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The role of photorespiration during H_2 photoproduction in *Chlorella prototheocoides* under nitrogen limitation

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Key message **Photorespiration in *Chlorella prototheocoides* plays an important role in photoprotection of photosystem (PS) II in the late phase of H_2 photoproduction, allowing PSII to supply more electrons to hydrogenase.**

Keywords H_2 photoproduction · Nitrogen limitation · Photodamage · Photorespiration

Introduction and aims

Under anaerobic conditions, many microalgae are able to produce hydrogen (H_2) in the light thanks to the activity of Fe–Fe hydrogenase, which is coupled to the reducing site of the photosynthetic electron transport chain and accepts electrons directly from reduced ferredoxin (Fd_{red}) (Ghirardi et al. 2007). In our previous work, we demonstrated that nitrogen (N) limitation was a decisive factor in the induction of H_2 photoproduction in *Chlorella prototheocoides*. Under nitrogen limitation (LN), the blocking of electron transfer and oxygen evolving complex injury resulted in reduced efficiency of photosystem (PS) II photochemical activity and attenuation of PSII oxygenic activity, which then favored a rapid establishment of anaerobiosis, followed by induction of high hydrogenase activity (Zhang et al. 2014). However, the mechanisms of long-term H_2 photoproduction by N-limited *C. prototheocoides* are still not clear.

Photorespiration is a consequence of the oxygenation of ribulose-1,5-bisphosphate (RuBP) catalyzed by RuBP carboxylase/oxygenase (Rubisco) (Wingler et al. 2000). Figure 1a shows the flexible interrelation between photorespiration and H_2 photoproduction. There is a delicate metabolic equilibrium between photorespiration and H_2 photoproduction. In this study, the role of photorespiration during H_2 photoproduction in *C. prototheocoides* under N limitation will be clarified. Understanding the essence of interactions between photorespiration and H_2 photoproduction would provide some new insights into mechanisms of long-term H_2 photoproduction in N-limited *C. prototheocoides*.

Methodological approach

Freshwater *C. prototheocoides* was used in the experiment. The algae were grown in regular TAP (Tris–Acetate–Phosphate) medium or modified TAP medium with a low concentration of ammonium (Zhang et al. 2014). The ammonium concentration in standard TAP medium was 7 mM, and in the modified TAP medium was about 0.35 mM. *C. prototheocoides* was pre-cultured in TAP medium at pH 7.2–7.4, 25 ± 1 °C for 5 days. Algae were illuminated under a 14 h:10 h (light:dark) photoperiod. The light intensity was 25–30 μmol m^{-2} s^{-1}.

Algal cells at stationary phase were centrifuged then resuspended. The TAP culture and LN culture were inoculated at a cellular concentration of about 8×10^6 cells mL^{-1} and 2.5×10^7 cells mL^{-1}, respectively. Then, the cultures were transferred into 20 mL glass tubes sealed

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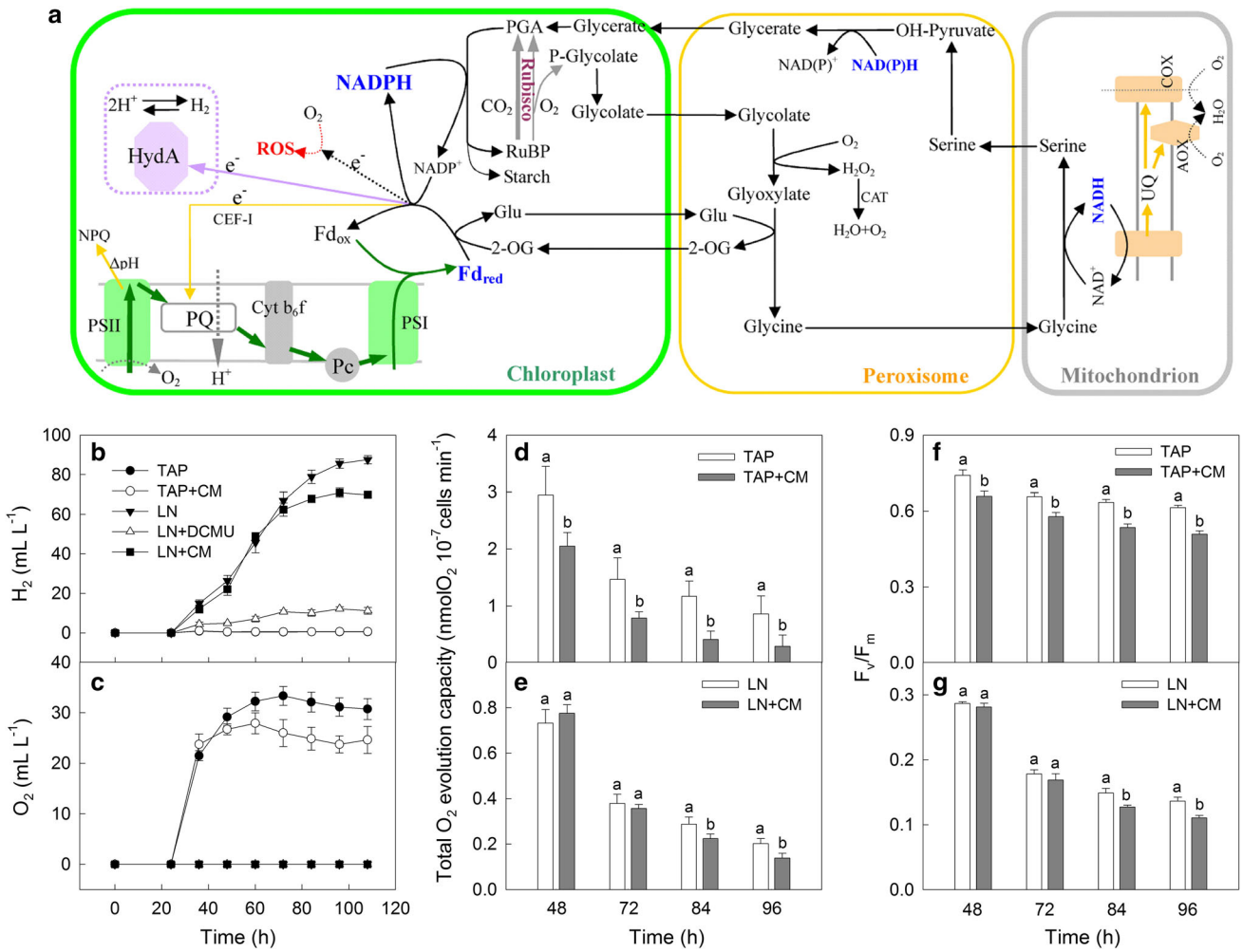

Fig. 1 **a** The flexible interrelation between photorespiration and H_2 photoproduction. **b–g** The photoproduction of H_2 and O_2, the total O_2 evolution capacity and the maximal photochemical efficiency of PSII (F_v/F_m) in *C. protothecoides* cultured in TAP or LN medium in the presence or absence of inhibitor (CM or DCMU). Mean ± SE of five replicates are presented. *Different letters* indicate a significant difference between the CM treatment and control at $P < 0.05$. *2-OG* 2-oxoglutarate, *AOX* alternative oxidase, *CAT* catalase, *CEF-I* cyclic electron flow around PSI, *CM* carboxymethoxylamine, *COX* cytochrome oxidase, *DCMU* 3-(3,4-dichlorophenyl)-1,1-dimethylurea, *Fd* ferredoxin, *Glu* glutamate, *HydA* hydrogenase, *LN* nitrogen limitation, *NPQ* non-photochemical quenching, *Pc* plastocyanin, *PGA* 3-phosphoglycerate, *PQ* plastoquinone, *PSII/I* photosystem II/I, *ROS* reactive oxygen species, *Rubisco* ribulose 1,5-biphosphate carboxylase/oxygenase, *RuBP* ribulose 1,5-biphosphate, *UQ* ubiquinone

with butyl rubber stoppers. The volume of head space in each vial was approximately 3.5 mL. Subsequently, sealed cultures were kept under continuous illumination from above at 35–40 μmol m^{-2} s^{-1}, at 25 ± 1 °C. After 24 h of incubation, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or 0.4 mM carboxymethoxylamine (CM) were added to the experimental tubes, and the headspaces of the vials were flushed with argon.

The H_2 and O_2 contents were analyzed using a gas chromatograph (GC112A, China) according to Zhang et al. (2014). Photosynthetic O_2 evolution capacity was measured with a Clark-type O_2 electrode (Hansatech Instruments, UK) according to Zhang et al. (2014). Maximal photochemical efficiency of PSII (F_v/F_m) was measured using an FMS-2 pulse modulated fluorometer (Hansatech Instruments, UK) according to Zhang et al. (2014).

Findings

The hydrogenase is inactivated by O_2 and thus its expression requires anaerobiosis (Ghirardi et al. 2007). No H_2 gas was detected in the TAP *C. protothecoides* culture during the process of incubation (Fig. 1b), because the produced O_2 (Fig. 1c) inhibited the activity of hydrogenase. *C. protothecoides* cells grown under LN conditions generated

a large amount of H_2 after 24 h of incubation (Fig. 1b), because the LN culture completely consumed atmospheric O_2 and was able to sustain anaerobiosis (Fig. 1c).

In LN *C. protothecoides* culture, the photoproduction of H_2 was not changed before 72 h of incubation, but was suppressed significantly after 72 h of incubation during H_2 photoproduction when the photorespiratory pathway was inhibited by carboxymethoxylamine (CM) (Fig. 1b), an inhibitor of the glycine decarboxylase complex (Corpas et al. 2004). So, what was the physiological function of photorespiration during H_2 photoproduction in *C. protothecoides*?

Treatment with CM did not change the total O_2 evolution capacity (Fig. 1e) and the maximal photochemical efficiency of PSII (F_v/F_m) (Fig. 1g) before 72 h of incubation in the LN culture, indicating that 0.4 mM CM had no direct effects on photosynthetic electron transport and photochemistry of PSII. The fact that 0.4 mM CM did not change the H_2 photoproduction before 72 h of incubation in the LN culture (Fig. 1b) and the fact that 0.4 mM CM did not alter in vitro hydrogenase activity (data not shown) suggest that the concentration of CM used in this study had no direct effect on the H_2 photoproduction. Therefore, the observed effects of CM treatments on H_2 photoproduction after 72 h of incubation in *C. protothecoides* were due to the inhibition of photorespiration.

Photorespiration results from the oxygenase reaction catalyzed by Rubisco and serves as a carbon recovery system (Wingler et al. 2000, Fig. 1a). We speculate that the photorespiration could affect H_2 photoproduction in the following ways: (1) consuming O_2, which is favorable for establishment of anaerobiosis and induction of hydrogenase; (2) consuming reducing equivalents and competing with hydrogenase for photosynthetic electrons, which would decrease the H_2 photoproduction; (3) protecting PSII, which is a direct electron source for H_2 photoproduction, from photodamage. It is also demonstrated by our observation that treatment with CM decreased the total O_2 evolution capacity (Fig. 1d) and F_v/F_m (Fig. 1f) significantly in the TAP culture during the experiment.

In LN *C. protothecoides* culture, the inhibition of the photorespiratory pathway did not increase the concentration of O_2 during the incubation (Fig. 1c), suggesting that the decrease in H_2 photoproduction in the late phase (after 72 h) of incubation (Fig. 1b) was not attributable to an increase in the O_2 level. The observation that the inhibition of the photorespiratory pathway did not increase H_2 photoproduction (Fig. 1b) suggests that the photorespiratory pathway might not compete for photosynthetic electrons with hydrogenase. Treatment with CM decreased the total O_2 evolution capacity (Fig. 1e) and F_v/F_m (Fig. 1g) significantly in the late phase (after 72 h) of H_2 photoproduction, which suggests that inhibition of photorespiration decreased photochemistry of PSII, leading to photodamage. PSII is a main source of electrons for hydrogenase, which is indicated by the fact that H_2 photoproduction was suppressed 87.2 % in LN culture when the photosynthetic electron transfer from Q_A^- to Q_B was inhibited by DCMU (Fig. 1b). The suppression in H_2 photoproduction (Fig. 1b) was synchronized with the decrease of photochemistry of PSII (Fig. 1e, g) in the late phase of H_2 photoproduction, which indicates that the decrease of H_2 photoproduction in the LN culture was attributable to severe photodamage of PSII when the photorespiratory pathway was inhibited.

Apart from photorespiration, plants have evolved other defense mechanisms to protect PSII (Niyogi 2000), such as non-photochemical quenching (NPQ) and cyclic electron flow around PSI (CEF-I). In LN *C. protothecoides* culture, the other defense mechanisms might protect PSII against photodamage in the early phase (before 72 h) of H_2 photoproduction. However, photorespiration might be induced to protect the PSII in the late phase (after 72 h) of H_2 photoproduction. This might be why the photoproduction of H_2 was not changed until 84 h of incubation in LN culture when the photorespiratory pathway was inhibited (Fig. 1b). Further studies are needed to address this question.

In conclusion, photorespiration plays an important role in photoprotection of PSII in the late phase of H_2 photoproduction in *C. protothecoides*, allowing PSII to supply more photosynthetic electrons to hydrogenase under N-limited conditions.

Author contribution statement LTZ and LL performed most of the experiments and wrote the manuscript. LTZ and JGL designed the study and revised the manuscript. MLH helped in measuring H_2 and O_2 contents. All authors have read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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