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Research article

Prediction of respective contribution of linear electron flow and PGR5-dependent cyclic electron flow to non-photochemical quenching induction

Ryoichi Sato [a], Hiroyuki Ohta [b,c], Shinji Masuda [b,c,*]

[a] Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, Japan
[b] Center for Biological Resources and Informatics, Tokyo Institute of Technology, Yokohama 226-8501, Japan
[c] Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo 152-8550, Japan

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ABSTRACT

In chloroplasts, regulated formation of the proton gradient across the thylakoid membrane (ΔpH) is important for controlling non-photochemical quenching (NPQ), which is crucial for plants to perform photosynthesis under fluctuating light conditions. The ΔpH is generated by two electron flows: the linear electron flow (LEF) and the cyclic electron flow (CEF). The *Arabidopsis* CEF mutant, *pgr5*, showed significantly lower NPQ values than those observed in WT, indicating that ΔpH, generated by the PGR5-dependent CEF, has a crucial role in controlling NPQ. However, the respective significance of LEF and CEF for ΔpH formation is largely unknown. Here we applied computer simulation to reproduce NPQ induction kinetics and estimate the respective contribution of LEF and PGR5-dependent CEF to the dynamics of ΔpH formation. The results indicate that the contribution of CEF to total ΔpH formation for induction of NPQ varies from 60–80%. The simulation also suggested a role of the PGR5-dependent CEF in accelerating electron transfer in the cytochrome b_6f complex.

1. Introduction

Plants convert sunlight energy to biochemical energy by photosynthesis, a process that sustains most biological activity on Earth. Therefore, controlling photosynthesis is critically important for almost all living organisms. On the Earth, sunlight is changeable in its intensity and spectral quality during the day, and plants have to perform photosynthesis with this fluctuating light (Li et al., 2009). Under high light conditions, plants switch on a photoprotective state in the photosynthetic machinery that safely dissipates the excess absorbed energy as heat (Horton et al., 1996). This dissipation of the excess absorbed energy can be monitored by a chlorophyll fluorescence parameter, non-photochemical quenching (NPQ) (Holt et al., 2004). NPQ has four components, which are termed qE (Johnson and Ruban, 2011), qZ (Nilkens et al., 2010), qT (Quick and Stitt, 1989), and qI (Müller et al., 2001). These components have different time scales for induction, indicating that acclimation to high light through NPQ is regulated in a stepwise manner (Nilkens et al., 2010). Among the four NPQ components, qE makes the greatest contribution to total NPQ induction, which is rapidly increased upon light illumination, and reversibly disappears in the dark (Johnson and Ruban, 2011). In the qE-dependent NPQ, chlorophyll fluorescence is quenched in LHCII antenna, although exact mechanisms of this quenching are still being debated (Ruban et al., 2012). qE is activated by an increment in the proton gradient across thylakoid membranes (ΔpH), and is regulated by PsbS, a subunit of photosystem II (PSII) (Johnson and Ruban, 2011). Specifically, acidification of the lumen side of the thylakoid membranes, by the increment in ΔpH formation, results in the protonation of several key amino-acid residues of PsbS that leads to induction of qE-dependent NPQ (Li et al., 2000). An *Arabidopsis* PsbS mutant (*npq4*) shows complete loss of the rapidly forming component of ΔpH-dependent NPQ induction (Li et al., 2000). The induction of qE is also affected by zeaxanthin concentration (Johnson and Ruban, 2011). The maximum qE induction requires not only high ΔpH but also accumulation of zeaxanthin

Abbreviations: PSI, photosystem I; PSII, photosystem II; b_6f, cytochrome b_6f complex; ΔpH, proton gradient across thylakoid membrane; NPQ, non-photochemical quenching; LEF, linear electron flow; CEF, cyclic electron flow; PAM, pulse-amplitude modulation; PQ, plastoquinone; PC, plastocyanin; Fd, ferredoxin; NADPH, nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate.

* Corresponding author. Center for Biological Resources and Informatics, Tokyo Institute of Technology, Yokohama 226-8501, Japan. Tel.: +81 45 924 5737; fax: +81 45 924 5823.
E-mail address: shmasuda@bio.titech.ac.jp (S. Masuda).

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(Niyogi et al., 1998). Zeaxanthin is generated and located in LHCII antenna under high-light condition; thus this carotenoid is considered to be quencher of qE-dependent NPQ (Ruban et al., 2012). In fact, accumulation level of zeaxanthin has relation to qE-dependent NPQ induction level (Johnson and Ruban, 2011). The accumulation level of zeaxanthin is regulated by violaxanthin de-epoxidase (Niyogi et al., 1998). This enzyme is located in thylakoid lumen and activated by lowering pH (Bratt et al., 1995). Hence, qE induction largely depends on the ΔpH formation.

The ΔpH is generated by photosynthetic electron flow. There are two types of photosynthetic electron flow in chloroplasts: linear electron flow (LEF) and cyclic electron flow (CEF) (Johnson, 2011). In the LEF, electrons are transferred from water to $NADP^+$ via PSII, plastoquinone (PQ), the cytochrome b_6f complex (b_6f), plastocyanin (PC), photosystem I (PSI), and ferredoxin (Fd) (Fig. 1) (Nelson and Ben-Shem, 2004). Water oxidation in PSII as well as electron transfer in b_6f result in generation of the ΔpH that is used for ATP synthesis. Electrons, passed through LEF, finally reduce $NADP^+$ to generate NADPH. The ΔpH is also generated by CEF, in which electrons go back to PQ from Fd. Thus, CEF contributes to ATP synthesis without $NADP^+$ reduction; therefore, a certain extent of CEF is important for maintaining the correct ATP/NADPH ratio for control of CO_2 fixation (Munekage and Shikanai, 2005).

There are two principal routes for CEF: the NADH dehydrogenase-like complex dependent pathway and the PGR5-PGRL1 complex dependent pathway (Hertle et al., 2013). The *Arabidopsis pgr5* mutant showed significant reduction in NPQ, indicating that the PGR5-dependent CEF has crucial roles for NPQ control through ΔpH generation (Munekage et al., 2002). In fact, the *pgr5* mutant is very sensitive to fluctuating light conditions (Suorsa et al., 2012). On the other hand, *Arabidopsis* mutants lacking NADPH dehydrogenase dependent CEF activity show normal induction of NPQ (Ishikawa et al., 2008). These results indicate that NPQ induction by CEF is mostly due to PGR5 activity, not NADPH dehydrogenase activity.

Although many components involved in NPQ have been elucidated, the respective contribution and dynamics of LEF and CEF activity during NPQ induction remain largely unknown. In this paper, we estimate the respective reaction dynamics of LEF and PGR5-dependent CEF for qE-dependent NPQ induction. We combine PAM analysis and computer simulation to calculate the induction dynamics of the two electron flows in generating the ΔpH.

2. Results & discussion

2.1. Kinetics of NPQ induction in wild type (WT) and the pgr5 mutant

To estimate the contribution of CEF to NPQ induction, we compared NPQ induction kinetics in WT and the *pgr5* mutant under different light intensities (Fig. 2). Under low light conditions, WT showed high NPQ induction at an early phase (~1 min after light illumination) (Fig. 2A), which rapidly decreased to low levels after ~2 min. In contrast, the *pgr5* mutant did not show such a high NPQ induction at the early phase (Fig. 2A) as shown previously (Munekage et al., 2002). The kinetics of NPQ induction in the *pgr5* mutant was similar to that in the *npq4* mutant. Given that the *npq4* mutant lacks the rapidly forming component of ΔpH-dependent NPQ induction (Johnson, 2011), the observed small NPQ increments in the *pgr5* and *npq4* mutants during the early phase reflect NPQ components other than ΔpH-dependent NPQ induction. The xanthophyll cycle carotenoids, such as zeaxanthin, do not accumulate in dark-adapted leaves (Johnson and Ruban, 2011), indicating that the increment of NPQ in WT during the first 1 min is due to ΔpH-dependent NPQ induction. Low NPQ induction in the *pgr5* mutant at the early phase suggests that the ΔpH generated by LEF is not enough to induce qE under low light conditions (Munekage et al., 2002).

Under high light conditions, WT showed high NPQ induction at the early phase (~1 min), which did not decrease, at least for 10 min (Fig. 2B). Under the high light conditions, the *pgr5* mutant showed small but significant NPQ induction at the early phase (~1 min) (Fig. 2B). The NPQ induction in the *pgr5* mutant indicates that LEF can induce the rapidly forming component of ΔpH-dependent NPQ induction to some extent under high light conditions. The NPQ induction in the *pgr5* mutant decreased within 2 min after illumination, and showed levels similar to the *npq4* mutant (Fig. 2B), indicating that some mechanisms relaxing ΔpH formation are activated in this time range.

To estimate the contribution of CEF and LEF to NPQ induction, we measured NPQ induction kinetics in WT and the *pgr5* mutant at the early phase in more detail (Fig. 3). In this experiment, NPQ was expressed as qE to directly show the extent of ΔpH formation at the early phase. To directly reflect the ΔpH-dependent NPQ induction at the early phase, qE was calculated by subtraction of the NPQ value of the *npq4* mutant from the NPQ value of the WT or the *pgr5*

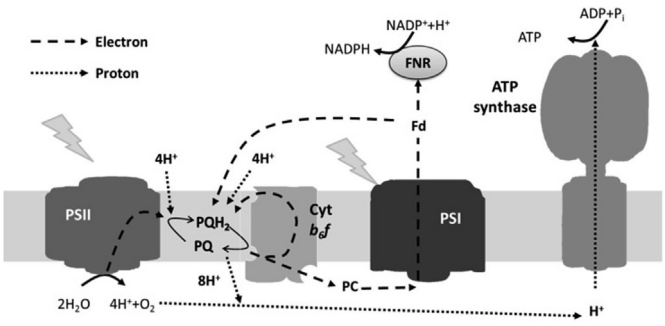

Fig. 1. A schematic representation of electron flow and ΔpH generation in the model. Proton and electron flows are represented by dotted and dashed lines, respectively.

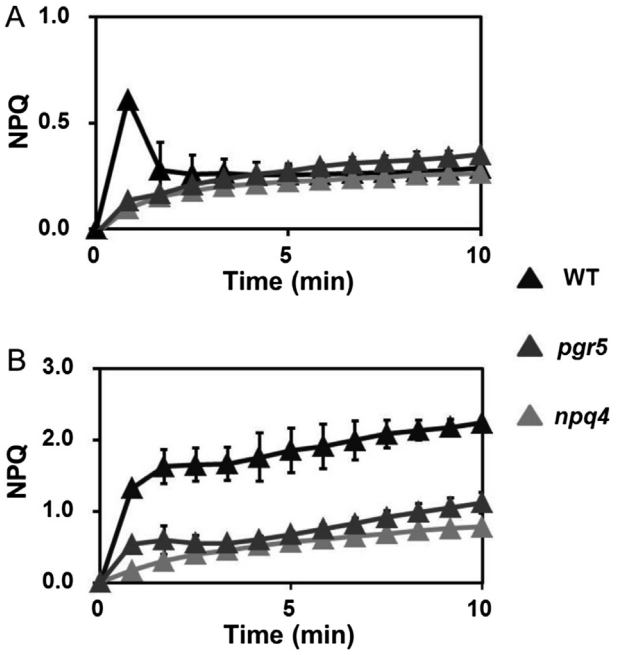

Fig. 2. Induction kinetics of NPQ in WT and *pgr5* and *npq4* mutants under (A) low light conditions (80 μmol m^{-2} s^{-1}) and (B) high light conditions (1200 μmol m^{-2} s^{-1}).