1 contained both the $psbA_1$ and $psbA_2$ genes. When the cells were cultivated under high light conditions the following results were found: (i) the O_2 evolution activity decreased faster in WT*1 cells than in WT*3 cells both in the absence and in the presence of lincomycin, a protein synthesis inhibitor; (ii) in WT*1 cells, the rate constant for the decrease of the O_2 evolution activity was comparable in the presence and in the absence of lincomycin; (iii) the D1 content revealed by Western blot analysis decayed similarly in both WT*1 and WT*3 cells and much slowly than O_2 evolution; (iv) the faster decrease in O_2 evolution in WT*1 than in WT*3 cells correlated with a much faster inhibition of the S_2-state formation; and (v) the shape of the WT*1 cells was altered. All these results were in agreement with a photo-inhibition process resulting in the loss of the O_2 activity much faster than the D1 turnover in PsbA1-PSII and likely to a greater production of reactive oxygen species under high light conditions in WT*1 than in WT*3.

In *Synechocystis* 6803 it has been found that the D1/Q130E mutant showed a decreased $^1O_2$ production concomitantly with a decreased rate of photodamage relative to the WT, whereas both $^1O_2$ production and photodamage were enhanced in the D1/Q130L mutant [60]. From these results and those discussed above, we expected that in *T. elongatus* the WT*3–E130Q cells would exhibit greater resistance to high light conditions than the WT*3 cells. Although the growth rates of WT*3–E130Q cells were slightly faster than WT*3 cells even with a light intensity equal to 80 μmol of photons m^{-2} s^{-1}, the resistance under a high light intensity (800 μmol of photons m^{-2} s^{-1}) was hardly affected [36].

The data in [36] and [60] can yet be reconciled. Firstly, the light intensity used may differ in the various experiments reported in the literature. This will influence the steady state concentration of $Phe_{D1}^{-\cdot}Q_A^{-\cdot}$ *vs.* $Phe_{D1}Q_A^{-\cdot}$ and thus the sensitivity of the $^1O_2$ yield on the E130Q mutation. At higher light intensities $Phe_{D1}^{-\cdot}Q_A^{-\cdot}$ is accumulated, the energy gap between $Phe_{D1}Q_A^{-\cdot}$ and $Phe_{D1}^{-\cdot}Q_A$ has little consequence on the $^1O_2$ yield, whereas the E_m of Phe/$Phe^{-\cdot}$ will influence the direct recombination rate in competition with the triplet route. Under lower light intensities, both the energy gap between $Phe_{D1}Q_A^{-\cdot}$ and $Phe_{D1}^{-\cdot}Q_A$ and the E_m value of Phe/$Phe^{-\cdot}$ matter. If we consider that the accumulated state is $Phe_{D1}Q_A^{-\cdot}$, the benefit of a larger energy gap between $Phe_{D1}Q_A^{-\cdot}$ and $Phe_{D1}^{-\cdot}Q_A$ (41 mV) in PsbA3/E130Q-PSII compared to that in PsbA3-PSII (26 mV) is likely offset by the disadvantage of a much lower value by 30 mV for the E_m of Phe/$Phe^{-\cdot}$. The case where $Phe_{D1}^{-\cdot}Q_A^{-\cdot}$ is photo-accumulated is unlikely because we would expect more photodamage something that is not observed. Finally, the fact that the downshift by 30 mV of the E_m of Phe/$Phe^{-\cdot}$ in PsbA3/E130Q-PSII did not decrease the rate of the photodamage of the cells when compared to WT*3 cells could suggest that in *T. elongatus* and in PsbA3-PSII the E_m of Phe/$Phe^{-\cdot}$ is not the only important parameter in the protection against photodamage. It is possible that the other 20 amino acid changes occurring within the PsbA1 to PsbA3 swap could play a totally different protective role.

The response of WT*1 and WT*3 cells to environmental changes should take into account that the $psbA_1$ and $psbA_3$ genes have not the

same promoter. In [36], both the *psbA*$_3$ gene and the gene encoding PsbA3/E130Q gene have the same promoter so that the effects of the E130 to Q130 substitution on the sensitivity to high light conditions were not complicated by different PsbA expressions depending on the environment conditions. Similarly, by using a heterologous expression system of the two D1 isoforms of PsbA of *Synechococcus elongatus* PCC 7942 (with D1:1 expressed under low light conditions and D1:2 up-regulated in high light or stress conditions) in the green alga *C. reinhardtii*, functional advantages of D1:1-PSII and D1:2-PSII at low and high light regimes, respectively, are revealed [61].

In our work [36,59] and in [24], the faster recovery under low light intensity of the O_2 activity in WT*3 cells than in WT*1 cells after an exposure of the cells to strong light intensity is difficult to rationalize with the observation that under the same low light intensity the PSII in WT (or 43-H) cells is constituted of PsbA1. These data could be rationalized, for example, by a model in which (i) the expression of the *psbA*$_3$ gene in 43H cells is down regulated by the expression of the *psbA*$_1$ gene and (ii) strong light intensities inhibit the transcription of *psbA*$_1$ which in turn cancels the down-regulation of the *psbA*$_3$ transcription.

We have observed that the lag phase of *psbA*$_3$-deleted mutant cells which still therefore contained both the *psbA*$_1$ and *psbA*$_2$ genes was shorter than that of WT*1 cells (that contain only the *psbA*$_1$ gene) under the illumination of 60 μmol photons m^{-2} s^{-1}. This result suggests that the *psbA*$_2$ gene present in the *psbA*$_3$-deleted mutant in [24] could be possibly transcripted during the lag phase.

9. PsbA3 and high light conditions

The consequence of the increase of the $E_m(Q_A/Q_A^{-\bullet})$ by ≈40 mV and of the $E_m(Phe/Phe^{-\bullet})$ by ≈17 mV is an increase by 23 mV of the energy gap between $Phe_{D1}^{-\bullet}Q_A$ and $Phe_{D1}Q_A^{-\bullet}$ in PsbA3-PSII when compared to PsbA1-PSII. This indicates that under a light intensity which results in an accumulation of $Q_A^{-\bullet}$ the thermal repopulation of the $Phe_{D1}^{-\bullet}Q_A$ state from the $Phe_{D1}Q_A^{-\bullet}$ state is more difficult in PsbA3-PSII than in PsbA1-PSII which is thus expected to favor the direct charge recombination between $P_{680}^{+\bullet}$ and $Q_A^{-\bullet}$. Therefore, since formation of $^3[P_{680}^{+\bullet}Phe_{D1}^{-\bullet}]$ may lead to the formation of $^3P_{680}$, this implies that PsbA3-PSII would be less prone to photo-damage than PsbA1-PSII. In addition, it has been suggested that the preferential expression of PsbA3 under strong light [24] resulted in a more efficient direct charge recombination between the donor side and the acceptor side thus also preventing the production of harmful $^1O_2$ from $^3P_{680}$ itself formed by charge recombination in the thermally repopulated $^3[P_{680}^{+\bullet}Phe_{D1}^{-\bullet}]$ state [13], but see [62] for a discussion. This reasoning relied on the assumption that the charge recombination occurred in the inverted region of the Marcus curve with a $E_m(Phe/Phe^{-\bullet})$ value and a $E_m(Q_A/Q_A^{-\bullet})$ value higher in PsbA3-PSII than in PsbA1-PSII. We have shown that, at least at cryogenic temperatures, such inverted region in Photosystem II exists [41]. Finally, if the Q_B/Q_BH_2 exchange is indeed faster in PsbA3-PSII than in PsbA1-PSII this gives a third advantage to PsbA3-PSII over PsbA1-PSII under high light conditions in which $Q_A^{-\bullet}$ is accumulated. This list is likely not exhaustive. For example, it was also found that in the PsbA1-PSII the quantum efficiency of photo-induced oxidation of side-pathway donors was lower which also indicates an advantage for PsbA3 under high light conditions [63].

All these different expectations were numerically assessed by a modeling of the electron transfer reactions in PSII similar to that done previously by Moser et al. [64] who relied on the knowledge provided the X-ray structure of the distances between the different redox cofactors in PSII and applied their empirical ruler that allows electron transfer rates to be calculated when the distance between the electron donor and acceptor and the driving force of the reaction are known [65]. We have followed a similar approach and compared the time courses of the redox changes of the main cofactors in PsbA1-PSII, PsbA3-PSII and PsbA3/E130Q-PSII. It appeared that in the three cases the quantum efficiency of $S_2Q_A^{-\bullet}$ formation was hardly affected which was consistent with the experimental results [36]. The simulated kinetics for $S_2Q_A^{-\bullet}$ charge recombination agreed with the experimental results, *i.e.* a similar kinetics in PsbA1-PSII and PsbA3-PSII [16] and a much slower charge recombination in PsbA3/E130Q-PSII [36]. In the same theoretical work, the simulated formations of singlet oxygen that were found to be ~60%, ~20% and ~7% in PsbA1-PSII, PsbA3-PSII, and PsbA3/E130Q-PSII, respectively, qualitatively correlated with the differential sensitivity to high light for PsbA1 *vs.* PsbA3, *e.g.* [59], and with the lack of detectable difference between PsbA3 and PsbA3/E130Q [36].

10. PsbA2-PSII and Tll0287 expression

A new hemoprotein has been found to be expressed when the *T. elongatus* genome has only the *psbA*$_2$ gene for D1 [66]. This hemoprotein was found in both the fraction containing the purified PsbA2-PSII core complex and the non-membrane protein pool. From MALDI-TOF/TOF spectrometry, N-terminal sequencing and MALDI-MS/MS analysis upon tryptic digestion, the new hemoprotein was identified to be the *tll0287* gene product with a molecular mass close to 19 kDa. Until now, *tll0287* was registered as a gene encoding a hypothetical protein with an unknown function. From the amino acid sequence and the EPR spectrum the 5th and 6th axial ligands of the heme iron are the H145 and likely either the Y93, Y159 or Y165, respectively. The amino acid sequence of Tll0287 and conserved residues are shown in Fig. 5. Homologous genes to *tll0287* are found in several cyanobacteria. Since the expression of the *psbA*$_2$ gene has been shown to occur under micro-aerobic conditions, under our cultivation conditions, *i.e.* in the presence of ≈20% O_2, the production of Tll0287 in WT*2 cells could be the answer to an oxidative stress. Future works would have to solve this question.

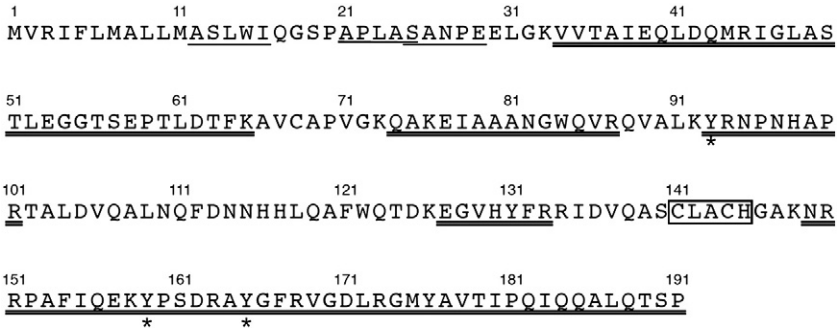

Fig. 5. Deduced amino acid sequence from the *tll0287* gene in *T. elongatus*. The underlined amino acid residues are those belonging to the main N-terminal sequences detected by using the Edman procedure. The frame shows the conserved heme-binding motif (CxxCH). The stars show the conserved tyrosine residues which are potential candidates to be the 6th heme iron axial ligand [66]. The doubly underlined amino acids are those belonging to the fragments identified with Mascot after tryptic digestion and MALDI-MS/MS.

Acknowledgements

We thank C. Azami, K. Brettel, N. Cox, S. Harada, H. Hayashi, J.L. Hughes, N. Ishida, E. Iwai, Y. Kato, K. Koyama, E. Krausz, M. Kusumi, T.-L. Lai, T. Manabe, T. Noguchi, S. Ogami, F. Rappaport, A.W. Rutherford, T. Shibamoto, H. Suzuki, R. Takahashi, S. Un, T. Watanabe and S. Yamamoto for their valuable participation to the works presented in this review. A. Krieger-Liszkay is acknowledged for the reading of the manuscript.

References

[1] Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, Crystal structure of oxygen-evolving Photosystem II at a resolution of 1.9 Å, Nature 473 (2011) 55–60.
[2] F.H.M. Koua, Y. Umena, K. Kawakami, J.-R. Shen, Structure of Sr-substituted Photosystem II at 2.1 angstrom resolution and its implications in the mechanism of water oxidation, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 3389–3894.
[3] B.A. Diner, F. Rappaport, Structure, dynamics, and energetic of the primary photochemistry of Photosystem II of oxygenic photosynthesis, Annu. Rev. Plant Biol. 53 (2002) 551–580.
[4] G. Renger, Light-induced oxidative water splitting in photosynthesis: energetics, kinetics, and mechanism, J. Photochem. Photobiol. B 104 (2011) 35–43.
[5] H. Dau, I. Zaharieva, M. Haumann, Recent developments in research on water oxidation by Photosystem II, Curr. Opin. Chem. Biol. 16 (2012) 3–10.
[6] B. Kok, B. Forbush, M. McGloin, Cooperation of charges in photosynthetic O_2 evolution—I. A linear four step mechanism, Photochem. Photobiol. 11 (1970) 457–475.
[7] P. Joliot, B. Kok, Oxygen evolution in photosynthesis, in: Govindjee (Ed.), Bioenergetics of Photosynthesis, Academic Press, New York, 1975, pp. 387–412.
[8] N. Cox, J. Messinger, Reflections on substrate water and dioxygen formation, Biochim. Biophys. Acta 1827 (2013) 1020–1030.
[9] A.K. Clarke, A. Soitamo, P. Gustafsson, G. Oquist, Rapid interchange between two distinct forms of cyanobacterial Photosystem II reaction-center protein D1 in response to photoinhibition, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 9973–9977.
[10] S.S. Golden, Light-responsive gene expression in cyanobacteria, J. Bacteriol. 177 (1995) 1651–1654.
[11] J. Komenda, H. Hassan, B.A. Diner, R.J. Debus, J. Barber, P.J. Nixon, Degradation of the Photosystem II D1 and D2 proteins in different strains of the cyanobacterium *Synechocystis* PCC 6803 varying with respect to the type and level of *psbA* transcript, Plant Mol. Biol. 42 (2000) 635–645.
[12] C.I. Sicora, S.E. Appleton, C.M. Brown, J. Chung, J. Chandler, A.M. Cockshutt, I. Vass, D.A. Campbell, Cyanobacterial *psbA* families in *Anabaena* and *Synechocystis* encode trace, constitutive, and UVB-induced D1 isoforms, Biochim. Biophys. Acta 1757 (2006) 47–56.
[13] P.B. Kós, Z. Deák, O. Cheregi, I. Vass, Differential regulation of *psbA* and *psbD* gene expression, and the role of the different D1 protein copies in the cyanobacterium *Thermosynechococcus elongatus* BP-1, Biochim. Biophys. Acta 1777 (2008) 74–83.
[14] P. Mulo, C. Sicora, E.-M. Aro, Cyanobacterial *psbA* gene family: optimization of oxygenic photosynthesis, Cell. Mol. Life Sci. 66 (2009) 3697–3710.
[15] C.I. Sicora, F.M. Ho, T. Salminen, S. Styring, E.-M. Aro, Transcription of a "silent" cyanobacterial *psbA* gene is induced by microaerobic conditions, Biochim. Biophys. Acta 1787 (2009) 105–112.
[16] M. Sugiura, Y. Kato, R. Takahashi, H. Suzuki, T. Watanabe, T. Noguchi, F. Rappaport, A. Boussac, Energetics in Photosystem II from *Thermosynechococcus elongatus* with a D1 protein encoded by either the $psbA_1$ or $psbA_3$ gene, Biochim. Biophys. Acta 1797 (2010) 1491–1499.
[17] E. Kiss, P.B. Kos, M. Chen, I. Vass, A unique regulation of the expression of the *psbA*, *psbD*, and *psbE* genes, encoding the D1, D2 and cytochrome b_{559} subunits of the Photosystem II complex in the chlorophyll d containing cyanobacterium *Acaryochloris marina*, Biochim. Biophys. Acta 1817 (2012) 1083–1094.
[18] T.C. Summerfield, J. Toepel, L.A. Sherman, Low-oxygen induction of normally cryptic *psbA* genes in cyanobacteria, Biochemistry 47 (2008) 12939–12941.
[19] X. Zhang, L.A. Sherman, Alternate copies of D1 are used by cyanobacteria under different environmental conditions, Photosynth. Res. 114 (2012) 133–135.
[20] J.W. Murray, Sequence variation at the oxygen-evolving centre of Photosystem II: a new class of 'rogue' cyanobacterial D1 proteins, Photosynth. Res. 110 (2012) 177–184.
[21] P. Mulo, I. Sakurai, E.-M. Aro, Strategies for psbA gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair, Biochim. Biophys. Acta 1817 (2012) 247–257.
[22] Y. Nakamura, T. Kaneko, S. Sato, M. Ikeuchi, H. Katoh, S. Sasamoto, A. Watanabe, M. Iriguchi, K. Kawashima, T. Kimura, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, M. Sugimoto, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, DNA Res. 9 (2002) 123–130.
[23] B. Loll, M. Broser, P.B. Kós, J. Kern, J. Biesiadka, I. Vass, W. Saenger, A. Zouni, Modeling of variant copies of subunit D1 in the structure of Photosystem II from *Thermosynechococcus elongatus*, Biol. Chem. 389 (2008) 609–617.
[24] J. Sander, M. Nowaczyk, J. Buchta, H. Dau, I. Vass, Z. Deak, M. Dorogi, M. Iwai, M. Rogner, Functional characterization and quantification of the alternative PsbA copies in *Thermosynechococcus elongatus* and their role in photoprotection, J. Biol. Chem. 285 (2010) 29851–29856.
[25] P. Moënne-Loccoz, B. Robert, M. Lutz, A resonance Raman characterization of the primary electron-acceptor in Photosystem II, Biochemistry 28 (1989) 3641–3645.
[26] P. Dorlet, L. Xiong, R.T. Sayre, S. Un, High field EPR study of the pheophytin anion radical in wild type and D1-E130 mutants of Photosystem II in *Chlamydomonas reinhardtii*, J. Biol. Chem. 276 (2001) 22313–22316.
[27] Y. Shibuya, R. Takahashi, T. Okubo, H. Suzuki, M. Sugiura, T. Noguchi, Hydrogen bond interactions of the pheophytin electron acceptor and its radical anion in Photosystem II as revealed by Fourier transform infrared difference spectroscopy, Biochemistry 49 (2010) 493–501.
[28] Y. Kato, M. Sugiura, A. Oda, T. Watanabe, Spectroelectrochemical determination of the redox potential of pheophytin a, the primary electron acceptor in Photosystem II, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 17365–17370.
[29] Y. Kato, T. Shibamoto, S. Yamamoto, T. Watanabe, N. Ishida, M. Sugiura, F. Rappaport, A. Boussac, Influence of the PsbA1/PsbA3 and Ca^{2+}/Sr^{2+} or Cl^-/Br^- exchanges on the redox potential of the primary quinone Q_A in Photosystem II as revealed by spectroelectrochemistry, Biochim. Biophys. Acta 1817 (2012) 1998–2004.
[30] S.A.P. Merry, P.J. Nixon, L.M.C. Barter, M. Schilstra, G. Porter, J. Barger, J.R. Durrant, D.R. Klug, Modulation of quantum yield of primary radical pair formation in Photosystem II by site-directed mutagenesis affecting radical cations and anions, Biochemistry 37 (1998) 17439–17447.
[31] K. Cser, I. Vass, Radiative and non-radiative charge recombination pathways in Photosystem II studied by thermoluminescence and chlorophyll fluorescence in the cyanobacterium *Synechocystis* 6803, Biochim. Biophys. Acta 1767 (2007) 233–243.
[32] F. Rappaport, M. Guergova-Kuras, P.J. Nixon, B.A. Diner, J. Lavergne, Kinetics and pathways of charge recombination in Photosystem II, Biochemistry 41 (2002) 8518–8527.
[33] A. Cuni, L. Xiong, R. Sayre, F. Rappaport, J. Lavergne, Modification of the pheophytin midpoint potential in Photosystem II: modulation of the quantum yield of charge separation and of charge recombination pathways, Phys. Chem. Chem. Phys. 6 (2004) 4825–4831.
[34] F. Rappaport, A. Cuni, L. Xiong, R. Sayre, J. Lavergne, Charge recombination and thermoluminescence in Photosystem II, Biophys. J. 88 (2005) 1948–1958.
[35] M. Sugiura, A. Boussac, T. Noguchi, F. Rappaport, Influence of Histidine-198 of the D1 subunit on the properties of the primary electron donor, P680, of Photosystem II in *Thermosynechococcus elongatus*, Biochim. Biophys. Acta 1777 (2008) 331–342.
[36] M. Sugiura, C. Azami, K. Koyama, A.W. Rutherford, F. Rappaport, A. Boussac, Modification of the pheophytin redox potential in *Thermosynechococcus elongatus* Photosystem II with PsbA3 as D1, Biochim. Biophys. Acta 1837 (2013) 139–148.
[37] L.B. Giorgi, P.J. Nixon, S.A.P. Merry, D.M. Joseph, J.R. Durrant, J.D. Rivas, J. Barber, G. Porter, D.R. Klug, Comparison of primary charge separation in the Photosystem II reaction center complex isolated from wild-type and D1–130 mutants of the cyanobacterium *Synechocystis* PCC 6803, J. Biol. Chem. 271 (1996) 2093–2101.
[38] T. Shibamoto, Y. Kato, M. Sugiura, T. Watanabe, Redox potential of the primary plastoquinone electron acceptor Q_A in Photosystem II from *Thermosynechococcus elongatus* determined by spectroelectrochemistry, Biochemistry 48 (2009) 10682–10684.
[39] H. Ishikita, K. Hasegawa, T. Noguchi, How does the Q_B site influence propagate to the Q_A site in Photosystem II? Biochemistry 50 (2011) 5436–5442.
[40] K. Saito, A.W. Rutherford, H. Ishikita, Mechanism of proton-coupled quinone reduction in Photosystem II, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 954–959.
[41] A. Boussac, F. Rappaport, K. Brettel, M. Sugiura, Charge recombination in $SnTyr_Z^{\bullet}Q_A^{-\bullet}$ radical pairs in D1 protein variants of Photosystem II: long range electron transfer in the Marcus inverted region, J. Phys. Chem. 117 (2013) 3308–3314.
[42] M. Sugiura, E. Iwai, H. Hayashi, A. Boussac, Differences in the interactions between the subunits of Photosystem II dependant on D1 protein variants in the thermophilic cyanobacterium *Thermosynechococcus elongatus*, J. Biol. Chem. 285 (2010) 30008–30018.
[43] A. Boussac, M. Sugiura, F. Rappaport, Probing the quinone binding site of Photosystem II from *Thermosynechococcus elongatus* containing either PsbA1 or PsbA3 as the D1 protein through the binding characteristics of herbicides, Biochim. Biophys. Acta 1807 (2010) 119–129.
[44] M. Sugiura, S. Ogami, M. Kusumi, S. Un, F. Rappaport, A. Boussac, Environment of Tyr_Z in Photosystem II from *Thermosynechococcus elongatus* in which PsbA2 is the D1 protein, J. Biol. Chem. 287 (2012) 13336–13347.
[45] A. Boussac, F. Rappaport, P. Carrier, J.-M. Verbavatz, R. Gobin, D. Kirilovsky, A.W. Rutherford, M. Sugiura, Biosynthetic Ca^{2+}/Sr^{2+} exchange in the Photosystem II oxygen evolving enzyme of *Thermosynechococcus elongatus*, J. Biol. Chem. 279 (2004) 22809–22819.
[46] N. Ishida, M. Sugiura, F. Rappaport, T.-L. Lai, A.W. Rutherford, A. Boussac, Biosynthetic exchange of bromide for chloride and strontium for calcium in the Photosystem II oxygen-evolving enzyme, J. Biol. Chem. 283 (2008) 13330–13340.
[47] G. Renger, C. Wolff, Existence of a high photochemical turnover rate at reaction centers of system-2 in Tris-washed chloroplasts, Biochim. Biophys. Acta 423 (1976) 610–614.
[48] J. Haveman, P. Mathis, Flash-induced absorption changes of primary donor of Photosystem II at 820 nm in chloroplasts inhibited by low pH or Tris-treatment, Biochim. Biophys. Acta 440 (1976) 346–355.
[49] M. Sugiura, F. Rappaport, K. Brettel, T. Noguchi, A.W. Rutherford, A. Boussac, Site-directed mutagenesis of *Thermosynechococcus elongatus* Photosystem II: the O_2 evolving enzyme lacking the redox active tyrosine D, Biochemistry 43 (2004) 13549–13563.
[50] M.J. Schilstra, F. Rappaport, J.H. Nugent, C.J. Barnett, D.R. Klug, Proton/hydrogen transfer affects the S-state-dependent microsecond phases of P_{680}^+ reduction during water splitting, Biochemistry 37 (1998) 3974–3981.
[51] P. Kuhn, H. Eckert, H.J. Eichler, G. Renger, Analysis of the $P_{680}^{+\bullet}$ reduction pattern and its temperature dependence in oxygen-evolving PSII core complexes from a thermophilic cyanobacteria and higher plants, Phys. Chem. Chem. Phys. 6 (2004) 4838–4843.

[52] G. Christen, G. Renger, The role of hydrogen bonds for the multiphasic $P_{680}^{+\bullet}$ reduction by Yz in photosystem II with intact oxygen evolution capacity. Analysis of kinetic H/D isotope exchange effects, Biochemistry 38 (1999) 2068–2077.
[53] C. Tommos, X.S. Tang, K. Warncke, C.W. Hoganson, S. Styring, J. Mc-Cracken, B.A. Diner, G.T. Bancock, Spin-density distribution, conformation, and hydrogen-bonding of the redox-active tyrosine Y_Z in Photosystem II from multiple electron magnetic resonance spectroscopies — implications for photosynthetic oxygen evolution, J. Am. Chem. Soc. 117 (1995) 10325–10335.
[54] S. Un, A. Boussac, M. Sugiura, Characterization of the tyrosine-Z radical and its environment in the spin-coupled $S_2TyrZ^{\bullet}$ state of Photosystem II from *Thermosynechococcus elongatus*, Biochemistry 46 (2007) 3138–3150.
[55] V. Petrouleas, D. Koulougliotis, N. Ioannidis, Trapping of metalloradical intermediates of the S-states at liquid helium temperatures. Overview of the phenomenology and mechanistic implications, Biochemistry 44 (2005) 6723–6728.
[56] J. Lavergne, Improved UV–visible spectra of the S-state transitions in the photosynthetic oxygen evolving system, Biochim. Biophys. Acta 1060 (1991) 175–188.
[57] E.M. Sproviero, J.A. Gascón, J.P. McEvoy, G.W. Brudvig, V.S. Batista, Quantum mechanics/molecular mechanics study of the catalytic cycle of water splitting in Photosystem II, J. Am. Chem. Soc. 130 (2008) 3428–3442.
[58] M.M. Nowaczyk, K. Krause, M. Mieseler, A. Sczibilanski, M. Ikeuchi, M. Rogner, Deletion of psbJ leads to accumulation of Psb27–Psb28 Photosystem II complexes in *Thermosynechococcus elongatus*, Biochim. Biophys. Acta 1817 (2012) 1339–1345.
[59] S. Ogami, A. Boussac, M. Sugiura, Deactivation processes in PsbA1-Photosystem II and PsbA3-Photosystem II under photoinhibitory conditions in the cyanobacterium *Thermosynechococcus elongatus*, Biochim. Biophys. Acta 1817 (2012) 1322–1330.
[60] A.U. Rehman, K. Cser, L. Sass, I. Vass, Characterization of singlet oxygen production and its involvement in photodamage of Photosystem II in the cyanobacterium *Synechocystis* PCC 6803 by histidine-mediated chemical trapping, Biochim. Biophys. Acta 1827 (2013) 689–698.
[61] D.J. Vinyard, J. Gimpel, G.M. Ananyev, M.A. Cornejo, S.S. Golden, S.P. Mayfield, G.C. Dismukes, Natural variants of Photosystem II subunit D1 tune photochemical fitness to solar intensity, J. Biol. Chem. 288 (2013) 5451–5462.
[62] A.W. Rutherford, A. Osyczka, F. Rappaport, Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: redox tuning to survive life in O_2, FEBS Lett. 586 (2012) 603–616.
[63] J.L. Hughes, N. Nicholas, A.W. Rutherford, E. Krausz, T.-L. Lai, A. Boussac, M. Sugiura, D1 protein variants in Photosystem II from *Thermosynechococcus elongatus* studied by low temperature optical spectroscopy, Biochim. Biophys. Acta 1797 (2010) 11–19.
[64] C.C. Moser, C.C. Page, P.L. Dutton, Tunneling in PSII, Photochem. Photobiol. Sci. 12 (2005) 933–939.
[65] C.C. Moser, P.L. Dutton, Protein Electron Transfer in Outline of Theory of Protein Electron Transfer, in: D.S. Bendal (Ed.), BIOS Scientific Publishers Ltd, Oxford, 1996, pp. 1–21.
[66] A. Boussac, K. Koyama, M. Sugiura, The Tll0287 protein is a hemoprotein associated with the PsbA2-Photosystem II complex in *Thermosynechococcus elongatus*, Biochim. Biophys. Acta 1827 (2013) 1174–1182.