Plastoquinol is more active than tocopherol in ROS scavenging during high light stress of *Chlamydomonas reinhardtii*

Beatrycze Nowicka[1], Jerzy Kruk[2]

Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Cracow, Poland: e-mail [1]beatrycze.nowicka@wp.pl, [2] jerzy.kruk@uj.edu.pl

Introduction

Chloroplasts are the main source of ROS in cells of photosynthetic organisms. High light (HL) stress is the main abiotic factor responsible for oxidative stress in plants. Two main processes responsible for ROS generation are electron 'leak' from the electron transport chain (mainly at PS I), leading to superoxide ($O_2^{\bullet-}$), and excitation energy transfer from long-lifetime excited states of chlorophyll, leading to singlet oxygen ($^1O_2$) formation in PS II [1,2]. To limit ROS production, several protective mechanisms have evolved, i.e. nonphotochemical quenching of excited chlorophyll, xanthophyll cycle, ROS detoxifying enzymes and low molecular antioxidants that are able to quench and scavenge ROS [3]. Important chloroplastic lipophilic antioxidants are plastoquinone (PQ)/plastoquinol (PQH_2) and tocopherols (Toc). Plastoquinol, an electron carrier, is also able to inhibit lipid peroxidation and scavenge $O_2^{\bullet-}$, as well as $^1O_2$, similarly to tocopherols [4,5].

In the present study, we have investigated levels of selected prenyllipids in *Chlamydomonas reinhardtii* during HL stress in control cultures and those containing pyrazolate (an inhibitor of *p*-hydroxyphenylpyruvate dioxygenase), FCCP (an uncoupler), D_2O (to extend $^1O_2$ lifetime) and inhibitors of PQ-pool reduction and oxidation, i.e. DCMU and DNP-INT, respectively.

Results

Chlorophyll concentration did not changed significantly during HL treatment, only in the presence of pyrazolate it decreased about 23% (data not shown).

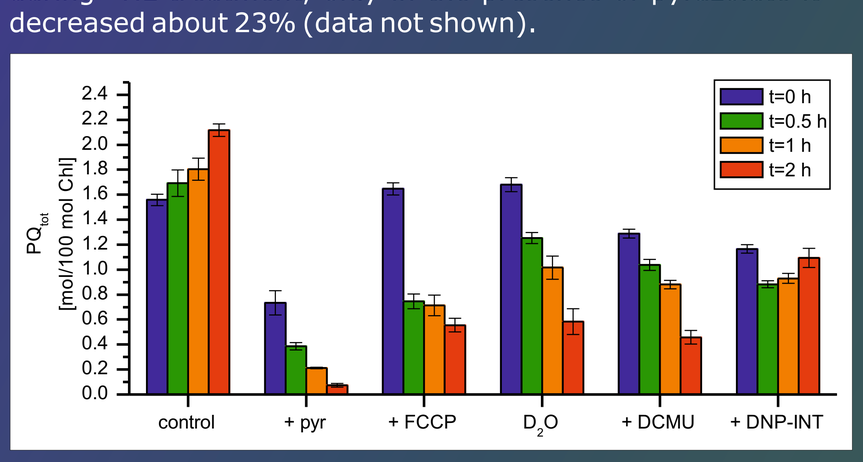

Fig. 1. The effect of high light on total plastoquinone (oxidized and reduced) content in the control culture, in the presence of 5 μM pyrazolate (pyr), 1 μM FCCP, in D_2O, 50 μM DCMU and 10 μM DNP-INT (n=3-4, ±SE), PQ_{tot}, total plastoquinone.

Before high light (HL) stress, the level of total PQ (PQ + PQH_2) in the control culture was about twice as high as that in the culture grown with pyrazolate (Fig.1). During HL exposure, PQ_{tot} increased only in the control (about 35%). In the presence of DNP-INT, it decreased transiently to increase back to the initial level. In other cultures, a significant decrease of the total level of PQ was observed. The decrease was most pronounced in the presence of pyrazolate (90%). In the case of FCCP, D_2O and DCMU, it was about 65%. For FCCP, the highest loss (55%) of total PQ was observed during the first 30 min of HL exposure, whereas in the other two cultures, the level of total PQ gradually decreased (Fig.1.)

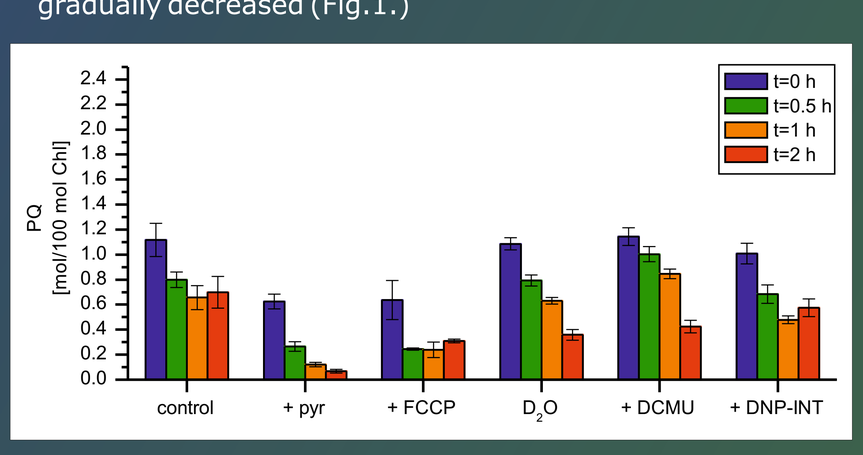

Fig. 2. The effect of high light on oxidized plastoquinone content in the control culture, in the presence of 5 μM pyrazolate (pyr),1 μM FCCP, in D_2O, 50 μM DCMU and 10 μM DNP-INT (n=3-4, ±SE), PQ, plastoquinone.

During HL stress, the level of oxidized PQ decreased in all the cultures (Fig. 2). In the case of control culture and DNP-INT, it decreased about 40%, for D_2O and DCMU ca. 60%, for FCCP - 50%, whereas in the presence of pyrazolate the decrease was about 90%.

Acknowledgement

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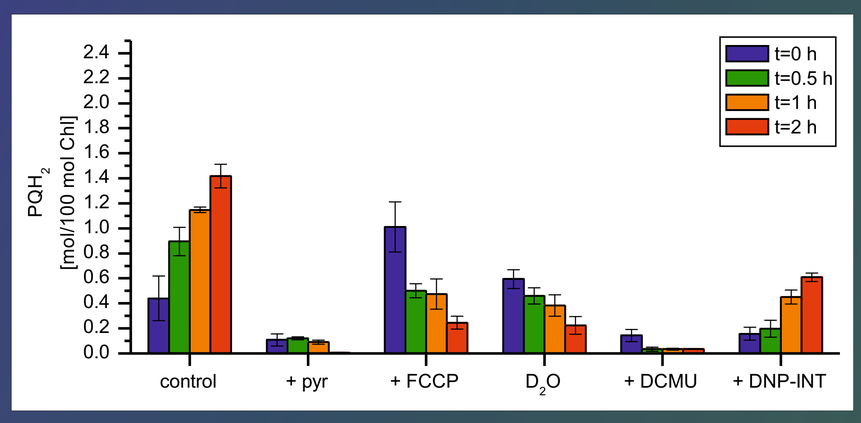

Fig.3. The effect of high light on plastoquinol content in the control culture, in the presence of 5 μM pyrazolate (pyr), 1 μM FCCP, in D_2O, 50 μM DCMU and 10 μM DNP-INT (n=3-4, ±SE), PQH_2, plastoquinol.

The content of PQH_2 in the control culture increased threefold during HL stress (Fig. 3). PQH_2 level increased also considerably in the presence of DNP-INT. In the other cultures, the decrease of PQH_2 content was observed. In the case of pyrazolate and DCMU, the low initial level of PQH_2 was observed that decreased further during HL stress. In D_2O, PQH_2 level decreased gradually. For FCCP, it decreased more than 50% during the first 30 min of light exposure. The levels of PQ and PQH_2 after 2 h of illumination were similar for FCCP and D_2O cultures.

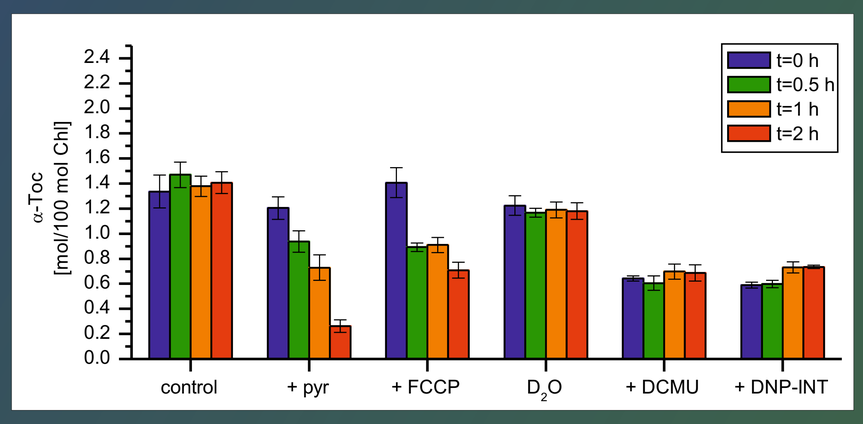

Fig. 4. The effect of high light on α-tocopherol content in the control culture, in the presence of 5 μM pyrazolate (pyr), 1 μM FCCP, in D_2O, 50 μM DCMU and 10 μM DNP-INT (n=3-4, ±SE), α-Toc, α-tocopherol.

In the case of α-Toc, HL stress was not affecting its level in the control culture, in D_2O, as well as in the culture with DCMU (Fig. 4). In the presence of DNP-INT, the small increase in α-Toc level was observed, while in the case of pyrazolate, α-Toc level decreased considerably. The loss of α-Toc was also observed in the culture with FCCP, especially during the first 30 min of illumination.

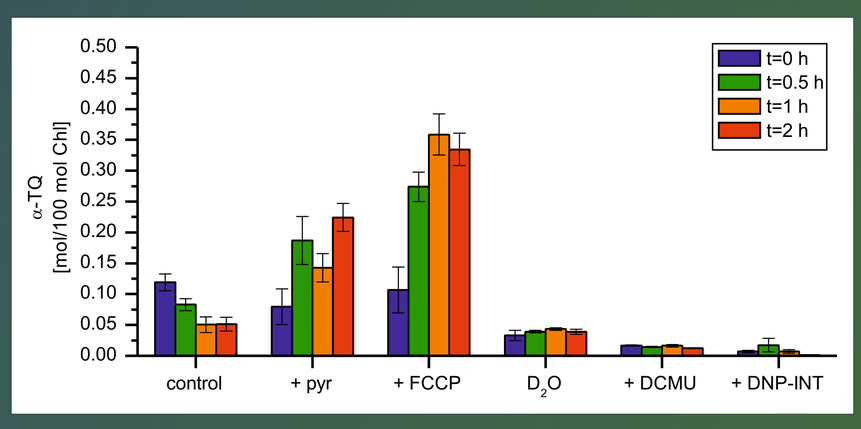

Fig.5. The effect of high light on α-tocopherolquinone content in the control culture, in the presence of 5 μM pyrazolate (pyr),1 μM FCCP, in D_2O, 50 μM DCMU and 10 μM DNP-INT (n=3-4, ±SE), α-TQ, α-tocopherolquinone.

In the investigated variants, the level of α-TQ decreased slightly in the control culture, while the increase was observed for pyrazolate and FCCP and this increase corresponded to 15% and 30% of α-Toc loss in these cultures, respectively (Fig. 5).

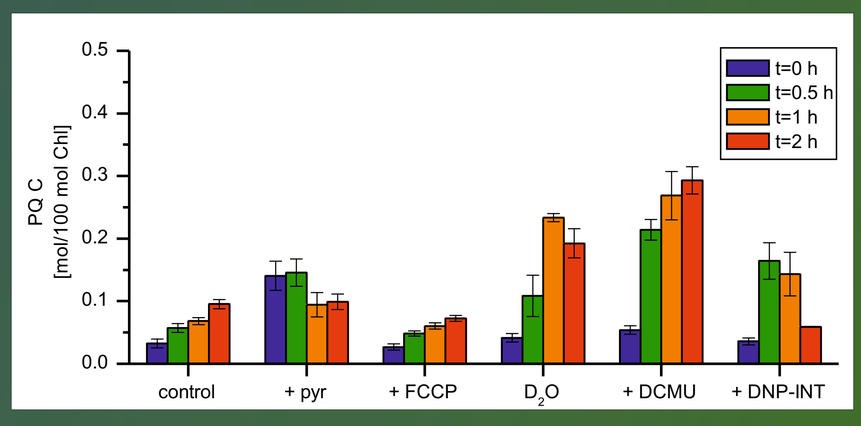

Fig.6. The effect of high light on plastoquinone C content in the control culture, in the presence of 5 μM pyrazolate (pyr),1 μM FCCP, in D_2O, 50 μM DCMU and 10 μM DNP-INT (n=3-4, ±SE), PQ C, plastoquinone C.

The initial level of PQ-C was similar in all the investigated cultures, except for pyrazolate, where it was fourfold higher (Fig. 6). For control and FCCP culture, its level increased about 2.5 times during HL stress. The significant increase was observed in cultures with D_2O and DCMU. For DCMU, PQ-C level increased 5.5 times, which corresponds to about 30% of total PQ loss.

In the case of both pyrazolate-FCCP and D_2O-FCCP cultures, Chl conc. decreased significantly (Fig. 7). In both cultures, total PQ and α-Toc levels dropped nearly to zero during HL stress and that was accompanied by the increase in α-TQ levels (Fig. 8).

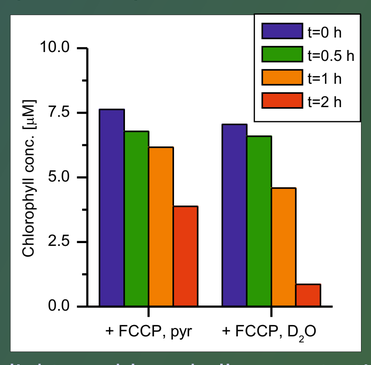

Fig. 7. The effect of high light on chlorophyll concentration in the presence of 5 μM pyrazolate (pyr) and 1 μM FCCP; in D_2O in the presence of 1 μM FCCP.

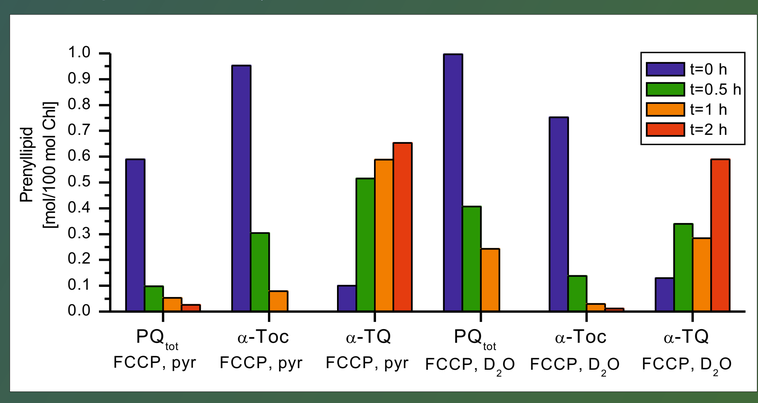

Fig. 8. The effect of high light on prenyllipids content in the presence of 5 μM pyrazolate (pyr) and 1 μM FCCP; in D_2O in the presence of 1 μM FCCP, α-Toc, α-tocopherol; α-TQ,α - tocopherolquinone, PQ_{tot}, total plastoquinone.

Conclusions

- The effect of HL stress on prenyllipids level observed for the control culture and that containing pyrazolate were comparable to those published recently [6].
- The control culture was able to acclimate to HL (no pigment loss) and this was accompanied by increase in PQ pool
- Inhibition of PQ and α-Toc biosynthetic pathways by pyrazolate resulted in slower growth of the culture and decreased level of PQ pool was observed, whereas the level of α-Toc remained unchanged
- PQ/PQH_2 and α-Toc are degraded under HL conditions - this is indicated by experiments with pyrazolate
- Addition of an uncoupler (FCCP) caused significant effect on prenyllipids level, especially during the first 30 min of HL stress, suggesting that proton gradient is required for short-time-responding protection mechanisms, such as nonphotochemical quenching
- The presented data indicate that PQ/PQH_2 is more effective in ROS detoxification than α-Toc
- In the presence of FCCP, α-TQ formation was enhanced, probably because of impaired regeneration reactions of α-Toc under uncoupled conditions
- Prolonged $^1O_2$ lifetime and inhibition of PQ reduction favour PQ-C formation. PQ-C is thought to be a product of reaction of $^1O_2$ with PQ.
- In the presence of pyrazolate + FCCP and D_2O + FCCP significant stress occurred and under such conditions a considerable amount of α-Toc was oxidized to α-TQ.

Materials and methods

Ch. reinhardtii was grown in HS medium at 25C in low light (70 μE $m^{-2}s^{-1}$). For experiments, 7-10 days old cultures were used where chlorophyll (Chl) conc. was 20-35 μg/ml. Day before HL exposure, Chl conc. was measured, then culture was set to Chl conc. of 6 μg/ml. In case of cultures with pyrazolate, pyrazolate was added. The cultures were grown with CO_2 (5% in air) for 18 h under 70 μE $m^{-2}s^{-1}$ illumination. Afterwards, the cultures were exposed to HL (2000 μE $m^{-2}s^{-1}$) for 2 hours. FCCP, DCMU or DNP-INT were added to the medium 10 min before HL exposure. In D_2O experiments, the cultures were centrifuged (3 min x 600*g*) and resuspended in D_2O-based HS medium.

For Chl measurements, cell suspension was centrifuged (5 min x 9000*g*), the pellet was extracted with acetone, centrifuged again (2 min x 9000*g*) and Chl conc. in the supernatant was determined according to [7]. The cell suspension for HPLC measurements was centrifuged (5 min x 9000*g*), the pellet was extracted with acetone, centrifuged again (2 min x 9000*g*), then the supernatant was evaporated and resuspended in methanol. HPLC analysis of PQ, PQH_2 and α-Toc was performed in the following system: C_{18} RP column, eluent - methanol:hexan (340:20, v/v), flow rate of 1,5 ml/min, absorption detection at λ=255 nm, fluorescence detection at λ_{ex} =290 nm, λ_{em}=330 nm. HPLC analysis of α-TQ and PQ C was performed using C_{18} RP column, platinum postcolumn (for reduction of quinones), eluent - methanol:water (99:1, v/v) or methanol, flow rate of 1 ml/min, fluorescence detection at λ_{ex} =290 nm, λ_{em}=330 nm.

References

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