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New steps in the regulation of photosynthesis: the influence of CF0-CF1 ATP synthase conductivity on the sensitivity of antenna down-regulation.

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Introduction

Acidification of the thylakoid lumen by electron transfer-driven proton transfer triggers the dissipation of excitation energy from the pigment beds associated with photosystem (PS) II, and thus, down-regulates light capture (see review in Kramer et al., 1999). In the simplest possible version of this model, in the absence of changes in the stoichiometry of H+/e- is constant (see Sackstder et al. 2000), g_{ATP}, or in the sensitivity of the antenna to lumen pH, a constant relationship should be observed between electron transfer and the initiation on down-regulation.

While this model may account, at least qualitatively, for down-regulation under many conditions, it runs into problems when photosynthesis is limited by the availability of PSI electron acceptors (Heber and Walker 1992, Asada 1996, Kramer et al. 1999). In this case, electron transfer chain (ETC) may become over-reduced before the lumen could be sufficiently acidified to initiate down-regulation, leading to rapid photoinhibition. Since plants are well-adapted to such conditions, it is clear that the simple model must be modified.

We hypothesized that g_{ATP} could be altered (e.g. by changes in substrate or product levels or by regulation) to change the relationship between electron transfer rate and proton motive force (*pmf*) thus changing the sensitivity of down-regulation. New spectroscopic tools developed in our laboratory (Kramer and Sacksteder 1998, Sacksteder et al. 2000, Sacksteder and Kramer 2000, Cruz et al. 2001), allow us to estimate the extent of *pmf* and g_{ATP} in intact plants under steady-state conditions, allowing us to test this hypothesis.

Materials and Methods

Nicotiana tabacum (tobacco) wild type (*Xanthi*) was grown in a greenhouse with a midday light intensity of about 900 μmol photons·m^{-2}·s^{-1}, as described previously (Sacksteder and Kramer 2000). Mutant tobacco plant, anti-SSu, in which an antisense gene was introduced against rbcS mRNA for the small subunit of Rubisco (Hudson et al. 1992) was grown in a growth chamber with a light intensity of 800 μmol photons·m^{-2}·s^{-1}, with a 8/16 hours day/night cycle, at 24/18°C, with 2000 ppm CO_2. Gas exchange measurements showed Rubisco activity in the mutant was about 15 % of wild type (not shown). Young, fully expanded, intact leaves were used for all the measurements. Steady-state rates of photosynthetic electron transfer and proton flux changes through the ATP synthase were estimated by following the absorbance changes upon rapid light-to-dark transitions, using our house constructed diffused optics kinetic spectrophotometer (DOFS) (Kramer and Sacksteder 1998). Constant actinic light was provided by a 1000-W xenon arc lamp (Oriel Instruments,

Stratford, CT). The transthylakoid electric field, Δψ, generated by movement of protons through the ATP synthase, and changes in the linear electron transfer chain, were measured via the electrochromic shift (ECS), and fluorescence changes (Sacksteder and Kramer 2000; Sacksteder et al. 2000). Saturation pulses were *ca*. 2 s of either 8000 or 4600 μmol photons·m^{-2}·s^{-1} white light and ϕ_{II} was calculated as (*Fm'-Fs*)/*Fm'* (Genty et al. 1990).

Results

Figure 1 A and B shows down-regulation (estimated as *Fm'/Fm*) as a function of electron transfer rates (estimated by i*ϕ_{ii}) at the range of [CO_2] (from 0 to 400 ppm) and light intensities (from 45 and 1300 μmol photons·m^{-2}·s^{-1}) in WT (Fig. 1A) and a low-Rubisco mutant (Fig. 1B) of tobacco. A very scattered relationship resulted from the interplay of two tendencies: 1) As light intensity increased, at constant [CO_2] *Fm'/Fm* tended to decrease as i*ϕ_{ii} increased. 2) On the other hand, at constant light intensity, changing [CO_2] tended to increase in *Fm'/Fm* as i*ϕ_{ii} was increased. This clearly demonstrates that the 'simple model' described above is incorrect.

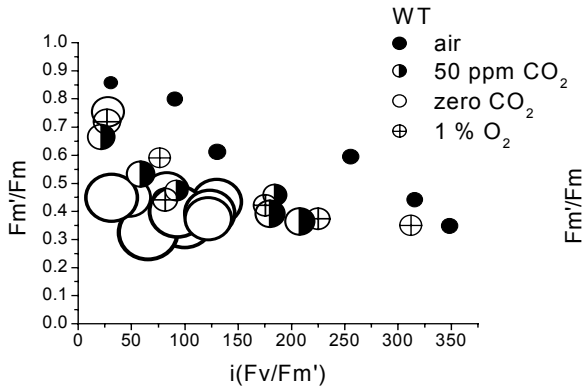


Fig. 1A

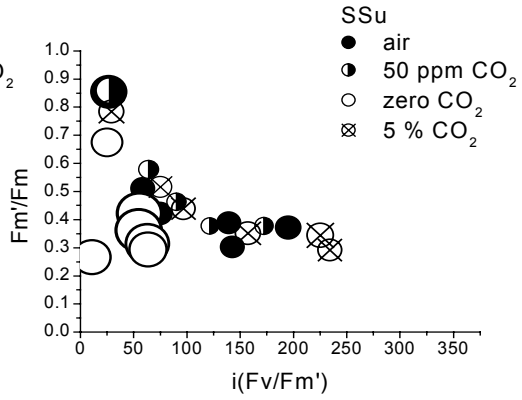


Fig. 1B

The diameter of the symbols in Fig. 1 was set proportional to the $t_{1/2}$ for decay of the ECS upon a light-dark transition from steady-state. The smallest diameter represented ca. 17 ms, while the largest represented 60 ms. This parameter should be a good estimator of $1/g_{ATP}$ since H^+ movement is pseudo-first order. In both WT and mutant plants, lowering [CO_2] increased $t_{1/2}$, indicating a decrease in g_{ATP}. In the mutant plant, at 400 ppm CO_2, down-regulation was more sensitive to electron transfer and $t_{1/2}$ for ECS was longer. Thus, restriction of carbon fixation by lowering either [CO_2] or Rubisco resulted in similar behaviors.

Moreover, the data in Figs. 1 A and B show a strong relationship between $t_{1/2}$ and the relationship between down-regulation and electron transfer. Apparently, as [CO_2] was lowered, g_{ATP} decreased, leading to a higher *pmf* (or ΔpH) and thus down-regulation at a given electron transfer rate.

An alternative interpretation is that the sensitivity of down-regulatory processes to lumen pH are, themselves, altered by [CO_2]. Figure 2 shows a nearly constant relationship between down-regulation (*Fm'*/*Fm*) and ECS_t, the extent of the ECS signal upon the light-dark transition, which we have previously found to be a good estimator of *pmf* (Cruz et al. 2001). This indicates that, to a first approximation, the relationship between electron transfer, but not that between *pmf* and antenna regulation, is altered by [CO_2].

As working models, we propose that the observed changes in g_{ATP} reflect: model 1)

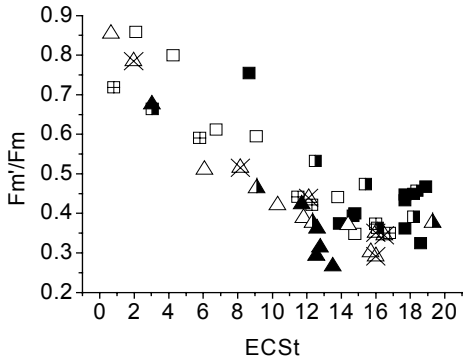


Fig. 2

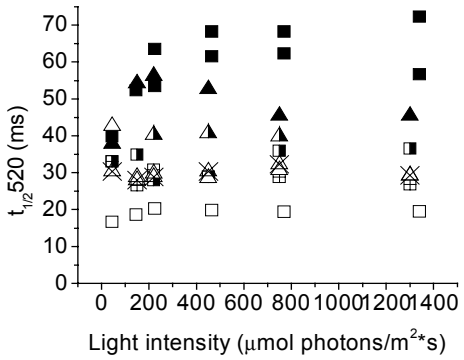
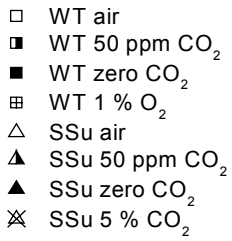


Fig. 3



alterations in substrate product (i.e. ATP, ADP and Pi) concentrations, or model 2) regulation of the ATP synthase. A remarkable feature of g_{ATP}, illustrated in Fig. 3, is its relative insensitivity to changes in light intensity, whereas [CO_2] had large effects over the entire range of light. Significant changes were only observed below 100 µmol photons·m^{-2} s^{-1}. At first glance, they appear to support model 2. However, earlier measurements have shown both assimilatory power and the ratio of ATP/ADP to also be relatively insensitive to light intensity above about 100 µmol photons·m^{-2}.s^{-1} (e.g. Heber et al. 1986).

Conclusions

The relationship between electron transfer rate and down-regulation depends upon whether photosynthetic flux is altered by changing light intensity or the turnover rate of Rubisco. We have shown that this alteration can be accounted for by changes in g_{ATP}, which alters the relationship between electron transfer and *pmf*, in turn affecting down-regulation by modulating lumen pH. It is yet unclear what process modifies g_{ATP}, though substrate/product levels or direct regulation are definite possibilities. In addition, in related work (see Kramer et al. in these Proceedings), we show that changing the fraction of *pmf* stored as ΔpH and Δψ can also influence the relationship between electron transfer and down-regulation.

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