Algal Research 18 (2016) 296–304

Contents lists available at ScienceDirect

Algal Research

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

Changes in the Photosystem II complex associated with hydrogen formation in sulfur deprived *Chlamydomonas reinhardtii*

Alena Volgusheva [c], Olaf Kruse [b], Stenbjörn Styring [a], Fikret Mamedov [a,*]

[a] *Molecular Biomimetics, Department of Chemistry — Ångström Laboratory, Box 523, Uppsala University, 751 20 Uppsala, Sweden*
[b] *Department of Biology,Center for Bitoechnology (CeBiTec) University of Bielefeld, 33501 Bielefeld, Germany*
[c] *Department of Biophysics, Faculty of Biology, Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 31 May 2016
Accepted 27 June 2016
Available online 12 July 2016

Keywords:
Photosystem II
Chlamydomonas reinhardtii
Sulfur deprivation
H_2 production
Fluorescence
Thermoluminescence

ABSTRACT

Redox properties of the acceptor side of Photosystem II were studied during H_2 gas production in cells of *Chlamydomonas reinhardtii*. Flash-induced variable fluorescence changes and thermoluminescence measurements were performed in wild type and Stm6 mutant cells during different stages of sulfur (S)-deprivation. Analysis of the fluorescence decay kinetics indicated that the forward electron transfer on the acceptor side of Photosystem II was dramatically slowed down during the O_2 evolution and O_2 consumption stages and was completely blocked in the anaerobic stage of S-deprivation, thus, indicating a complete reduction of the PQ-pool. During the H_2 formation stage, the forward electron transfer kinetics in the µsec and msec time scale re-appeared indicating partially restored electron flow from Q_A^- to Q_B and the PQ-pool. Thermoluminescese measurements fully confirmed the fluorescence kinetic analysis. Activation of hydrogenase in the H_2 formation stage is responsible for re-oxidation of the PQ pool and reactivation of the electron flow which was found to be faster and more efficient on the Stm6 mutant due to the higher amount of functionally preserved Photosystem II.
© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Sun and water are the most abundant natural resources available in the biosphere. The only organisms which can utilize these two resources are those possessing oxygenic photosynthesis. A variety of green algae and cyanobacteria are able to use photosynthetic reactions to produce hydrogen (H_2) gas, one of the most promising future renewable energy carriers [1,2]. This means that electrons and protons extracted from water by Photosystem II (PSII) [1] are utilized by hydrogenases for H_2 synthesis [3,4]. H_2 has the high energy content and clean combustion back to water, making it the ideal solar fuel [5].

The hydrogenase enzyme of green algae belongs to the di-iron type of hydrogenases and possesses the highest turnover rate (few thousands per second) among many species [6,7]. However, its activation requires the absence of oxygen, making the direct photosynthetic H_2 formation very short lived. The reason is that the oxygen binds to the active site of enzyme and irreversibly inhibits its activity [8,9]. Thus, separation of PSII and hydrogenase activities either in space or time is necessary for the sustained H_2 evolution over the prolonged period of time. The first option is not easy since algal hydrogenase is localized in the stroma of chloroplast. Separation in time is more straightforward and the two-stage process based on the sulfur (S)-deprivation was shown to result in sustained, at least for several days, light-inducible H_2 evolution in *Chlamydomonas reinhardtii* [10].

Since sulfur is an essential component of the protein biosynthesis, S-deprivation induces dramatic changes in the algal cell metabolism. Upon application of the S-deprivation procedure, cell division is heavily impaired and accumulation of endogenous protein and starch peaks in the first few hours, resulting in significant morphological changes. Furthermore, the assimilation of CO_2 is ceased and the level of Rubisco becomes almost undetectable after 48 h [10,11].

Termination of CO_2 fixation during S-deprivation was shown to have a significant effect on the primary photosynthetic reactions. The amount of all photosynthetic complexes involved in the electron transfer from water to $NADP^+$ is gradually decreased in the following order: Cytochrome b_6f complex > PSI > PSII. PSII is the most affected complex during S-deprivation [10,11].

PSII is a multisubunit protein complex localized in the thylakoid membranes of cyanobacteria, green algae and plants which uses light energy to extract electrons from water and to reduce the PQ-pool [12]. The latest crystal structure reported from thermophile cyanobacteria defines the PSII core complex with 20 protein subunits and all redox cofactors at 1.9 Å resolution [13]. After excitation of the primary electron donor in PSII, a Chl tetramer, P680, charge separation takes place

Abbreviations: Chl, chlorophyll; PQ, plastoquinone; PSII, photosystem II; Q_A and Q_B, primary and secondary plastoquinone acceptors in photosystem II; TAP, tris-acetate-phosphate medium; Y_Z and Y_D, redox active tyrosines in photosystem II..

* Corresponding author.
E-mail address: fikret.mamedov@kemi.uu.se (F. Mamedov).

http://dx.doi.org/10.1016/j.algal.2016.06.025
2211-9264/© 2016 Elsevier B.V. All rights reserved.

between P680 and pheophytin molecule within few picoseconds. After this primary event electron is transferred to the first quinone acceptor, Q_A in about 200 psec. Charge separated state is then stabilized by the reduction of $P680^+$ by a redox active tyrosine residue on the D1-protein, Y_Z in the nsec time range. The charge separation is further stabilized when on the acceptor side of PSII electron is transferred from Q_A^- to the secondary quinone acceptor Q_B (microseconds to milliseconds depending on the occupancy of the Q_B-site). Double reduced and protonated Q_B (after two consecutive charge separations) leaves PSII in the plastoquinole form and is replaced by the oxidized PQ molecule [12]. On the donor side of PSII, $Y_Z^\bullet$ is reduced by the $CaMn_4O_5$-cluster, a catalytic site where the water oxidation occurs. Four consecutive charge separations are needed to oxidize two water molecules to molecular O_2 in the so-called S-cycle. Depending on the S-state, $Y_Z^\bullet$ re-reduction occurs in μsec to msec time range [14].

During cultivation of *C. reinhardtii* under the S-deprivation conditions, the amount of PSII in the thylakoid membrane is decreasing. This was underlined by the decrease of the absorption changes associated with Q_A reduction, and the decrease of the D1-protein level [10]. The loss of PSII centers was accompanied by the decrease in the overall rate of O_2 evolution. In sealed bioreactors this results in the anaerobic conditions in the culture due to the unchanged or sometimes even enhanced respiration which overtake the decreasing PSII activity. A few hours after the establishments of anaerobic conditions, the H_2 evolution could be detected indicating that hydrogenase was expressed and activated. However the residual PSII activity was reported to be important for this process indicating a direct link between water oxidation and H_2 formation [3,15].

We recently quantified changes in the PSII amount during different stages of the S-deprivation procedure in the wild type (WT) and the **S**tate **T**ransition **M**utant 6 (Stm6) of *C. reinhardtii* [4]. The Stm6 mutant with a perturbed cyclic electron flow was first described in [16]. The mutant is lacking the Moc1 gene involved in the assembly of the mitochondrial respiratory chain and has been shown to have elevated H_2 production under the S-deprived conditions [17]. In this work we present the detailed analysis of the flash-induced fluorescence decay kinetics and thermoluminescence measurements in both strains and we show that decrease in the PSII amount during S-deprivation and H_2 evolution is accompanied with regulation of the electron transfer on the acceptor side of PSII [4].

2. Materials and methods

2.1. Cell growth conditions and sulfur depletion

C. reinhardtii WT cc406 and Stm6 mutant strains [17] were grown photoheterotrophically in TAP medium, pH 7.0, in Erlenmeyer flasks at 25 °C under continuous illumination (70 $\mu E\ m^{-2} \times s^{-1}$) and constant shaking. After reaching the mid-logarithmic phase (25–30 μg Chl ml^{-1}), the cells were pelleted by centrifugation (3000 ×*g* for 2 min). The pellet was washed three times in S-depleted medium (pH 7.7) and resuspended in the same medium (TAP–sulfur) to a final concentration of Chl about 15–17 μg Chl ml^{-1}. The samples at this time point are defined as the *control* samples in all experiments. This was followed by 130 h of incubation in sealed bioreactors at 25 °C under the same light and mixing conditions. Aliquots of the suspension were taken from the bioreactors at the defined time points and dark adapted for 5 min at 20 °C before any measurements were performed as described in [4].

2.2. Measurement of O_2 exchange and H_2 production

Respiration in the dark and light induced O_2 evolution were measured at 25 °C with a Clark type O_2 electrode as described in [4]. The dissolved O_2 concentration in the algal suspension was measured with an oxygen analyzer MAPK-302T, Russia, which was placed aseptically in the bioreactor.

H_2 concentration was determined using a Clarus 500 gas chromatograph (Perkin Elmer, Shelton, CT, USA) complete with Molecular Sieve 5A 60/80 Mesh column, Perkin Elmer, and Ar as a carrier gas.

2.3. Fluorescence and thermoluminescence measurements

Flash-induced changes in the chlorophyll fluorescence yield (variable fluorescence decay kinetics) were measured as with the FL3300 dual-modulation fluorometer (Photon System Instruments, Czech Republic) in the 150 μs–100 s time range. Actinic flash duration was 30 μs [4]. The measurements were done in the absence or presence of 20 μM DCMU.

Thermoluminescence signal was detected with TL200/PMT thermoluminescence system (Photon System Instruments). Aliquots of cell suspension were cooled down to −10 °C and were excited by the actinic flash of 50 μs duration. The sample then was then heated to 60 °C at the heating rate of 1 °C/s. The measurements were done in the absence or presence of 20 μM DCMU.

Measurements of samples, taken from the anaerobic and H_2 production stages were performed under anaerobic conditions. All presented numerical data are mean values of experiments done at least in triplicate. Error bars represent standard deviation.

3. Results

3.1. Characterization of the S-deprived cultures

Fig. 1A shows that under photoheterotrophic conditions the growth rates of the WT and Stm6 mutant strains were almost identical. After three and a half days the concentration of cells in both cultures reached ca 12×10^6 cells/ml (arrow in Fig. 1A). The total Chl content ($a + b$) per cell was also similar at this time point (Table 1, control). However, the Chl *a*/*b* ratio value was slightly lower in the Stm6 mutant if compared to the WT, 2.41 and 2.62 respectively (Table 1, control) suggesting a relative increase of the LHCII antenna in the Stm6 mutant.

When photoheterotrophic cell growth in TAP medium has reached the mid log phase (ca 12×10^6 cells/ml, arrow in Fig. 1A), cells were collected, washed 3 times, resuspended in the TAP–sulfur medium and placed in the sealed bioreactors to induce anaerobiosis. During first 72 h of S-deprivation under these conditions cell concentration was unaffected in both WT and Stm6 mutant (Table 1). The total Chl amount was also not changed in the WT cells but somewhat decreased in the Stm6 mutant (by 9%, Table 1). The Chl *a*/*b* ratio, however, slightly increased in both WT and Stm6 cells, which indicates some rearrangement in the antenna complexes and relative decrease of the LHCII during S-deprivation. These data agree in general with the results obtained earlier [11].

Changes in the rates of dark respiration and light-induced O_2 evolution during S-deprivation in both strains are shown in Fig. 1B and C. In the WT culture the rate of O_2 evolution significantly decreased from 266 μmol O_2 (mg Chl)$^{-1} \times h^{-1}$ in the control sample to 49 μmol O_2 (mg Chl)$^{-1} \times h^{-1}$ after 72 h of S-deprivation. In the meantime, the rate of O_2 consumption increased from 33 to 39 μmol O_2 (mg Chl)$^{-1} \times h^{-1}$ (Fig. 1B). Thus, both rates became quite similar by the end of the experiment.

In the Stm6 mutant the initial rate of O_2 evolution was lower than in the WT and it decreased from 160 to 21 μmol O_2 (mg Chl)$^{-1} \times h^{-1}$ during 72 h of S-deprivation. The O_2 consumption first quickly increased from 51 to 73 μmol O_2 (mg Chl)$^{-1} \times h^{-1}$ during the first 24 h of S-deprivation and then stayed on about the same level until the end of experiment (Fig. 1C). It is also important to notice that after 24 h of S-deprivation the rate of O_2 consumption became higher than the rate of O_2 evolution in the Stm6 mutant (Fig. 1C) explaining the much faster activation of hydrogenase activity as described earlier [15].

Differences in the initial respiration and O_2 evolution rates between the WT and Stm6 mutant resulted in fact that in the Stm6 mutant the

Download English Version:

https://daneshyari.com/en/article/8086749

Download Persian Version:

https://daneshyari.com/article/8086749

Daneshyari.com