CHAPTER THREE

Alternative and cyclic electron flow: Rate and role in potato leaves

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Abstract Alternative, $J_{\text{Alt}}$ and PSI cyclic, $J_{\text{Cyc}}$, electron transport rates (ETR) were measured in potato leaves. Insignificant $J_{\text{Alt}}$ and $J_{\text{Cyc}}$ rates were detected during light-limited photosynthesis. The result shows that the requirement of the potato ATP synthase is still close to $12\text{H}^+/3\text{ATP}$, not $14\text{H}^+/3\text{ATP}$. During light-saturated photosynthesis ETR through PSII and PSI were significantly faster than ETR for PGA reduction. We suggest that the excess PSI ETR, as well as the excess PSII ETR, are energy-dissipating cycles, not coupled with proton translocation.

Keywords Leaf, cyclic, alternative, electron transport

Introduction

Provided that the Q-cycle is operating and the requirement of ATP synthase is $12\text{H}^+/3\text{ATP}$, only a small ATP deficiency may be caused by requirements for starch synthesis and other secondary metabolism. The discovery of 14 subunits III in the CF$_3$ of the ATP synthase (Seeleit et al. 2000) indicated the possible requirement of $14\text{H}^+/3\text{ATP}$. In this case the proton deficiency of at least 17% must be covered by an additional $\text{H}^+$-coupled electron flow, such as cyclic electron flow around PSI, $J_{\text{Cyc}}$, or linear electron flow to alternative acceptors other than CO$_2$, $J_{\text{Alt}}$. In this work, using wild type and transgenic potato plants with altered expression of plastidic NADP-MDH, we measured $J_{\text{Alt}}$ and $J_{\text{Cyc}}$ with the aim of seeing whether this relatively large gap exists in the ATP/NADPH budget.

Materials and methods

Plants. Tubers of transgenic potato with altered expression levels of chloroplast NADP-malate dehydrogenase (Solanum tuberosum L. cv Désirée, antisense 102, sense 4, Backhausen et al. 1998) were grown in a growth cabinet. Full-grown leaves attached to the plant were used in experiments. The experimental routine consisted of light and CO$_2$ response measurements at different O$_2$ concentrations, essentially as described in (Laisk et al. 2002).
Gas exchange measurements. In the two-channel fast-response leaf gas exchange measurement system (Fast-Est, Tartu, Estonia) the leaf was enclosed in a leaf chamber of 32 mm diameter, gas flow rate of 0.5 mmol s\(^{-1}\). In experiments leaf temperature was between 22\(^\circ\)C and 23\(^\circ\)C. CO\(_2\) uptake and transpiration were measured, dissolved carboxylation site CO\(_2\) concentration was calculated considering the mesophyll liquid phase diffusion resistance. Oxygen evolution was measured in the same flow-through system at the background concentration of 50 \(\mu\)mol O\(_2\) mol\(^{-1}\) using a Zr-cell analyzer Ametek S-3A (Thermox, Pittsburgh, PA, USA). The simultaneous O\(_2)/CO\(_2\) measurements were calibrated with an error of <1% (Oja et al. 2007). The rate of electron transport for PGA reduction, \(J_{C}\), was calculated considering RuBP carboxylation and oxygenation (Laisk et al. 2002).

Chl fluorescence measurements. Chl fluorescence was measured with PAM-101 and ED-101 emitter-detector (H. Walz, Effeltrich, Germany), applying corrections for PSI fluorescence, crosensitivity, detector saturation and fluorescence unsaturation during the saturation pulses. PSII electron transport, \(J_{F}\), was calculated on the basis of the quantum yield of PSII considering excitation partitioning to PSII, found from CO\(_2\) exchange and fluorescence measurements at strictly limiting PADs (Laisk et al. 2002).

Leaf transmittance at 810 nm. A new single-beam spectrophotometer FE-810 (Fast-Est, Tartu, Estonia) having the noise level of 0.1% of the typical full redox signal was designed for the measurement of leaf transmittance changes. The nonlinear 810 nm signal change per e\(^{-}\) arriving at P700\(^{+}\) and redox-equilibrated PC\(^{+}\) was calibrated from the oxidative titration (Oja et al. 2003). Electron transport rate through PSI, \(J_{F}\), was calculated from the post-illumination re-reduction rate of PSI donor side electron carriers.

**Results**

No significant differences between the differently MDH-expressing potato plants in the growth rate and routinely measured photosynthetic parameters of leaves were observed, indicating that the actual NADP-MDH electron flow rates were smaller than the full enzyme capacities even in the MDH-deficient line (Backhausen et al. 1998).

Evaluating the alternative reduction rates from simultaneous measurements of CO\(_2\) uptake and O\(_2\) evolution. The low O\(_2\) concentration of 50 \(\mu\)mol L\(^{-1}\) blocked RuBP oxygenation and Mehler-type O\(_2\) reduction during these measurements. We expected that the reduction of oxaloacetate plus nitrite along with PGA would cause faster O\(_2\) evolution compared to CO\(_2\) uptake. Contrary to the expectation, both rates varied in an exactly equal manner at all light intensities and CO\(_2\) concentrations independent of the genetic treatment (Fig. 1).

PSII electron transport. The calculated PSII ETR, \(J_{F}\), was very close to the electron transport supporting PGA reduction, \(J_{C}\), at limiting PAD, but at high PADs \(J_{F}\) continued to increase, saturating at a significantly higher rate than \(J_{C}\) (Fig. 2). The difference \(J_{F}-J_{C}\) close to zero at limiting light, was typically 30–40 \(\mu\)mol e\(^{-}\) m\(^{-2}\) s\(^{-1}\) at light saturation. The difference \(J_{F}-J_{C}\) remained constant or slightly decreased when CO\(_2\) and O\(_2\) concentrations were decreased. Since no alternative reductions were detected from the parallel O\(_2)/CO\(_2\) exchange measurements, the fast flux \(J_{F}-J_{C}\)

![Fig 1 Simultaneous measurements of CO\(_2\) uptake and O\(_2\) evolution in a potato leaf. Light (open symbols) and CO\(_2\) responses (filled symbols) of both rates were measured simultaneously and steady-state O\(_2\) evolution rate was plotted against CO\(_2\) uptake rate](image-url)
did not arrive at the PSI acceptor side, but most likely was a dissipative cycle around PSII, activated at light saturation.

**PSI electron transport.** At limiting PADS $J_1$ was closely equal to $J_C$, indicating no cyclic electron transport (Fig. 2). At high PADS, when $J_C$ became light-saturated, $J_1$ continued to increase, exceeding $J_C$ by 45–60 µmol e$^{-}$ m$^{-2}$ s$^{-1}$ without correlation with the expression of MDH. The difference $J_1 - J_C$ remained fast and even increased when the linear rate, $J_C$, was limited by low CO$_2$ and O$_2$ concentrations.

**Discussion**

In the simultaneous O$_2$/CO$_2$ exchange measurements we eliminated RuBP oxygenation and Mehler-type O$_2$ reduction using almost completely O$_2$-free gas. We expected to see excess O$_2$ evolution serving for nitrite plus oxaloacetate reduction, but we did not detect any excess O$_2$ evolution, independent of the expression of MDH. Since CO$_2$ evolution from the Krebs cycle was not inhibited by anoxia during the short exposures, there could still be some very slow reduction of oxaloacetate if CO$_2$ evolution from the Krebs cycle became inhibited proportionally to the oxaloacetate reduction, so that the resulting change in the net CO$_2$ uptake was independent of the electron acceptor, whether it was PGA or oxaloacetate (Oja et al. 2007). Such a slow $J_{cab}$ could cover the ATP necessity for starch and protein synthesis in chloroplasts, but not compensate for the missing 17% of ATP if 14H$^+$/3ATP is the requirement of the chloroplast ATP synthase.

PSI electron flow rates, $J_F$, calculated from the post-illumination kinetics of the deconvoluted 810 nm signal, were very close to the PGA reduction rates, $J_C$, eliminating PSI cyclic electron transport during light-limited photosynthesis. Since the highest light-limited photosynthetic rates were 50–80% of the light-saturated rates, certainly no PSI cyclic electron transport was observed that could cover the proton deficiency of 17%, expected if 14H$^+$/3ATP were required. Therefore, either the number of CF$_0$ subunits was still 12 in the chloroplast ATP synthase of potato, but not 14 as observed in spinach chloroplasts (Seeleert et al. 2000), or the number of CF$_0$ subunits does not necessarily indicate the proton requirement per turn of the rotor, which is still 12H$^+$/3ATP.

**Energy-dissipative cycle around PSI during light-saturated photosynthesis.** The difference $J_F - J_C$, nearly zero when photosynthesis was light-limited, significantly increased when photosynthesis became light-saturated. When CO$_2$ and O$_2$ concentrations were varied, the rate $J_F - J_C$ was about constant, not proportional to the linear rate $J_C$, as expected if the cyclic rate supported a certain fraction of proton pumping. We suggest that the relatively fast cyclic electron transport observed at light saturation is a proton-uncoupled energy-dissipative cycle around PSI. As to the possible mechanism, there is a transmembrane reducing equivalent transfer system in higher plant chloroplasts, involving a membrane-anchored thioredoxin-like protein HCF164 faced to thylakoid lumen side. Chloroplast thioredoxin m is the source of reducing equivalents for reduction of HCF164 and the candidates for electron acceptors are the subunit PSI-N, as well as Cyt f and Rieske FeS protein (Motohashi and Hisabori 2006). Although its rate has not yet been measured in vitro, this is the only known transmembrane electron transfer in vitro.
transport pathway that can short-circuit electrons from the acceptor side to the donor side of PSI uncoupled from proton translocation.

In potato leaves at the light saturation of photosynthesis our Chl fluorescence measurements confirmed the presence of extra electron flow through PSII, \( J_C - J_F \) that could not be the flow to alternative acceptors. We have suggested that this extra PSII ETR, not arriving at the acceptors beyond PSI, is an energy-dissipative cycle around PSII (Laisk et al. 2006).

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References


