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Role of cyclic electron transport around photosystem I in regulating proton motive force☆

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ABSTRACT

In addition to ΔpH formed across the thylakoid membrane, membrane potential contributes to proton motive force (*pmf*) in chloroplasts. However, the regulation of photosynthetic electron transport is mediated solely by ΔpH. To assess the contribution of two cyclic electron transport pathways around photosystem I (one depending on PGR5/PGRL1 and one on NDH) to *pmf* formation, electrochromic shift (ECS) was analyzed in the *Arabidopsis pgr5* mutant, NDH-defective mutants (*ndhs* and *crr4-2*), and their double mutants (*ndhs pgr5* and *crr4-2 pgr5*). In *pgr5*, the size of the *pmf*, as represented by ECS_t, was reduced by 30% to 47% compared with that in the wild type (WT). A g_H^+ parameter, which is considered to represent the activity of ATP synthase, was enhanced at high light intensities. However, g_H^+ recovered to its low-light levels after 20 min in the dark, implying that the elevation in g_H^+ is due to the disturbed regulation of ATP synthase rather than to photodamage. After long dark adaptation more than 2 h, g_H^+ was higher in *pgr5* than in the WT. During induction of photosynthesis, g_H^+ was more rapidly elevated in *pgr5* than that in the WT. Both results suggest that ATP synthase is not fully inactivated in the dark in *pgr5*. In the NDH-deficient mutants, ECS_t was slightly but significantly lower than in the WT, whereas g_H^+ was not affected. In the double mutants, ECS_t was even lower than in *pgr5*. These results suggest that both PGR5/PGRL1- and NDH-dependent pathways contribute to *pmf* formation, although to different extents. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. Introduction

In the thylakoid membranes of chloroplasts, light-driven electron transport is coupled with the establishment of a proton (H^+) gradient (ΔpH). ΔpH, together with the membrane potential (Δψ), constitutes the total proton motive force (*pmf*), which is ultimately used to drive chloroplast F_0F_1-ATP synthase (ATP synthase). Whereas both components of the *pmf* contribute to ATP synthesis [1,2], only ΔpH can induce the energization-dependent quenching (qE) component of non-photochemical quenching (NPQ) of chlorophyll fluorescence via acidification of the thylakoid lumen [3,4]. Through this qE mechanism, absorbed excessive light energy is safely dissipated as heat from the light-harvesting complexes of photosystem (PS) II. In Mitchell's hypothesis, ΔpH and Δψ are thermodynamically and kinetically equivalent [5,6]. In the mitochondria, almost all of the *pmf* is stored in the form of Δψ because of the low permeability of the mitochondrial inner membrane to ions. In contrast, studies in electrode-impaled giant chloroplasts have indicated that virtually all of the Δψ component of the *pmf* is rapidly dissipated under continuous illumination [7–9], suggesting that the contribution of ΔpH to *pmf* predominates in chloroplasts. Kramer and co-workers argued against this idea by demonstrating that a substantial fraction of *pmf* can be stored as Δψ during steady-state photosynthesis [10,11]. However, a recent study using specific inhibitors of ΔpH and Δψ formation and a mutant lacking the induction of qE came to a different conclusion [12].

The electrochromic shift (ECS) is a bandshift phenomenon in the absorption spectra of some photosynthetic pigments and depends on the presence of an electric field formed across the thylakoid membrane [1]. ECS can be used to probe the trans-thylakoid *pmf* in intact leaves [13]. During steady-state photosynthesis, the size of the *pmf* is determined by the balance between its generation, which depends on photosynthetic electron transport, and its relaxation, which depends mainly on ATP synthase activity [14]. Applying a dark pulse by switching off the actinic light for 1 s during steady-state photosynthesis collapses the light-dependent H^+ influx into the thylakoid

Abbreviations: AL, actinic light; ECS, electrochromic shift; ETR, electron transport rate; Fd, ferredoxin; NDH, NADH dehydrogenase-like complex; NPQ, non-photochemical quenching; PGR5, PROTON GRADIENT REGULATION 5; PGRL1, PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE 1; *pmf*, proton motive force; PS, photosystem; Φ_{PSII}, PSII quantum yield; $P700^+$, oxidized PSI reaction center; PQ, plastoquinone; qE, energization-dependent NPQ

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lumen and can thus be used to elucidate the rate of H^+ efflux via ATP synthase [15]. The amplitude of the light-dark difference in the ECS signal (ECS_t) represents the total size of the *pmf* formed in the light. In addition, the H^+ conductivity of ATP synthase (g_H^+) can be calculated from the kinetics of the initial ECS decay.

Formation of *pmf* depends on both linear and PSI cyclic electron transport. In linear electron transport, *pmf* formation is coupled with O_2 evolution and reduction of $NADP^+$. In contrast, PSI cyclic electron transport generates *pmf* without net accumulation of NADPH by recycling electrons from ferredoxin (Fd) or NADPH to the plastoquinone (PQ) pool [16]. In *Arabidopsis*, PSI cyclic electron transport consists of two partly redundant pathways [17]: The main pathway depends on PGR5 (PROTON GRADIENT REGULATION 5) and PGRL1 (PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE) proteins [18,19] and is sensitive to antimycin A [20,21]. The PGR5/PGRL1-dependent pathway likely corresponds to PSI cyclic electron transport that was discovered by Arnon and co-workers [22]. The other pathway is insensitive to antimycin A and is mediated by the chloroplast NADH dehydrogenase-like (NDH) complex [23–25]. In the *pgr5* mutant, induction of qE is severely impaired, suggesting that PGR5/PGRL1-dependent PSI cyclic electron transport contributes markedly to ΔpH formation, which is needed to induce qE [18,26]. In contrast, a defect in chloroplast NDH does not affect qE induction [23,24]. Despite the minor contribution of chloroplast NDH to qE, double mutants defective in the two PSI cyclic electron transport pathways have severe phenotypes of photosynthesis and plant growth [17]. These results suggest the important role of NDH-dependent PSI cyclic electron transport in the *pgr5* mutant background. How does chloroplast NDH alleviate the *pgr5* phenotype? The exact molecular mechanism is still unclear.

The *pgr5* mutant is defective in *pmf* formation via PSI cyclic electron transport [17,18,26]. ECS analysis has estimated that 13% of ATP output in *Arabidopsis* depends on PGR5/PGRL1-dependent PSI cyclic electron transport [27]. Unexpectedly, however, ECS analysis has also revealed that g_H^+ is enhanced in the *pgr5* mutant [27]. Upregulation of ATP synthase may compensate partially for the reduced ATP synthesis, but it should seriously disturb the regulation of photosynthetic electron transport that is induced via low lumen pH at high light intensities in the wild type (WT) [17,18,26,28,29]. Neither the molecular mechanism nor the physiological meaning of the g_H^+ phenotype of the *pgr5* mutant is clear. In this study, we extended the ECS analysis of *Arabidopsis* mutants defective in PSI cyclic electron transport to further characterize the mysterious g_H^+ phenotype of the *pgr5* mutant and to directly analyze the contribution of chloroplast NDH to *pmf* in the *pgr5* mutant background.

2. Material and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana WT (ecotype Columbia *gl1*) and mutants were grown in soil for 8 to 12 weeks under growth chamber conditions (50 μmol photons m^{-2} s^{-1}, 8 h light/16 h dark cycles at 23 °C).

2.2. Chlorophyll fluorescence measurements

Chlorophyll fluorescence parameters were measured with a MINI-PAM (pulse-amplitude modulation) portable chlorophyll fluorometer (Walz, Effeltrich, Germany) in ambient air at room temperature (25 °C), as described previously [30]. NPQ and the quantum yield of PSII (Φ_{PSII}) were calculated as $(Fm - Fm')$ / Fm' and $(Fm' - Fs)$ / Fm', respectively. Relative ETR was calculated as $\Phi_{PSII} \times$ PFD (photon flux density).

2.3. ECS analysis

The ECS signal was monitored as the absorbance change at 515 nm by using a DUAL-PAM-100 (Walz, Effeltrich, Germany) equipped with a P515/535 emitter-detector module (Walz). Plants were first dark adapted for 15 min and then the detached leaves were analyzed. Except in the analysis in Fig. 3, the ECS signal was obtained after 2 to 3 min of illumination at different actinic light (AL) intensities, and a 1-s dark pulse was applied three times (30 s apart) for technical replicates. In Fig. 2C and Supplementary Fig. S2, longer illumination of AL (15 to 16 min) was used to record steady-state ECS signals. ECS_t, which represents the difference in total *pmf* between light and dark, was estimated from the total amplitude of the rapid decay of the ECS signal during the dark pulse. All ECS_t levels were normalized against the 515-nm absorbance change induced by a single turnover flash (ECS_{ST}), as measured on dark-adapted leaves before recording. This normalization accounted for changes in leaf thickness and chloroplast density between leaves [31]. g_H^+, which reflects the proton conductivity of ATP synthase, was estimated by fitting the first 300 ms of the decay curve with a first-order exponential decay kinetic as the inverse of the decay time constant, as described by Avenson et al. [27].

3. Results

3.1. Effects of PGR5-dependent PSI cyclic electron transport on formation and relaxation of pmf

ECS_t (ECS_t /ECS_{ST}) represents the total size of the *pmf* formed in the light. The contribution of PGR5-dependent PSI cyclic electron transport to *pmf* formation is supported directly by a lower ECS_t observed in the *Arabidopsis pgr5* mutant [27] and in rice *PGR5* knockdown lines [26]. To analyze the impact of the *pgr5* defect on *pmf* in a broader range of light intensities (46 to 1076 μmol photons m^{-2} s^{-1}), the light-intensity dependence of ECS signal parameters were compared between WT and *pgr5* mutant plants (Fig. 1). Consistent with the findings of a previous report by Avenson et al. [27], ECS_t was dramatically reduced in *pgr5* plants at light intensities of 138 μmol photons m^{-2} s^{-1} or more (Fig. 1A). As in rice [26], however, the difference was subtle at a low light intensity of 46 μmol photons m^{-2} s^{-1}, consistent with the normal plant growth at 50 μmol photons m^{-2} s^{-1} [32]. In addition, an increase in the ECS decay rate (g_H^+), which likely reflects the H^+ conductivity of chloroplast ATP synthase, is observed in the *Arabidopsis pgr5* mutant [27]. Consistently, we observed more rapid decay of ECS signals in *pgr5* than in the WT at high light intensities (Supplementary Fig. S1), from which g_H^+ was calculated. At light intensities of 320 μmol photons m^{-2} s^{-1} or more we observed a significant increase in g_H^+, reflecting increased H^+ conductivity of ATP synthase [27], in *pgr5* mutants compared with the WT, whereas g_H^+ changed little in the WT (Fig. 1B). Faster proton efflux from the lumen through the ATP synthase, as indicated by higher g_H^+, partly explains the lower *pmf* in *pgr5* at light intensities higher than 320 μmol photons m^{-2} s^{-1}. At 138 μmol photons m^{-2} s^{-1}, however, the small elevation in g_H^+ cannot solely explain the drastic reduction in ECS_t observed in *pgr5* mutants (Fig. 1).

In Fig. 1, the leaves were exposed to actinic light (AL) at each light intensity for 2 to 3 min. Because three dark pulses were applied at each AL intensity, the first dark pulse was after 2-min and the last one was after 3-min exposure of AL. Induction of the Calvin cycle may influence the ECS signal in this period after long dark adaptation. To assess this possibility, ECS signals were compared after short (2 to 3 min) and long (15 to 16 min) AL exposure (Supplementary Fig. S2). At 138 μmol photons m^{-2} s^{-1}, ECS_t was slightly lower after a long AL exposure than after a short AL exposure in both the WT and *pgr5*, and this process was accompanied by a slight increase in g_H^+. The 2 to 3-min AL exposure may not be long enough to monitor the steady-state levels of g_H^+ at 138 μmol photons m^{-2} s^{-1}, which was not statistically different between *pgr5* and the WT (Supplementary Fig. S2D). This is inconsistent with an independent measurement, in which g_H^+ was slightly but significantly higher in *pgr5* than that in the WT (Fig. 1B). The difference between the genotypes was not statistically significant after long

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