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Corrigendum to "Influence of Histidine-198 of the D1 subunit on the properties of the primary electron donor, P_{680}, of photosystem II in *Thermosynechococcus elongatus*"

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ABSTRACT

Two mutants, D1-H198Q and D1-H198A, have been previously constructed in *Thermosynechococcus elongatus* with the aim at modifying the redox potential of the $P_{680}^{\bullet+}/P_{680}$ couple by changing the axial ligand of P_{D1}, one the two chlorophylls of the Photosystem II primary electron donor [Sugiura et al., Biochim. Biophys. Acta 1777 (2008) 331–342]. However, after the publication of this work it was pointed out to us by Dr. Eberhard Schlodder (Technische Universität Berlin) that in both mutants the pheophytin band shift which is observed upon the reduction of Q_A was centered at 544 nm instead of 547 nm, clearly showing that the D1 protein corresponded to PsbA1 whereas the mutants were supposedly constructed in the *psbA*$_3$ gene so that the conclusions in our previous paper were wrong. O_2 evolving mutants have been therefore reconstructed and their analyze shows that they are now correct mutants which are suitable for further studies. Indeed, the D1-H198Q mutation downshifted by ≈3 nm the $P_{680}^{\bullet+}/P_{680}$ difference absorption spectrum in the Soret region and increased the redox potential of the $P_{680}^{\bullet+}/P_{680}$ couple and the D1-H198A mutation decreased the redox potential of the $P_{680}^{\bullet+}/P_{680}$ couple all these effects being comparable to those which were observed in *Synechocystis* sp. PCC 6803 [Diner et al., Biochemistry 40 (2001) 9265–9281 and Merry et al. Biochemistry 37 (1998) 17,439–17,447]. We apologize for having presented wrong data and wrong conclusions in our earlier publication.

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1. Introduction

The light-driven oxidation of water in Photosystem II (PSII) is a key step in photosynthesis, the process that is the main input of energy into biology and is thus responsible not only for the production of biomass including food, fibre and fuels (fossil and non-fossil) but also putting O_2 into the atmosphere. PSII in cyanobacteria is made up of 17 membrane proteins and 3 extrinsic proteins. Altogether these 20 subunits bear 35 chlorophylls, 2 pheophytins (Phe), 2 hemes, 1 non-heme iron, 2 plastoquinones (Q_A and Q_B), a Mn_4CaO_5 cluster, 2 Cl^-, 12 carotenoids and 25 lipids [1,2].

The excitation resulting from the absorption of a photon is transferred to the photochemical trap P_{680}, which is composed of four chlorophyll *a* molecules, P_{D1}/P_{D2} and Chl_{D1}/Chl_{D2}, and two pheophytin *a* molecules, Phe_{D1}/Phe_{D2}. Charge separation then occurs. After some picoseconds the positive charge is mainly stabilized on P_{D1} but this is often termed P_{680}^{+}. P_{680}^{+} then oxidizes Y_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] for reviews. The Mn_4CaO_5 cluster 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, *e.g.* [5–9].

A consensus has emerged that primary charge separation in PSII occurs between Chl_{D1} and $Pheo_{D1}$, *e.g.* [10,11]. In a few ps, the $P_{680}^{\bullet+}Pheo_{D1}^{\bullet-}$ state is then formed with 80% of the cation residing on P_{D1} and 20% on P_{D2} [3], see also [12] for similar calculated values based on the recent high-resolution PSII structure [1]. Then, the pheophytin anion transfers the electron to the quinone, Q_A. P_{680}^{+} is reduced by Tyr_Z, at position 161 of the D1 protein. $Tyr_Z^{\bullet}$ is in turn reduced by the Mn_4CaO_5 cluster.

Abbreviations: PSII, Photosystem II; Chl, chlorophyll; MES, 2–(*N*–morpholino) ethanesulfonic acid; P_{680}, chlorophyll dimer acting as the second electron donor; Q_A, primary quinone acceptor; Q_B, secondary quinone acceptor; 43H, *T. elongatus* strain with a His–tag on the C terminus of CP43; WT*3, cells containing only the *psbA*$_3$ gene; $Pheo_{D1}$, pheophytin; P_{D1} and P_{D2}, Chl monomer of P_{680} on the D1 or D2 side, respectively; E_m, redox potential *versus* SHE; TL, Thermoluminescence; DCMU, 3–(3,4–dichlorophenyl)–1,1–dimethylurea; PPBQ, phenyl *p*–benzoquinone; DCBQ, 2,6 dichloro-1,4 benzoquinone; β–DM, *n*–dodecyl–β–maltoside; Cm, chloramphenicol; Gm, gentamycin; Sm, streptomycin; Sp, spectinomycin.

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An extraordinary feature of PSII amongst reaction centre complexes is the high redox potential of the $P^{\bullet+}_{680}/P_{680}$ couple which is required to drive water oxidation, *e.g.* [13]. Possible reasons for the elevated value compared to Chl*a in vitro* include protein-pigment interactions, such as H-bonding, *e.g.* [12,14–16], pigment-cofactor interactions [17–21], a low dielectric environment [22] and puckering of pigment molecules [23].

The importance of the amino-acid ligand, D1-His198, on the functional properties of P_{D1} has been examined by mutagenesis in the mesophilic cyanobacterium *Synechocyctis* sp. PCC 6803 [10]. Of the 11 mutants examined only 3 were found to be photoautotrophic: H198Q, H198A and H198C mutants. Interestingly, in the D1-H198A mutant the Mg atom was conserved despite the lack of any proteinaceous ligand. The likely substitution of the His ligand by a water molecule had nevertheless some influence on the $P^{\bullet+}_{680}/P_{680}$ absorption difference spectrum which was down-shifted by ≈2 nm in the Soret region. This spectroscopic change was accompanied by a decrease of ≈80 mV in the estimated mid-point redox potential of the $P^{\bullet+}_{680}/P_{680}$ couple. In the D1-H198Q mutant, in which glutamine could possibly act as a ligand to Mg, the $P^{\bullet+}_{680}/P_{680}$ absorption difference spectrum was downshifted by 3–4 nm in the Soret region but the redox potential was almost unchanged in PSII core complexes. Thus, the correlation between the spectral changes and the redox properties of P_{680} were not obvious in these mutants [10]. This prompted us to study the role of the D1 axial ligand to P_{D1} in the thermophilic cyanobacterium *Thermosynechococcus elongatus* from which fully active PSII can be purified and importantly for which the PSII structure likely identical to that one of *T. vulcanus* is known [1,2]. To achieve this aim we have described the development of a mutagenesis system for the construction of D1 mutants in *T. elongatus* and the characterization of D1-H198Q and D1-H198A mutants by low-temperature fluorescence, thermoluminescence, oxygen-evolution, FTIR spectroscopy and time-resolved absorption spectroscopy has been reported [24].

The two mutants, D1-H198Q and D1-H198A, were constructed in a strain (WT*3) in which the *psbA*$_1$ and *psbA*$_2$ gene were previously deleted so that this strain expressed only the *psbA*$_3$ gene. However, after the publication of this earlier work [24] and in order to investigate the Qy region of the $P^{\bullet+}_{680}/P_{680}$ difference spectra, it was observed by Eberhard Schlodder from the Technische Universität in Berlin (Max Volmer Lab, Str 17 Juni 135, D-10623 Berlin, Germany) that in both mutants the pheophytin band shift which is observed upon the reduction of Q_A was centered at 544 nm instead of 547 nm clearly showing that the D1 protein corresponded to PsbA1 whereas the mutants were constructed in the *psbA*$_3$ gene. The red shift by ≈3.0 nm of the C550 band–shift for the PsbA3–PSII sample, relative to the PsbA1–PSII sample, reflects the stronger hydrogen bond to the ^{1}13–keto of the $Pheo_{D1}$ from the carboxylate group of E130 in PsbA3–PSII than from Q130 in PsbA1–PSII, as previously shown [25,26].

Since to determine at which step the contamination of the strains occurred in our earlier work was impossible we decided to reconstruct the two mutants and to redo the most important experiments with new control experiments in order to add a *corrigendum* on our previous conclusions instead of presenting a simple retraction.

2. Materials and methods

The site-directed mutant strains PsbA3/H198A and PsbA3/H198Q were reconstructed from *T. elongatus* 43-H cells [27]. First, site-directed mutations were done in the *psbA*$_3$ gene in the 43H strain, *i.e.* in the strain which contains the three *psbA* genes, by using a spectinomycine (Sp)/streptomycine (Sm) resistant cassette in a site-directed plasmids as described in [24]. Segregation of all the *psbA*$_3$ copies in genome was confirmed by digestion of *psbA*$_3$ with *Pvu* II after PCR amplification of the mutated region by using the *P3* (5′- CCAGGCACTCA ACTGGAGTTGTGAACGGTT -3′) and *P4* primers (5′ CCACCGAAACCGAA TCTGCCAACTACGGTT −3′) (Fig. 1).

Then, both the H198A and H198Q mutants were knocked out by substitution of gentamycine (Gm) resistant cassette and chloramphenicol (Cm) resistant cassette for the *psbA*$_1$ - *psbA*$_2$ genes, respectively. Fully segregated *psbA*$_1$/*psbA*$_2$-deletion mutants were selected by growth on either Gm or Cm for H198A and H198Q, respectively. Their genotype were confirmed by PCR analysis using the *P1* primer (5′ GGGCACCACTC GAATGGTTTGCTCGTGG −3′) and *P2* primer (5′ ACCTCTCTAGTGATAA GTAGTGATAAGTCC −3′) as shown in Fig. 1A and D. Plasmid DNA were introduced into *T. elongatus* cells by electroporation (BioRad gene pulser). The segregated cells were selected as single colonies on DTN agar plates containing 25 μg Sp mL^{-1}, 10 μg Sm mL^{-1}, 40 μg Km mL^{-1} and 5 μg Cm mL^{-1}.

Cells were cultivated and the PSII complexes were purified as previously described [28]. Absorption changes were measured with a lab–built spectrophotometer [29] where the absorption changes are sampled at discrete times by short analytical flashes. These analytical flashes were provided by an optical parametric oscillator pumped by a neodymium:yttrium–aluminum garnet laser (Nd:YAG, 355 nm), which produces monochromatic flashes (1 nm full–width at half–maximum) with a duration of 5 ns. Actinic flashes were provided by a second neodymium:yttrium–aluminum garnet laser (Nd:YAG, 532 nm), which pumped an optical parametric oscillator producing monochromatic saturating flashes at 695 nm (1 nm full–width at half–maximum) with a duration of 5 ns. The path length of the cuvette was 2.5 mm. PSII was used at 25 μg of Chl mL^{-1} in 1 M betaine, 15 mM $CaCl_2$, 15 mM $MgCl_2$, and 40 mM MES (pH 6.5). PSII were dark–adapted for ≈1 h at room temperature (20–22 °C) before the addition of 0.1 mM phenyl *p*–benzoquinone (PPBQ) dissolved in dimethyl sulfoxide.

Thermoluminescence (TL) glow curves were measured with a lab–built apparatus [30,31]. PSII core complexes were diluted to 10 μg Chl mL^{-1} in 1 M betaine, 40 mM MES, 15 mM $MgCl_2$, 15 mM $CaCl_2$, pH 6.5 and then dark–adapted for 1 h at room temperature. Before loading the sample, 50 μM DCMU were added to the dark–adapted samples. The samples were illuminated at 0 °C by using a saturating xenon flash. Immediately after the flash, the samples were heated at the constant heating rate indicated in the legend of the figure and TL emission was detected. It was checked that, after dilution, the 3 PSII samples had a similar OD at 673 nm.

The O_2 activity was measured at 25 °C by using a Clark type oxygen electrode (Hansatech) with continuous saturating white light through infrared and water filters. The activity was measured over a period of 1.5 min in the presence of 0.5 mM 2,6–dichloro–*p*–benzoquinone (dissolved in dimethyl sulfoxide) as an electron acceptor.

Cw-EPR spectra were recorded using a standard ER 4102 (Bruker) X-band resonator with a Bruker Elexsys 500 X-band spectrometer equipped with an Oxford Instruments cryostat (ESR 900). The PSII samples at 1.1 mg of Chl mL^{-1} were loaded in the dark into quartz EPR tubes and further dark-adapted for 1 h at room temperature. Then, the samples were frozen in the dark to 198 K and then transferred to 77 K in liquid N_2. Illuminations with visible light for approximately 5–10 s with a 800 W tungsten lamp filtered by water and infrared cut-off filters at temperatures close to 200 K were done in a non-silvered dewar in ethanol cooled down with dry ice. No artificial electron acceptors were added to probe the redox state of the PSII electron acceptor side. Prior to the measurements all the samples were degassed at 198 K.

3. Results and discussion

3.1. Intactness of the mutant PSII

D1-H198Q and D1-H198A PSII core complexes exhibited an O_2 evolving activity of ≈3300 μmol O_2 (mg Chl)$^{-1}$ h^{-1} and ≈3000 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, respectively, thus confirming that these mutations did not affect the formation of a PSII with a high activity. The EPR results shown in Fig. 2 also show that the S_2 multiline signal induced by 200 K

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