Electronic Supplementary Information

Hydrogen photoproduction in green algae *Chlamydomonas reinhardtii* under magnesium deprivation

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Flash-induced Chl fluorescence decay kinetics in cells of aerobic culture of *C. reinhardtii* under Mg-deprivation – The decay of flash-induced variable Chl fluorescence provides detailed information about the electron transfer reactions in PSII [1-3]. Fig. 1A shows typical fluorescence decay in *C. reinhardtii* cells incubated for seven days in the complete or Mg-free medium at pH 7.0. Illumination of the cells suspension with a single-turnover saturating flash results in the reduction of Q_A, which leads to the increased fluorescence yield (F_M). Subsequent dark re-oxidation of Q_A^- results in relaxation of the fluorescence yield and could be characterized by three decay phases with different lifetimes: the fast phase (τ ~100-600 μs), the middle phase (τ ~2-15 ms) and the slow phase (τ ~1-3 s). The first phase reflects Q_A^- to $(Q_B)/Q_B^-$ electron transfer, the middle phase reflects electron transfer to Q_B which first has to bind to the Q_B site and the slow phase reflect recombination with the S_2-state of the water oxidizing complex in PSII (WOC) [1-4].

In our control samples (Fig. 1A, Table 1, TAP), the fast phase (τ ~300 μs) contributed to 73% of the total Chl fluorescence decay and indicates efficient electron transfer from Q_A^- to Q_B. The middle phase (τ ~46 ms, A_2 16%) with the relative amplitude of about 16% reflects the Q_B binding and is quite slow compared to the literature data (2-15 ms) for chloroplasts and thylakoid membranes [1, 5]. This difference may be due to the fact that algae cells possess more reduced PQ-pool due to the presence of chlororespiratory pathways, if compared to plant chloroplasts [6]. Similar results were observed in experiments with whole *C. reinhardtii* cells, where the lifetime of the middle phase was found to be ~35 ms [7]. The slow phase (τ ~8 s, 11%), is associated with the recombination between Q_A^- and the donor side in PSII reaction centers incapable of transferring an electron from Q_A^- to Q_B.

In starved cells (Fig. 1A, Table 1, TAP-Mg), the lifetime of the fast phase was doubled if compared to the control cells (TAP), indicating significantly slower electron transfer from Q_A^- to Q_B under the Mg-deprivation. The amplitude of this phase was also decreased by about 20%. The

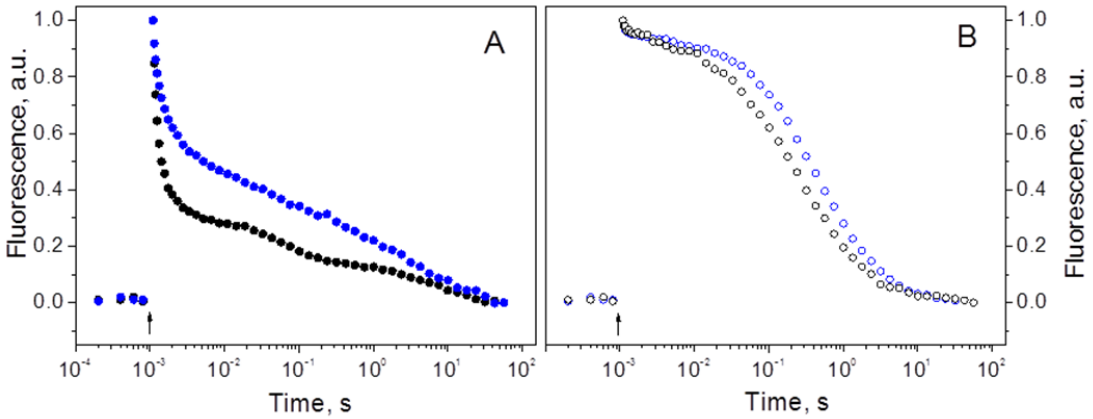

Figure 1. *Normalized flash-induced variable Chl fluorescence decay traces from C. reinhardtii cells. Measurements has been done after seven days incubation of cells in complete (TAP, ●) and Mg-free (TAP-Mg, ●) medium at pH 7.0. Fluorescence decay was measured in the absence (A) and in the presence of 20 μM DCMU (B, ○, ○).*

Table 1. *Analysis of the flash-induced fluorescence decay kinetics in C. reinhardtii. Cells were incubated for 7 days in complete (TAP) and Mg-depleted (TAP-Mg) medium at pH 7.0. The data were obtained from the fitting of the fluorescence relaxation curves shown in Figure 3. The numbers represent the lifetime (s) and amplitude (A) ± SE of the estimation. np – not present.*

Medium	**Fast phase**		**Middle phase**		**Slow phase**	
	τ, µs	**A, %**	**τ, ms**	**A, %**	**τ, s**	**A, %**
No addition						
TAP	229±80	73±4	45.7±0.7	16±1	7.51±1.3	11±0.7
TAP-Mg	587±60	51±2	81.3±1.2	24±2	8.54±0.9	25±1
+DCMU						
TAP	np	np	110±0.1	49±2	1.29±0.1	51±3
TAP-Mg	np	np	232±0.1	55±3	2.42±0.2	45±2

lifetime of the middle component in starved cells also demonstrated nearly two-fold increase while its amplitude was about two times greater than in the control. These changes in the electron transfer from Q_A^- to $(Q_B)/Q_B^-$ and Q_B-binding are related to the accumulation of reduced PQ in the membrane and decrease in the apparent equilibrium constant between Q_A and Q_B [1,4,7,8]. As a consequence, the amplitude of the slow component increased by 14% suggesting increase in the relative amount of non Q_B-reducing centers of PSII under the Mg-deprivation (Table 1).

In presence of DCMU, an inhibitor which blocks the electron transfer between Q_A and Q_B, the fluorescence relaxation reflects re-oxidation of Q_A^- via the recombination pathway with the donor side of PSII and can be fitted with two exponential components (Fig. 1B, Table 1). The origin of the first component (middle phase) could be assigned to the fast charge equilibrium between the $CaMn_4O_5$-cluster in the S_2-state and $Tyr_Z^\bullet$. The second component is associated with the slow charge recombination between Q_A^- and the S_2-state itself [1,3]. The lack of Mg in the growth medium resulted in the two-fold increase in the fluorescence lifetime of both components (Table 1). These results also demonstrate more reduced state of the thylakoid membrane and, as a consequence, an increased stability of the S_2-state of WOC under Mg-deprivation.

Thermoluminescence characteristics of aerobic culture of *C. reinhardtii* under Mg-deprivation – Thermoluminescence measurements are very useful complement to the fluorescence measurements and help to understand changes in the electron transport in PSII [9-12]. In our experiments, algal suspension was cooled to -10 °C and then illuminated by a short flash to induce a single charge separation in PSII. The generated charge separated states are stabilized as $S_2Q_B^-$ and $S_2Q_A^-$ pairs. The $S_2Q_A^-$ state is dominating in the presence of DCMU. Subsequent increasing of temperature of the sample leads to the charge recombination and can be observed as luminescence at different temperatures such as so-called B-band at 30-40 °C, which arises from the recombination of Q_B^- with the S_2 state or Q-band at 10-20 °C, which arises from the recombination of Q_A^- with the S_2-state of WOC [9-12].

At pH 7.0, the maximum peak temperature, T_M, of the B-band was obtained at 35 °C for the control cells (Fig. 2A). In the Mg-deprived cells, T_M of the B-band was shifted by 10 °C toward the lower temperature (Fig. 2B). This indicates decrease in the free energy of activation associated with the recombining species. The amplitude of the B-band for Mg-deprived cells was 24% higher if compared to the control.

It has been shown, that the B-band measured on whole cells could be deconvoluted into two

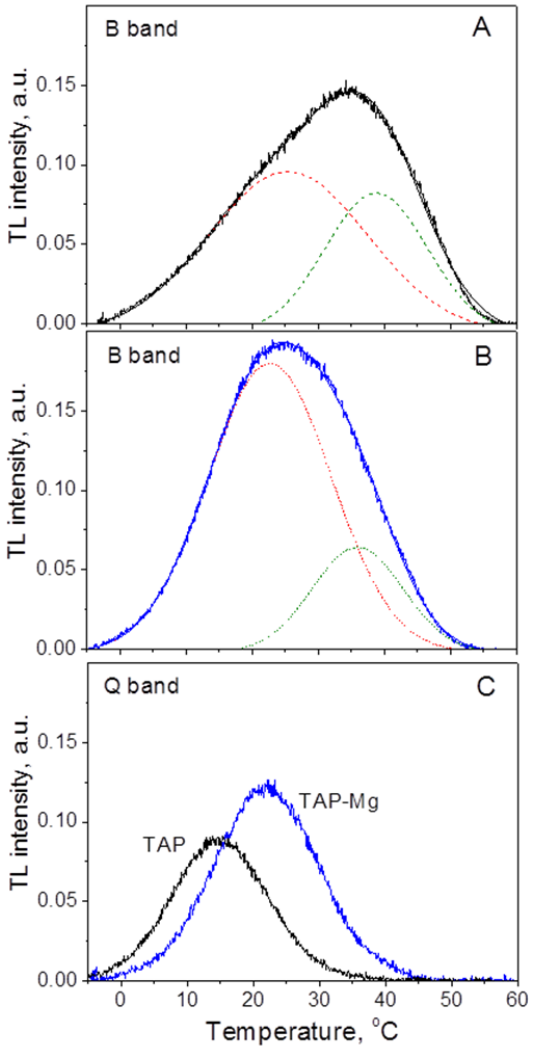

Figure 2. *Effect of Mg-deprivation on thermoluminescence glow curves measured from C. reinhardtii cells. (A) Thermoluminescence curves measured from the control cells (TAP) and (B) after seven days of Mg-starvation (TAP-Mg) at pH 7.0 in the absence (A, B) and the presence of 20 μM DCMU (C). The dashed lines represent deconvolution of the Gaussian sub-components.*

major peaks (Gaussian sub-bands) with different T_M and amplitudes [13]. We performed similar deconvolution, Fig. 2A and B, dashed lines. Two components were deconvoluted from the band from control cells, with T_M at ~39 °C with the amplitude of 47% (green dashed line) and T_M at ~25 °C with the amplitude of 53% (red dashed line, Fig. 2A). These components are consistent with the $S_2Q_B^-$ and $S_2Q_A^-$ recombination bands respectively and represent the "clean" B- and Q-band. This analysis indicates that our flash illumination at -10 °C resulted in 47% of the PSII centers in the $S_2Q_B^-$ state and 53% in the $S_2Q_A^-$ state. This distribution is determined by the flash temperature i.e. not complete electron transfer from Q_A^- to Q_B (most probably restrained Q_B binding at lower temperature) and existing redox equilibria on the acceptor side of PSII. In the Mg-deprived cells (Fig. 2B, dashed lines) the amplitude of $S_2Q_B^-$ component decreased to 20%, while the amplitude of $S_2Q_A^-$ component increased to 80%. The appearance of the dominating $S_2Q_A^-$ component is additional evidence that the PQ-pool was more reduced and the redox equilibrium was shifted towards Q_A^- in the Mg-deprived cells [9-12,14].

In the presence of DCMU, T_M of the Q-band was found to be 14 °C and 21 °C in the control and Mg-deprived cells respectively (Fig. 2C). Thus, T_M in the Mg-deprived cells was shifted by 7 °C toward higher temperature. This suggests higher stabilization energy for the $S_2Q_A^-$ recombination pair and can be explained by the modification of the redox potential of Q_A [15,16]. This modification of the Q_A redox potential is again, most likely, a consequence of the more reduced state of the thylakoid membrane under the Mg-deprivation.

Conclusion – Analysis of the Q_A^- re-oxidation kinetics showed that the decline in the electron transport in the Mg-deprived cells have occurred due to the limitations on the acceptor side of PSII. In particular, Mg-deprivation led to the slower electron transport from Q_A^- to Q_B and promoted the formation of the non Q_B-reducing centers in PSII (Fig. 1, Table 1).

Fluorescence decay data were confirmed by the analysis of the thermoluminescence glow curves (Fig. 2). These measurements also showed inhibition of the electron transport on the acceptor side of PSII in the Mg-deprived cells. Appearance of the dominating Q-band ($S_2Q_A^-$ recombination component) in the Mg-deprived cells is another evidence for the block in the electron transfer from Q_A^- (Fig. 2B).

Thermoluminescence measurements also showed changes in the redox potential of Q_A and/or Q_B (Fig. 2). The deconvoluted B-band, associated with the $S_2Q_B^-$ recombination, was downshifted by 2-3 °C in the Mg-deprived cells (Fig. 2A and B), while the Q-band, associated with $S_2Q_A^-$ recombination, was upshifted by 7 °C (Fig. 2C). This result suggests that the lack of Mg changed the redox potential of both Q_B and Q_A into lower and higher potential forms, respectively. It is known that the opposite shift of the B- and Q-bands is indicative of the changes associated with the acceptor side of PSII, whereas violations of the WOC lead to the shift of the bands in the same direction [17]. It is worth to mention that fluorescence decay measurements in the presence of DCMU also indicated active donor side of PSII (Fig. 2B). Smaller temperature gap between $S_2Q_A^-$ and $S_2Q_B^-$ recombination due to the Mg-deprivation suggests that the redox potential of Q_B become similar to the redox potential of Q_A with equilibrium shifted towards Q_A and, consequently, accumulation of the non Q_B-reducing centers under the Mg-deprived conditions.

Taken together, the fluorescence and thermoluminescence measurements show serious limitations on the acceptor side of PSII which are indicative of the reduced PQ-pool.

Methods – Flash-induced variable fluorescence decay kinetics was monitored with a FL3000 dual modulation fluorometer (Photon Systems Instruments, Brno, Czech Republic) as described in [18]. The fluorescence detection began 100 µs after the single actinic flash (30 µs duration). Samples at a Chl concentration of 10 µg/ml were dark adapted for 3 min before the fluorescence detection. Measurements were done in the presence or absence of 20 µM of DCMU. The experimental data were fitted with three exponential decay components as described in [1].

Thermoluminescence bands were measured with TL200/PMT thermoluminescence system (Photon System Instruments, Brno, Czech Republic). Cells suspension was concentrated to ~30 µg Chl ml^{-1} and dark adapted for 3 min at 20 °C. The thermoluminescence curves were recorded from -8 to +60 °C at a heating rate of 1 °C s^{-1}. Measurements were done in the presence or absence of 20 µM DCMU.

References

1. F. Mamedov, H. Stefansson, P.-Å. Albertsson, S. Styring, *Biochemistry*, 2000, **39**, 10478–10486.
2. G. Renger, H.-J. Eckert, A. Bergmann, J. Bernarding, B. Liu, A. Napiwotzki, F. Reifarth, H.J. Eichler, *Austral. J. Plant. Physiol.*, 1995, **22**, 167–181.
3. I. Vass, D. Kirilovsky, A.L. Etienne, *Biochemistry*, 1999, **38**, 12786-12794.
4. A. Volgusheva, S. Styring, F. Mamedov, *Proc Natl Acad Sci USA*, 2013, **110**, 7223-7228.
5. S.A. Desouky, M.A. Usama, W.A. Ahmed, *J. Environ. Stud.*, 2011, **7**, 1–8.
6. F.A. Wollman, P. Delepelaire, *J. Cell Biol.*, 2005, **98**, 1–7.
7. Z. Perrine, R. Sayre, *Biochemistry*, 2011, **50**, 1454-1464.
8. H. Gong, Y. Tang, J. Wang, X. Wen, L. Zhang, C. Lu, *Biochim. Biophys. Acta*, 2008, **1777**, 488-495.
9. I. Vass, Govindjee, *Photosynth. Res.* 1996, **48**, 117–126.
10. E, Tyystjärvi, I. Vass, in *Chlorophyll a fluorescence: a signature of photosynthesis*, G.C. Papageorgiou, Govindjee, eds., Kluwer Academic Dordrecht; 2004, 363–388.
11. J.M. Ducruet, *J. Exp. Bot.*, 2003, **54**, 2419–2430.
12. P.V. Sane, *Methods Mol. Biol.*, 2004, **274**, 229–248.
13. T. Pocock, P.V. Sane, S. Falk, N.P. Hüner, *Biochem. Cell Biol.*, 2007, **85**, 721–729.
14. A.W. Rutherford, Govindjee, Y. Inoue, *Proc Natl Acad Sci USA*, 1984, **81**, 1107–1111.
15. G.N. Johnson, A.W. Rutherford, A. Krieger, *Biochim. Biophys. Acta*, 1995, **1229**, 202-207.
16. M. Roncel, I. Yruela, D. Kirilovsky, F. Guerrero, M. Alfonso, R. Picorel, J.M. Ortega, *Biochim. Biophys. Acta*, 2007, **1767**, 694-702.
17. A.G. Ivanov, P.V. Sane, V. Hurry, G. Oquist, N.P. Hunter, *Photosynth. Res.*, 2009, **51**, 85-96.
18. F. Mamedov, M.M. Nowaczyk, A. Thapper, M. Rögner, S. Styring, *Biochemistry*, 2007, **46**, 5542-5551.