Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

The D1-173 amino acid is a structural determinant of the critical interaction between D1-Tyr161 (Tyr_Z) and D1-His190 in Photosystem II

Miwa Sugiura [a,b,c,*], Yui Ozaki [a], Masato Nakamura [b], Nicholas Cox [d], Fabrice Rappaport [e], Alain Boussac [f,**]

[a] Proteo-Science Research Center, Ehime University, Bunkyo-cho, Matsuyama Ehime, 790-8577, Japan
[b] Department of Chemistry, Graduate School of Science and Technology, Ehime University, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan
[c] PRESTO, Science and Technology Agency (JST), 4-1-8, Honcho, Kawaguchi, Saitama 332-0012, Japan
[d] Max-Planck-Institut für Chemische Energiekonversion, Stiftstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany
[e] Institut de Biologie Physico-Chimique, UMR 7141 CNRS and Université Pierre et Marie Curie, 13 rue Pierre et Marie Curie, 75005 Paris, France
[f] iBiTec-S, CNRS UMR 8221, CEA Saclay, 91191 Gif-sur-Yvette, France

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 20 August 2014
Accepted 26 August 2014
Available online xxxx

Keywords:
Photosystem II
PsbA
D1
Thermosynechococcus elongatus
Tyr_Z
Proline

ABSTRACT

The main cofactors of Photosystem II (PSII) are borne by the D1 and D2 subunits. In the thermophilic cyanobacterium *Thermosynechococcus elongatus*, three *psbA* genes encoding D1 are found in the genome. Among the 344 residues constituting the mature form of D1, there are 21 substitutions between PsbA1 and PsbA3, 31 between PsbA1 and PsbA2, and 27 between PsbA2 and PsbA3. In a previous study (Sugiura et al., J. Biol. Chem. 287 (2012), 13336-13347) we found that the oxidation kinetics and spectroscopic properties of Tyr_Z were altered in PsbA2PSII when compared to PsbA(1/3)-PSII. The comparison of the different amino acid sequences identified the residues Cys144 and Pro173 found in PsbA1 and PsbA3, as being substituted in PsbA2 by Pro144 and Met173, and thus possible candidates accounting for the changes in the geometry and/or the environment of the Tyr_Z/His190 phenol/imidizol motif. Indeed, these amino acids are located upstream of the α-helix bearing Tyr_Z and between the two α-helices bearing Tyr_Z and its hydrogen-bonded partner, D1/His190. Here, site-directed mutants of PSII, PsbA3/Pro173Met and PsbA2/Met173Pro, were analyzed using X- and W-band EPR and UV-visible time-resolved absorption spectroscopy. The Pro173Met substitution in PsbA2-PSII *versus* PsbA3-PSII is shown to be the main structural determinant of the previously described functional differences between PsbA2-PSII and PsbA3-PSII. In PsbA2-PSII and PsbA3/Pro173Met-PSII, we found that the oxidation of Tyr_Z by $P_{680}^{+\bullet}$ was specifically slowed during the transition between S-states associated with proton release. We thus propose that the increase of the electrostatic charge of the Mn_4CaO_5 cluster in the S_2 and S_3 states could weaken the strength of the H-bond interaction between $Tyr_Z^{\bullet}$ and D1/His190 in PsbA2 *versus* PsbA3 and/or induce structural modification(s) of the water molecules network around Tyr_Z.

1. Introduction

The light-driven oxidation of water in Photosystem II (PSII) is the first step in the photosynthetic production of most of the earth's-biomass, fossil fuels and O_2. The PSII super-complex in cyanobacteria is made up of 17 membrane protein subunits and 3 extrinsic proteins, capping the site of water splitting catalysis (PsbY was not present in [1] but observed in [2]). The homologous membrane proteins D1 and D2 form the center of the super-complex. They bind all key cofactors/redox centers involved in primary charge separation, electron transfer and water splitting/plastoquinone reduction catalysis [1]. The excitation resulting from the absorption of a photon is transferred to the photochemical trap (reaction center) that undergoes charge separation. The reaction center consists of four chlorophyll *a* molecules, P_{D1}/P_{D2} and Chl_{D1}/Chl_{D2}, and two pheophytin *a* molecules, Phe_{D1}/Phe_{D2}. The resulting positive charge is then stabilized on P_{680} (an excited state of mainly P_{D1}/P_{D2} character). $P_{680}^{+\bullet}$ then oxidizes a nearby redox active tyrosine, Tyr_Z, the Tyr161 of the D1 polypeptide, which in turn oxidizes an inorganic 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 accumulates

Abbreviations: PSII, Photosystem II; Chl, chlorophyll; MES, 2-(N-morpholino) ethanesulfonic acid; P_{680}, chlorophyll dimer acting as the electron donor; Q_A, primary quinone acceptor; Q_B, secondary quinone acceptor; Cm, chrloramphenicol; Sm, streptmycin; Sp, spectinomycin; 43H, *T. elongatus* strain with a His-tag on the C terminus of CP43; PQ, plastoquinone 9; WT*1, WT*2, WT*3, cells containing only the $psbA_1$, $psbA_2$, $psbA_3$ gene, respectively; $Pheo_{D1}$, pheophytin; P_{D1} and P_{D2}, Chl monomer of P_{680} on the D1 or D2 side, respectively; PPBQ, phenyl *p*-benzoquinone; β-DM, *n*-dodecyl-β-maltoside; NIR, near-infrared; CW, continuous wave; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight

* Correspondence to: M. Sugiura, Proteo-Science Research Center, Ehime University, Bunkyo-cho, Matsuyama Ehime, 790-8577, Japan.
** Corresponding author.
E-mail addresses: miwa.sugiura@ehime-u.ac.jp (M. Sugiura), alain.boussac@cea.fr (A. Boussac).

http://dx.doi.org/10.1016/j.bbabio.2014.08.008
0005-2728/

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, O_2 is released and the S_0 state is regenerated [4–9].

Cyanobacterial species have multiple *psbA* variants encoding for the D1 protein, *e.g.* [10–22]. These gene homologues are known to be differentially transcribed depending on the environmental conditions, see [23] for a recent discussion. The genome of the thermophilic cyanobacterium *Thermosynechococcus elongatus* contains three different *psbA* genes [24]. Among the 344 residues of the matured PsbA proteins, 21 differ between PsbA1 and PsbA3, 31 between PsbA1 and PsbA2 and 27 between PsbA2 and PsbA3. The $psbA_1$ gene is constitutively expressed under "general" laboratory conditions, while the $psbA_3$ gene is transcribed under light stress conditions such as high light or UV light conditions [14,25,26]. The transcription of the $psbA_2$ gene has been reported to increase under micro-aerobic conditions [16]. Notably, D1 transcription/regulation may vary among cyanobacteria. In *T. elongatus*, the differential transcription patterns of the three genes coding for the D1 subunit raise the possibility that, in this species, the regulation at the transcription level represents an acclimation mechanism where the functional properties of PSII are adjusted to cope with the increased photon flux, *e.g.* the interchange of the PsbA1 and PsbA3 variants modifies the redox potential of $Pheo_{D1}$ [23]. Changes in the already identified properties of the redox cofactors depending on the D1 variant constituting PSII in *T. elongatus* have been recently reviewed [27].

The effects of the PsbA(1/3)/PsbA2 substitution are much less documented. In a previous study [28], we showed, based on the analysis of the kinetic and spectroscopic properties of $Tyr_Z/Tyr_Z^{\bullet}$ and $P_{680}/P_{680}^{+\bullet}$ that the protein structure in the vicinity of the Tyr_Z, likely the hydrogen bond between the phenol group of Tyr_Z and D1/His190, is modified in PsbA2-PSII as compared to PsbA(1/3)-PSII. This change is critical to the function of Tyr_Z as its rapid reversible oxidation by $P_{680}^{+\bullet}$ relies on the shuttling of its phenolic proton to the nitrogen (2.46 Å between the O and N) of D1/His190 forming the stabilized neutral tyrosyl radical [29]. An increase in the distance between the acid/base residues is expected to alter the dynamics of the proton coupled electron transfer processes associated with the oxidation of Tyr_Z [30]. Proline is known to constrain the specific angles of the NH-C = O peptide bond and thus to possibly change the orientation of the downstream helix, *e.g.* [31]. Thus we hypothesized that the Cys144 to Pro144 and Pro173 to Met173 substitutions in PsbA2 from PsbA1 and PsbA3, located upstream of the α-helix bearing Tyr_Z and between the two α-helices bearing Tyr_Z (helix C in Fig. 1) and its hydrogen-bonded partner, D1/His190 (helix D in Fig. 1) were responsible for the differences in the structure of the Tyr_Z-His190 couple (Fig. 1). PsbA2 in *T. elongatus* represents one of the few photosynthetic species already sequenced in which the D1-173 amino acid is not a Pro. These include PsbA0 in *Anabaena* (*alr*3742) with a Met and D1-4 in *Gloeobacter violaceus* PCC 7421 (*glr*1706) with a Ser. Therefore, in order to assess the hypothesis according to which substituting Met173 for Pro173 in PsbA2 for PsbA3 accounts for the structural changes in the vicinity of Tyr_Z and thus for the functional characteristics described above, we used these two observables and compared the sets of pairs: the PsbA3/Pro173Met and PsbA2/Met173Pro pair and the PsbA2-PSII and PsbA3-PSII pair.

In this work, using EPR spectroscopy and time resolved absorption spectroscopy, site directed-mutants of the amino acid residue at position 173 in PsbA2 and PsbA3 were studied by reengineering PsbA2 to mimic the PsbA3 structure for Tyr_Z and *vice versa*. In the PsbA3/Pro173Met-PSII and PsbA2/Met173Pro-PSII mutants, we show that the Pro173Met substitution in PsbA2-PSII *versus* PsbA3-PSII is the main structural parameters that affects the electron transfer between Tyr_Z and P_{680}.

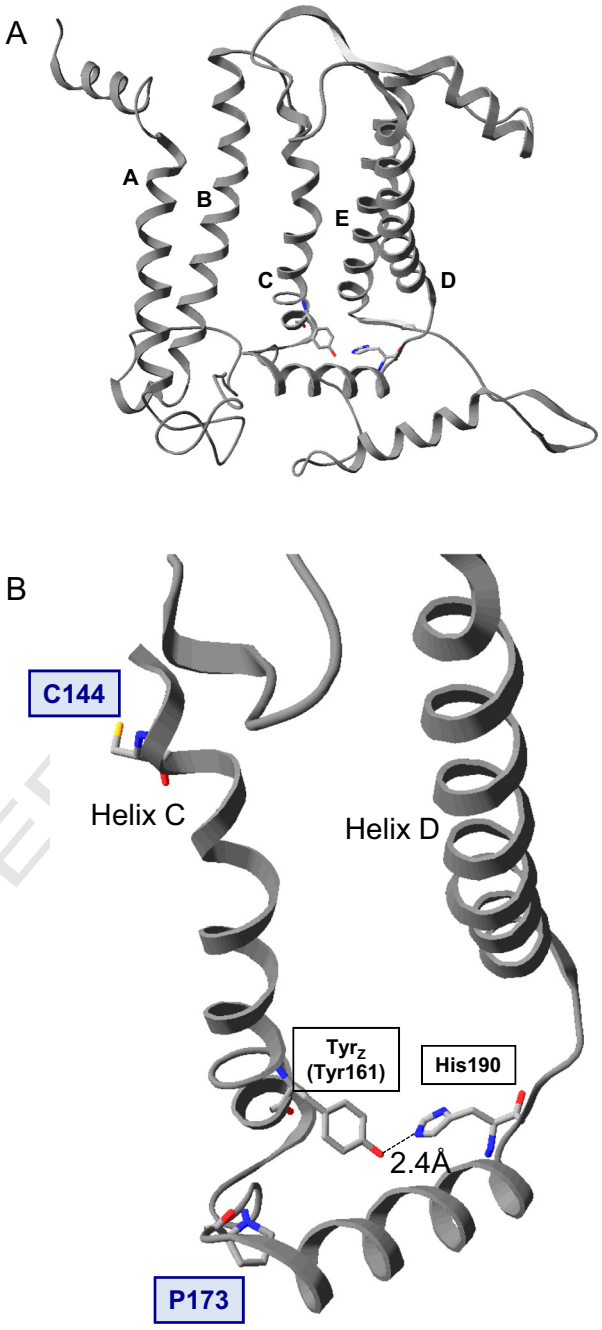

Fig. 1. Structure of D1 helices (A) and around Tyr_Z and His190 (B) from the 1.9 Å resolution structure model of Umena et al. [1]. Tyr_Z (D1/Tyr161) and His190 are belonging to helix C and helix D, respectively. The H-bond distance between Tyr_Z and His190 is 2.4 Å. Pro173 and Cys144 in PsbA2 and PsbA3 were substituted. Figures were drawn with Swiss Pdb Viewer with PDB 3ARC.

2. Materials and methods

2.1. T. elongatus mutant strains

In this study, purified PSII from *T. elongatus* WT*2 [28] and WT*3 [32] cell strains were used as controls. WT*2 and WT*3 are both His-tagged strains on the C-terminus of CP43 [33] with PsbA2 and PsbA3 as the D1 protein by deletion of $psbA_1/psbA_3$ and $psbA_1/psbA_2$, respectively [28,32]. Fig. 2A shows a map of the constructed plasmid DNA, pUPsbA2/Cm for making the PsbA2/Met173Pro site directed mutant in *T. elongatus*. For the expression of the $psbA_2$ gene under the control of the $psbA_3$ promoter instead of the $psbA_2$ promoter, a 1291 bp DNA

Please cite this article as: M. Sugiura, et al., The D1-173 amino acid is a structural determinant of the critical interaction between D1-Tyr161 (Tyr_Z) and D1-His190 in Photosystem II, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbabio.2014.08.008