Electronic Supplementary Information

Photosystem ratio imbalance promotes direct sustainable H_2 production in Chlamydomonas reinhardtii

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Low temperature fluorescence spectra at 77 K were recorded with FluoroLog-3 spectrofluorometer from Horiba/Jobin Yvon equipped with the finger dewar filled with liquid N_2. Aliquots of cell suspension of the C3 mutant of C. reinhardtii were withdrawn from bioreactors at the different time points of H_2 production (see Fig. 2) and frozen under anaerobic conditions in 4 mm diameter quartz EPR tubes before the measurements. Emission spectra were collected with excitation light of 440 nm in the range of 625 – 800 nm and the results are shown in Fig. 1S.

Deconvolution of the spectral components in the control sample show 3 fluorescence peaks at 685 nm, 710 nm and 750 nm (Fig. 1S, A). In C. reinhardtii, similarly to other oxygenic photosynthetic organisms, the F685 peak originates from the PSII reaction center and associated antenna, while the F710 nm peak originates from the PSI reaction center and associated antenna (1-3). F750 peak most probably is arising from the long-wavelength light harvesting antenna associated with PSI (LHCI) (3)

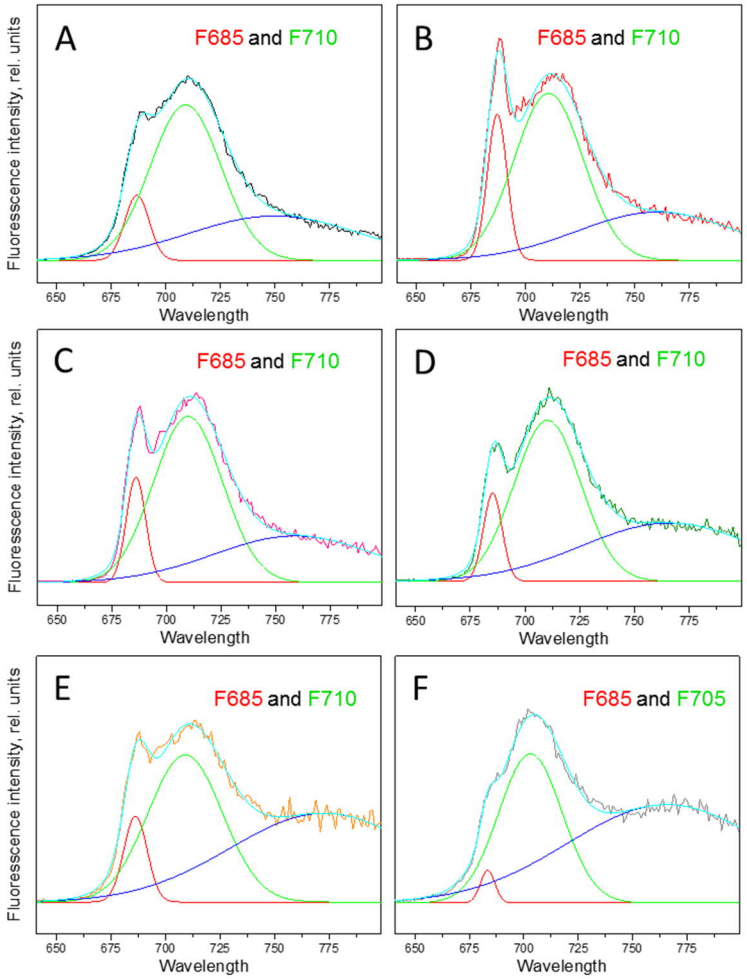

Figure 1S. Chl fluorescence emission spectra from H_2 producing C. reinhardtii cells after 0 hrs of incubation in bioreactors (control, A), 1 day (B), 6 days (C), 12 days (D), 20 days (E) and 30 days (F). Gaussian deconvolution shows contributions from the PSII peak (red, F685), PSI peak (green, F710) and from peak at 750-770 nm (blue).

which is disconnected from the PSI reaction center and present in the thylakoid membranes of C3 mutant.

State transition in photosynthesis is known to balance the energy distribution between PSII and PSI by lateral movement and association of the LHCII with either PSII or PSI (1, 2). The overall shape of the fluorescence emission with the dominating F710 peak indicates that cells of the C3 mutant are mostly present in State 2 where part of the PSII antenna LHCII is moved and associated with PSI (1, 2). The overall intensity of fluorescence emission has decreased during the H_2 production stage which is in agreement with the decrease of total amount of Chl (Table 1). Interestingly, after one day of incubation, the F685 peak intensity increased relatively to the F710 peak (Fig. 1S, B). This is correlated with the temporal increase of the total Chl amount during this period of anaerobic incubation (Table 1). The most likely reason for this is that the part of LHCII antenna is re-associated with the PSII reaction center during first 6 days of H_2 production as a consequence of reactivation of the PSII mediated electron transfer. Then the F685 peak started to decrease and after 30 days was observed only as a shoulder of the PSI peak (Figs. 1S, C-F). This reflect the overall decrease in the amount of PSII which also correlates with our EPR data (Table 1, Fig, 3A). Interestingly, the PSI peak was shifted to 705 nm after 30 days of H_2 production (Fig. 1S, F).

Thermoluminescence measurements were done with TL200/PMT system (Photon System Instruments, Czech Republic) as described before (4). Aliquots of cell suspension of the C3 mutant of C. reinhardtii were withdrawn from bioreactors at the different time points of H_2 production (Fig. 2) and measured under anaerobic conditions. The sample was cooled down to -5 °C and excited by actinic flash of 50 μsec duration. The sample was then heated to +50 °C with the hating rate of 1 °C/sec.

Thermoluminescence measurements are shown in Fig. 2S. Two bands were observed after application of a single flash in the control C3 mutant cells – at 19 °C and 36 °C (Fig. 2S, B). The last one represent the B_1-band (the Q_B^- – S_2 state recombination) and the dominating latter one is a modified

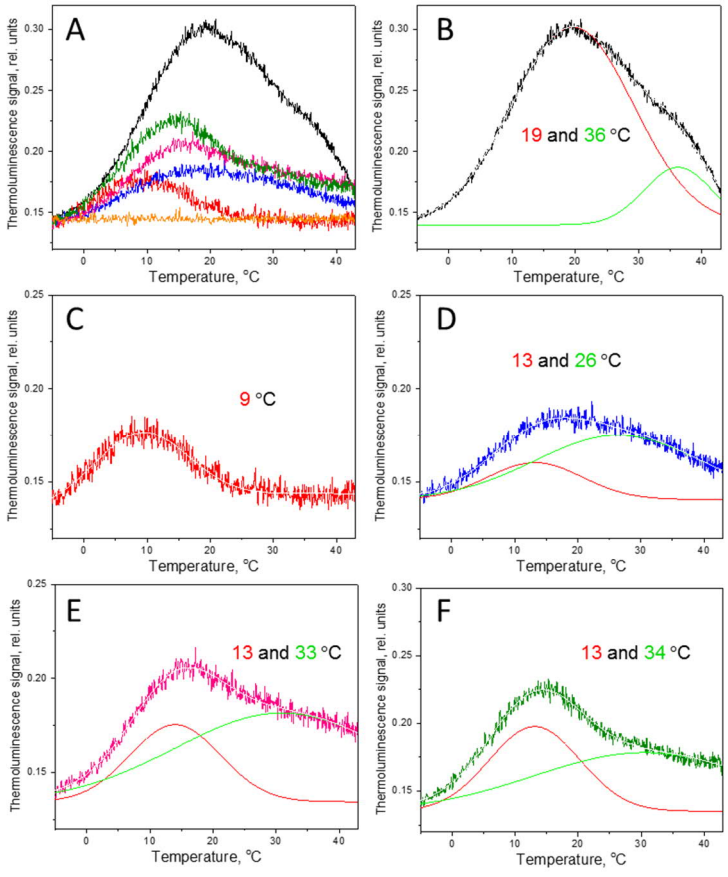

Figure 2S. Thermoluminescence curves from the H_2 producing C. reinhardtii cells after 0 hrs of incubation in bioreactors (control, A, B, black traces), 1 day (A, C, red), 2 days (A, D, blue), 6 days (A, E, pink), 12 days (A, F, green), 20 days (A, orange). Gaussian deconvolution shows contributions from the Q-band (red) and B_1-band (green).

Q-band (the Q_A^- – S_2 state recombination) (4-6). Presence of these two bands reflects more reduced state of the PQ pool in the C3 mutant if compared to WT (Fig. 1D). After 1 day of incubation in bioreactors only Q-band was observed (Fig. 2S, C). This reflects establishment of the anaerobic conditions and full reduction of the PQ pool in the thylakoid membrane in the beginning of H_2 production. In the control samples this band was observed only after addition of DCMU (Fig. 1D) when $Q_A^- \rightarrow Q_B$ electron transfer is completely blocked. However, after 2 days the B_1-band reappeared and was present during next 10 days of H_2 production together with the Q-band which was still persistent (12 days in total, Figs. 1S D-F). The appearance of the B_1-band demonstrate the presence of bound Q_B in PSII after 2 days of H_2 production and therefore, some extent of the forward electron transfer from PSII. It is also noticeable that the total thermoluminescence signal was decreasing with the prolonged H_2 production and after 20 days was below our detection limit (Fig. 1S, A, orange trace). This is due to the decrease in the PSII amount which was also shown by EPR measurements (Fig. 3A). Thus, our thermoluminescence measurements complemented and confirmed our analysis of the flash-induced fluoresce decay kinetics from these samples quite well (Fig. 3C).

Thus, fluorescence emission and thermoluminescence measurements show that in the beginning of H_2 production process the C3 mutant cells are present in State 2 and have the reduced PQ pool. This make sense due to the decreased amount of PSI in this mutant (Fig. 1B). During the first 1-2 days of H_2 production, PSII is reactivated as shown by the temporal increase of the F685 peak (Fig. 1S, B) leading to the increased supply of electron by PSII. This resulted in full reduction of the PQ pool as shown by thermoluminescence data (Fig. 2S, C). After that, the increased amount of PSI (Fig. 3B) helped to reactivate the electron transfer from PSII as shown by reappearance of the B_1-band (Fig. 2S, D-F). This fine tuning of PSII and PSI activities allowed to sustain the H_2 production for several weeks in a row.

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